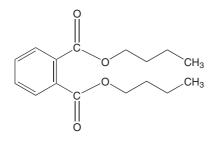
Dibutyl Phthalate

David R Wallace

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 84-74-2
- SYNONYMS: Dibutyl-1,2-benzenedicarboxylate; o-Benzenedicarboxylic acid; Dibutyl ester; DBP
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Plasticizer and softener; Aromatic dicarboxylic acid ester
- CHEMICAL FORMULA: C₁₆H₂₂O₄
- Chemical Structure:



Uses

Dibutyl phthalate has multiple uses in a variety of materials. Primary uses for dibutyl phthalate are to soften and increase plastic flexibility, for example, in shower curtains, raincoats, food wraps, and car interiors to name a few. It has been used in insect repellents and as a solvent for perfume oil and resins. Dibutyl phthalate can be used as a plasticizer in nitrocellulose lacquers, elastomers, explosives, nail polish, and solid rocket propellants. Other uses include perfume fixative, textile lubricating agent, safety glass additive, printing inks, and adhesives.

Exposure Routes and Pathways

Exposure to dibutyl phthalate is usually by inhalation. It is known to leech out from finished plastics into blood, milk, and other food materials and, therefore, can be ingested orally. Dermal and ocular exposures are also possible.

Toxicokinetics

In rats, greater than 90% of dibutyl phthalate was excreted in the urine within 48 h following i.v. or oral dosing, but elimination in the feces was low. No accumulation was observed in tissues 24 h after exposure. Dibutyl phthalate was hydrolyzed rapidly by the rat liver esterases. Monobutyl phthalate (MBP) was a common metabolite in different species. The glucuronide conjugate was also detected in rat, hamster, and guinea pig together as well as a small amount of phthalic acid and unchanged compound. Omega or omega-1 oxidation products of MBP were also detected in the urine.

Mechanism of Toxicity

Dibutyl phthalate acts as an uncoupler of oxidative phosphorylation in rats.

Acute and Short-Term Toxicity (or Exposure)

The majority of the studies focusing on dibutyl phthalate exposure has centered on acute exposure via inhalation, ingestion, and dermatological contact.

Animal

Exposures to dibutyl phthalate have caused photophobia, conjunctivitis, edema, and keratitis. Increase in mean liver weight and testicular atrophy have been observed in rats exposed to dibutyl phthalate. The LD_{50} in rats is 8–10 g kg⁻¹ (oral) and 4 g kg⁻¹ (intraperitoneal). Aerosol (2 h) exposure of dibutyl phthalate at a concentration of 250 mg m⁻³ in mice produced symptoms of irritation to the upper respiratory tract and eyes. Increased concentrations resulted in bronchospasms causing difficulty in breathing, ataxia, weakness, convulsions, and eventually death. The LC_{50} in mice was determined to be 25 g m^{-3} . Dibutyl phthalate has weak estrogenic activity in a number of assays and may act as an anti-androgen.

Human

Dibutyl phthalate has low acute toxicity based on animal studies. It can cause an immediate stinging and burning sensation upon contact by splashing. Following ingestion, it can also cause dizziness and nausea. Contact with the skin has been reported to result in contact dermatitis.

Chronic Toxicity (or Exposure)

Information regarding the long-term, chronic, exposure to dibutyl phthalate is lacking. There is no information on human carcinogenicity and only limited effects in animals. No information is available on human teratogenicity, but animal studies have shown a reduced body weight. No information on dibutyl phthalate effects on the human reproductive system is available.

Animal

Rats exposed to 0.5 mg m^{-3} for $6 \text{ h} \text{ day}^{-1}$ for 6 days exhibited significantly higher brain and lung weights, smaller overall body weights compared to control groups. The Environmental Protection Agency (EPA) has classified dibutyl phthalate as 'group D' carcinogen, in that no definitive carcinogenic characteristics have been reported.

Human

Generalized symptoms of chronic exposure dibutyl phthalate include pain, numbness, spasms, weakness, and finally, polyneuritis. In an American Conference of Governmental Industrial Hygienists (ACGIH) study of 147 Russian workers exposed to several dibutyl esters for a period of 0.5–19 years and an air concentration of 1.7–66 mg m⁻³ reported significant adverse effects. By the seventh year of work, reports of pain, numbness, and muscle spasms were reported. These symptoms were followed by weakness in the extremities and a 32% rate of polyneuritis.

In Vitro Toxicity Data

Short-term studies with human cells have yielded negative results. Long-term mutagenicity tests have provided inconclusive results.

Clinical Management

Induced emesis is not recommended if the victim has any signs of esophageal or gastrointestinal tract irritation or burns, or decreased sensory response, depressed gag reflex, or impending shock. Activated charcoal slurry with or without saline cathartic or sorbitol can be given in cases of oral exposures. Skin decontamination should be done with repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water

for at least 15 min. Treatment is supportive and symptomatic and no specific antidote is available.

Environmental Fate

Dibutyl phthalate is considered nonhazardous for air, sea, and road freight. No other reports on dibutyl phthalate are available.

Exposure Standards and Guidelines

Occupational Safety and Health Administration permissible exposure level, the National Institute for Occupational Safety and Health recommended exposure limit, and the ACGIH threshold limit value has been set at $5.0 \,\mathrm{mg}\,\mathrm{m}^{-3}$ for an 8–10 h day per 40 h week. Although dibutyl phthalate is not subject to EPA emergency planning requirements, if dibutyl phthalate is released in a quantity exceeding 10 pounds over 24 h, the appropriate local, state, and federal authorities must be notified.

See also: Fragrances and Perfumes; Nitrocellulose; Toxicity Testing, Dermal.

Further Reading

- ACGIH (1991) Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th edn. Cincinnati, OH: American Conference of Government Industrial Hygienists.
- Sittig M (1991) Handbook of Toxic and Hazardous Chemicals, 3rd edn. Park Ridge NJ: Noyes Publications.

Relevant Websites

http://www.intox.org – Canadian Centre for Occupational Health and Safety. Cheminfo: Dibutyl phthalate.

http://www.osha.gov – Occupational Safety and Health Administration, US Department of Labor Health Guidelines.

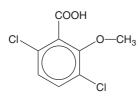
http://www.epa.gov – US Environmental Protection Agency Technology Transfer Network Air Toxics Website: Dibutyl phthalate.

Dicamba

Xun Song

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 1918-00-9
- SYNONYMS: Banlen; Banvel 480; Brush buster; Compound B dicamba; Velsicol compound 'r'; Velsicol 58-CS-11; Banvel herbicide; Banvel 4WS; Banfel[®]; Banvel[®]; Banvel CST[®]; Banvel D[®]; Banvel XG[®]; Mediben[®]
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Benzoic acid, 3,6-dichloro-2-methoxy-



Uses

Dicamba is mainly used as herbicide to control weeds, dock, bracken, and brush. Dicamba is frequently applied with other herbicides including atrazine, glyphosate, imazethapyr, ioxynil, and mecoprop.

Exposure Routes and Pathways

Dicamba is available as an odorless, white or brown, crystalline solid. Exposure to dicamba may occur through oral, dermal, or inhalation route.

Toxicokinetics

Dicamba is known to be well absorbed orally. Minimal absorption occurs through the skin. Following ingestion in animals, dicamba is readily distributed in all organs and systems. When given as a food supplement to rats, most dicamba was excreted unchanged in the urine while a small proportion was metabolized into glucuronic acid conjugates. The half-life of elimination in rats was estimated to be 0.83 h.

Mechanism of Toxicity

There is little evidence of dicamba toxicity in mammals. In plants its primary action is to act as a growth regulator.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for dicamba is 757–1707 mg kg⁻¹ in rats, 1190 mg kg⁻¹ in mice, 2000 mg kg⁻¹ in rabbits, and 566–3000 mg kg⁻¹ in guinea pigs. In rabbits, the dermal LD_{50} is greater than 2 g kg⁻¹ while the inhalation LC_{50} in rats is greater than 200 mg l⁻¹.

Human

Symptoms of poisoning with dicamba include loss of anorexia, vomiting, muscle weakness and spasms, bradycardia, shortness of breath, central nervous system effects (excitation or depression), incontinence, and cyanosis. Inhalation can cause irritation of the nasal passages and lungs and loss of voice. Recovery from severe overdose is generally complete within 2–3 days. Dicamba can cause severe, permanent corrosive damage to the eyes. Dicamba may cause skin burns.

Chronic Toxicity (or Exposure)

Animal

Prolonged dietary dicamba exposure at high dosages in rats led to changes in liver and decreased body weights. Dicamba was negative in reproductive, teratogenic, and carcinogenic tests.

Human

Little is known regarding chronic effects of dicamba in humans but animal studies suggest little potential for chronic toxicity.

In Vitro Toxicity Data

Dicamba is not mutagenic.

Clinical Management

There is no specific antidote; therefore, the treatment is symptomatic and supportive. Skin decontamination should be done with repeated washing with soap. Exposed eyes should be irrigated with copious amounts of water (at room temperature) for at least 15 min. Emesis can be induced if initiated within 30 min of ingestion. Ipecac can be used to induce emesis. Emesis is not encouraged if the patient is comatose or convulsing. Activated charcoal slurry with or without saline cathartic and sorbitol may be used.

Environmental Fate

Dicamba is moderately persistent in soil (half-life is 1–4 weeks). Microbial degradation is predominant. Degradation increases with temperature, increasing moisture, and low pH. When soil moisture increases above 50%, however, dicamba degradation is reduced. Photodegradation occurs to a limited extent. Some dicamba residues volatilize from plant surfaces. Dicamba does not bind to soil particles and is highly water soluble and therefore mobile. Groundwater contamination is possible. In surface waters, microbial degradation is predominant.

Photodegradation can also occur. Dicamba does not significantly bioaccumulate.

Ecotoxicology

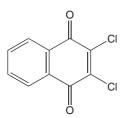
Dicamba is practically nontoxic to birds (LD_{50} in mallard ducks was >2 g kg⁻¹). The 8 day dietary LC_{50} in mallards and quail was >10 000 ppm. Dicamba is also of low toxicity to fish. The LC_{50} (96 h) for dicamba was 100–135 mg l⁻¹ in rainbow trout, bluegill, grass shrimp, fiddler crab, and sheepsheadminnow. The LC_{50} (48 h) was 110 mg l⁻¹ in *Daphnia magna*. Dicamba is not toxic to bees.

Dichlone

Xun Song

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 117-80-6
- SYNONYMS: Dichloronaphthoquinone; 2,3-Dichloro-1,4-naphthoquinone; Phygon; Algistat; Quintar; Sanquinon; Miraclear
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Naph-thoquinone
- CHEMICAL STRUCTURE:



Uses

Dichlone is primarily used as a fungicide. It is especially effective for brown rot of stone fruit and scab on apples and pears. Dichlone is also used to control blue algae. There are no registered uses for dichlone in the United States.

Exposure Routes and Pathways

Exposure to dichlone may occur through oral and dermal routes.

Exposure Standards and Guidelines

The reference dose for dicamba is 0.045 mg kg^{-1} day⁻¹.

See also: Pesticides; Pollution, Water.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://www.epa.gov – US Environmental Protection Agency and National Pesticide Information Center.

Toxicokinetics

Toxicokinetic data for dichlone are limited. It has been demonstrated that dichlone is poorly absorbed from the gastrointestinal tract.

Mechanism of Toxicity

The exact mechanism of toxicity is not clear. *In vitro* studies suggested that incubation of dichlone with normal human erythrocytes induced rapid loss of intracellular potassium, increased the osmotic fragility, and inhibited the Na⁺,K⁺-ATPase. Dietary dichlone exposure caused inhibition of glycolysis in rat liver. Dichlone can inhibit pyruvate and succinate dehydrogenases. Dichlone was also reported to cause oxidative stress and swelling of mitochondria.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute dermal LD_{50} in rabbits was 5 g kg^{-1} . The oral LD_{50} in rats was 1.3 g kg^{-1} . Dichlone is a skin irritant.

Human

Dichlone has relatively low toxicity in humans. It is irritating to the skin and mucous membranes. Irritation of the cornea may occur. Ingestion of large doses usually results in prompt emesis. Large doses may cause central nervous system depression, coma, and death.

Chronic Toxicity (or Exposure)

Animal

Little toxicity was noted in rats given a diet of 1500 ppm dichlone for 2 years. Dietary exposure of dogs to dichlone (500 ppm) for 1 year elicited slight liver changes.

Human

Based on animal studies, dichlone exposure is not expected to lead to chronic toxicity. Little is known, however, regarding long-term exposures in humans.

Clinical Management

If a poisoning is suspected, one should not wait for symptoms to develop. Immediate medical attention should be sought. Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become obtunded, comatose, or convulsing. Emesis is most effective if initiated within 30 min. An activated charcoal cathartic may also be employed. Use of any fat should be avoided since these agents may increase the irritant effects If contaminated with dichlone the affected area should be washed vigorously with soap. Contaminated clothing should be removed and discarded.

Ecotoxicology

Dichlone is toxic to fish. Dichlone is relatively nontoxic to bees. The LC_{50} in *Daphnia magna* is 0.014 ppm.

Exposure Standards and Guidelines

The reference dose for dichlone is $0.08 \text{ mg kg}^{-1} \text{ day}^{-1}$.

See also: Pesticides.

Further Reading

- Babich H, Palace MR, Borenfreund E, et al. (1994) Naphthoquinone cytotoxicity to bluegill sunfish BF-2 cells. *Archives of Environmental Contamination and Toxic*ology 27: 8–13.
- Pritsos CA, Pisani DE, and Pardini RS (1985) Inhibition of liver glycolysis in rats by dietary dichlone (2,3-dichloro-1,4-naphthoquinone). Bulletin of Environmental Contamination and Toxicology 35: 23–28.

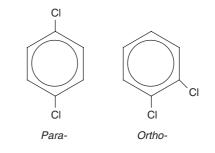
Dichlorobenzene

Elmar Udarbe Zamora

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: 1,2-DCB (CAS 9-5501); 1,3-DCB (CAS 541-73-1); 1,4-DCB (CAS 106-46-7)
- SYNONYMS: 1,2-DCB: Benzene-1,2-chloro-, o-Dichlorobenzene, 1,2-Dichlorobenzene; 1,3-DCB: Benzene-1,3-chloro-, m-Dichlorobenzene, 1,3-Dichlorobenzene; 1,4-DCB: Benzene-1,4-chloro-, p-Dichlorobenzene, 1,4-Dichlorobenzene, AI13-0050, p-Dichloricide, Evola, Globol, Paracide, Para crystals, Parazene, Paradow, Paramoth, Paranuggets, Paradi, PDC, PDCB, p-Dichlorobenzol, Persia-parazol, Santochlor, p-Chlorophenyl chloride
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Chlorinated aromatic hydrocarbon
- CHEMICAL FORMULA: C₆H₄Cl₂

• CHEMICAL STRUCTURE:



Uses

The dichlorobenzenes are used as moth repellants, insecticides, miticides, fumigants, disinfectants, space and air deodorizers, to prevent breakup of stoneware and molding, for molding resins and surface coatings. They are also used as chemical intermediates, for example, in the manufacture of polyphenyl sulfide. These are available as mothballs, flakes, cake, crystals, and as a 100% concentrate. In some 1,4-dichlorobenzene commercial preparations, 1,2- and 1,3-dichlorobenzenes usually occur in minute amounts.

Exposure Routes and Pathways

The most common way to be exposed to 1,4-dichlorobenzene is by inhaling the vapors from mothballs and toilet deodorizers. However, this route $(35 \,\mu g \, day^{-1})$ is not expected to lead to substantial effects. Ingestion and dermal or ocular contact are the other most common routes of exposure.

Toxicokinetics

1,4-Dichlorobenzene is well absorbed orally and by inhalation. The highest concentrations are found in the adipose tissue. It is rapidly oxidized to phenolic compounds and metabolized to sulfate and glucuroate conjugates. The major metabolite is 2,5-dichlorophenol. From 91% to 97% is excreted in the urine within 5 days.

Mechanism of Toxicity

In some species, 1,4-dichlorobenzene can cause nephrotoxicity associated with hyaline droplets. This syndrome requires the presence of $\alpha 2\mu$ -globulin protein.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dichlorobenzene is an eye, skin, and upper respiratory tract irritant in animals. It also affects the liver and kidneys and is considered a potential carcinogen. The oral LD_{50} is 500 mg kg⁻¹ in rats and more than $2 g kg^{-1}$ in rabbits. The lowest-observed-adverseeffect level (LOAEL) is 155 mg kg⁻¹ per 2 years of intermittent exposure in rats and mice. 1,4-Dichlorobenzene and a primary metabolite were negative in the mouse *in vivo* micronucleus test.

Human

Dichlorobenzene has low acute toxicity. However, people exposed to high levels for relatively short periods of time potentially can develop malaise, nausea, vomiting, headaches, and irritation of eyes, respiratory tract, and the skin (burning sensation upon contact). Central nervous system (CNS) depression may occur at high concentrations that are extremely irritating to the eyes and nose. The TDLo in humans is 300 mg kg^{-1} .

Chronic Toxicity (or Exposure)

Animal

Rats, rabbits, and guinea pigs exposed through inhalation over a long period of time have reduced food consumption and body weights. CNS signs include tremors and respiratory/dermal effects seen were nasal and ocular discharges. The liver and kidneys increased in weight and showed degenerative and necrotic cellular changes. Livers of rats exposed orally $(300 \text{ mg kg}^{-1} \text{ day}^{-1})$ for prolonged periods were found to exhibit cellular degeneration, cloudy swelling, and focal necrosis. 1,2-DCB was found to be toxic to Fisher rats. As noted before, the nephrotoxicity is dependent on presence of species-specific expression of $\alpha 2\mu$ -globulin protein.

Human

People exposed to levels above the maximum contaminant level goal (MCGL) for prolonged periods of time can potentially have abnormalities in the blood (e.g., anemia), in the CNS, develop skin lesions, experience appetite loss, and have liver damage. Protein, bilirubin, and blood is present in the urine. A woman exposed to *p*-dichlorobenzene for 6 years developed nervous system effects, including severe muscle incoordination, weakness in all limbs, and decreased reflex responses. While 1,4-dichlorobenzene is a carcinogen in rats, mechanistic information suggests that it has little carcinogenic potential in humans.

In Vitro Toxicity Data

Dichlorobenzenes can covalently bind to nucleic acids. In a concentration dependent manner, 1,2-dichlorobenzene and *p*-dichlorobenzene were found to be estrogenic in the yeast estrogen screen with relative potencies in relation to B-estradiol of 2.2×10^{-7} for 1,4-dichlorobenzene and 1.04×10^{-8} for 1,3-di-chlorobenzene. No mutagenic potential was observed in the gene mutation assay using mouse lymphoma cells.

Clinical Management

The affected skin or eyes should be immediately flushed with plenty of water for 15 min. The affected skin should be washed with soap and water. Activated charcoal slurry with or without saline cathartic can be given if dichlorobenzene is ingested. Vomiting should not be induced. The victim should be moved to fresh air when exposed through inhalation. Humidified oxygen (100%) can be supplemented with assisted ventilation. If the victim is unconscious, nothing should be given by mouth, airway, breathing, and circulation should be stabilized, and the victim should be brought to the hospital immediately. Since many of the reported toxicities are due to chronic exposure, treatment is mostly symptomatic and supportive.

Environmental Fate

Most 1,4-dichlorobenzene found in the environment is due to its use as a toilet deodorizer and moth repellant. Since the solid easily vaporizes, much of 1,4-dichlorobenzene is released into the air. Dinitrobenzenes in water readily evaporate. DCB adsorbs to soil and is relatively resistant to degradation.

Ecotoxicology

This chemical was found to be present in fish (400 ppm). It may also accumulate in plants.

Other Hazards

It is incompatible with aluminum and its alloys and could react with plastics, rubber, or coatings. It reacts violently with oxidizing agents like chlorine and permanganate. In cases of spills or leakage, all flammable material or all sources of ignition are removed to prevent fire. The spill is dampened with 60–70% alcohol. The spill is removed by using a paper dampened with 60–70% alcohol, which is disposed in a suitable container. All contaminated clothing are washed with 60–70% alcohol and then washed in soap and water.

Exposure Standards and Guidelines

The MCLG is the level of a contaminant in drinking water below which there is no known or expected risk to health. The Environmental Protection Agency sets the MCLG for *o*-dichlorobenzene at 0.6 ppm.

The National Institute for Occupational Safety and Health sets the recommended exposure limit at 1.7 ppm in an 8 h threshold limit value – timeweighted average (TLV – TWA).

The Occupational Safety and Health Administration (OSHA) sets the permissible exposure limit for *p*-dichlorobenzene as 75 ppm for an 8 h TWA.

The American Conference for Governmental Industrial Hygienists sets the TLV – TWA for *p*-dichlorobenzene at 10 ppm.

The National Fire Protection Assocation rates 1,4dichlorobenzene as moderately hazardous to health and moderately flammable.

See also: Pesticides.

Further Reading

Barter JA and Sherman JH (1999) An evaluation of the carcinogenic hazard of 1,4-dichlorobenzene based on internationally recognized criteria. *Regulatory Toxicology and Pharmacology* 29: 64–79.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dichlorobenzenes.

Dichloroethanes

Madhusudan G Soni and Harihara M Mehendale

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- SYNONYMS: Chlorinated hydrochloric ether; Ethylidene chloride; Ethylidine dichloride
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Chlorinated hydrocarbon
- CHEMICAL STRUCTURE:



Uses

1,2-Dichloroethane is utilized as a solvent, pesticide, fumigant, gasoline additive, and in the synthesis of vinyl chloride. 1,1-Dichloroethane is utilized in relatively small quantities, primarily in the chemical, agricultural, and petroleum industries. In the past, 1,1-dichloroethane was used as an anesthetic.

Exposure Routes and Pathways

Inhalation through contaminated air and ingestion of contaminated water are common routes of exposure in humans.

Toxicokinetics

Dichloroethanes are readily absorbed through the lungs following inhalation exposure in both humans and experimental animals. Absorption after oral ingestion in experimental animals is rapid, complete, and essentially linear. Studies in animals have shown that dichloroethanes are well absorbed through the skin following dermal exposure. Dichloroethanes are metabolized to a variety of chlorinated metabolites, some of which (e.g., chloroacetaldehyde and acetyl chloride) are reactive species and are more toxic than the parent compounds. The relatively greater rate of metabolism of 1,1-dichloroethane relative to 1,2dichloroethane is not consistent with the relatively higher toxicity, mutagenicity, and carcinogenicity of 1,2-dichloroethane. Therefore, it is possible that there is alternative route of metabolism. Dichloroethanes appear to be rapidly distributed in humans. In rats, dichloroethanes are readily distributed throughout body tissue after inhalation or oral ingestion. The highest concentrations were found in fat. Following inhalation exposure in rats, elimination occurred primarily via the excretion of soluble metabolites and unchanged parent compound in urine and carbon dioxide in the expired breath. Urinary metabolites accounted for 84% of the absorbed dose, fecal accounted for 2%, and carbon dioxide accounted for $\sim 7\%$. Following oral exposure, urinary metabolites accounted for 60%, unchanged in the breath accounted for 29%, and carbon dioxide in breath accounted for 5% of the administered 150 mg kg^{-1} dose.

Mechanism of Toxicity

The mechanism of action of dichloroethane-induced toxicity is not fully elucidated. By most criteria, 1,1-dichloroethane is less toxic than 1,2-dichloroethane. Studies of the possible mutagenicity and carcinogenicity of 1,1-dichloroethane have been negative or inconclusive. In contrast, 1,2-dichloroethane is carcinogenic in rats and mice and mutagenic in several test systems, particularly in the presence of activating enzymes, such as hepatic glutathione transferases and to a lesser extent by hepatic microsomal cytochrome P450 enzymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

An acute oral LD_{50} of 680 mg kg⁻¹ has been reported for rats. In mice the reported LD_{50} values for male and female were 489 and 413 mg kg⁻¹, respectively. No adverse clinical effects were noted in rats, rabbits,

or guinea pigs exposed to 1000 ppm 1,1-dichloroethane for 13 weeks, which followed a prior 13-week exposure to 500 ppm. However, under the same conditions, renal injury was apparent in cats. Short-term animal studies indicate that the liver and kidneys are the principal target organs of 1,2-dichloromethane. Lowest reported effect levels for ingestion and inhalation were 49–82 mg kg⁻¹ body weight per day (increases in liver weight in rats exposed for 13 weeks) and 202 mg m⁻³ (effects on liver and kidney function in rats exposed for 12 months), respectively. According to available evidence, 1,2-dichloromethane does not adversely affect the reproductive or development process in animals except at maternally toxic levels. Results of in vivo studies in rats, mice, and insects were consistently positive for genotoxic activity.

Human

Very limited human toxicity data are available for dichloroethanes. Symptoms observed were central nervous system depression, corneal opacity, bronchitis, respiratory distress, myocardial lesions, hemorrhagic gastritis and colitis, increased blood clotting time, hepatocellular damage, renal necrosis, and histopathological changes in brain tissue. Death was most often attributed to cardiac arrhythmia. In the past, 1,1-dichloroethane was used as an anesthetic at levels of ~25 000 ppm. This use was discontinued when it was discovered that cardiac arrhythmias might be induced. In persons with impaired pulmonary function, especially those with obstructive airway diseases, the breathing of dichloroethane might cause exacerbation of symptoms to its irritant properties.

Chronic Toxicity (or Exposure)

Animal

In chronic toxicity studies, liver and kidneys were the principal target organs. Exposure to 1,2-dichloroethane by gavage for 78 weeks induced a significant increase in the incidence of tumors at several sites in both rats and mice. Inhalation exposure of rats or mice did not show significant increases in tumor incidence. However, repeated dermal or intraperitoneal application of 1,2-dichloroethane resulted in an increase in lung tumors in mice.

Human

No information is available on the chronic (longterm) effects of 1,1-dichloroethane in humans. Epidemiological studies regarding the carcinogenic effects of 1,2-dichloroethane are not conclusive, due to concomitant exposure to other chemicals.

In Vitro Toxicity Data

In several *in vitro* assays in prokaryotes, fungi, and mammalian (including human) cells, 1,2-dichloroethane has been consistently genotoxic.

Clinical Management

Respiratory and cardiovascular function should be supported, and the victim should be moved to fresh air and given artificial respiration; if breathing is difficult, oxygen should be given. In case of contact with material, the eyes should be flushed immediately with running water for at least 15 min; the skin should be washed with soap and water. Contaminated clothing and shoes should be removed, and isolated at the site. In case of oral exposure, emesis should not be induced. A charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered.

Environmental Fate

1,2-Dichloroethane released to the environment partitions to the atmosphere. In the atmosphere, reaction with photochemically produced hydroxyl radicals is the primary degradation mechanism of 1,2-dichloroethane. 1,2-Dichloroethane released to soil or water surfaces is expected to volatilize quickly. Biodegradation occurs slowly in water and soil surfaces. 1,2-Dichloroethane is not expected to undergo hydrolysis and photolysis.

Dichloroethylene, 1,2-

Sachin S Devi and Harihara M Mehendale

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 540-59-0 (sym); CAS 156-59-2 (cis); CAS 156-60-5 (trans)
- SYNONYMS: Ethene, 1,2-dichloro-; Ethylene, 1,2dichloro-; 1,2-Dichloroethene; Acetylene dichloride; Dioform; *sym*-Dichloroethylene; 1,2-DCE
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organic compounds
- CHEMICAL FORMULA: C₂H₂Cl₂
- CHEMICAL STRUCTURE:



Exposure Standards and Guidelines

The US Occupational Safety and Health Administration regulatory level in workplace air is 1 ppm for an 8 h day, 40 h week. US Environmental Protection Agency has set a limit in water of 0.005 mg l^{-1} . The US National Institute for Occupational Safety and Health recommends that it would be prudent to handle 1,2-dichloroethane in the workplace as if it was a human carcinogen.

See also: Gasoline; Pesticides; Vinyl Chloride.

Further Reading

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Uses

1,2-Dichloroethylene is used as a direct solvent for perfumes, dyes, gums and waxes, oils, fats, lacquers, thermoplastics, phenols, and camphor; as a chemical intermediate for chlorinated compounds; and as an agent in retarding fermentation. 1,2-Dichloroethylene is also used as a low-temperature solvent for heat-sensitive substances in the extraction of caffeine, fats, and natural rubber, as well as in organic synthesis for polymers and telomers and as a coolant in refrigeration plants. Miscellaneous applications include use as a dry cleaning solvent, cleaning solution for printed circuit boards, and use in food packaging, adhesives, and germicidal fumigants.

Background Information

1,2-Dichloroethylene is a colorless, volatile liquid with an ether-like, slightly acrid odor. The commercial product is available as either the *cis* or *trans* isomer or a mixture of the two isomers. The *trans* isomer has an odor threshold concentration of 17 ppm of air.

Exposure Routes and Pathways

- Breathing 1,2-dichloroethylene that has leaked from hazardous waste sites and landfills.
- Drinking contaminated tap water or breathing vapors from contaminated water while cooking, bathing, or washing dishes.
- Breathing 1,2-dichloroethylene, touching it, or touching contaminated materials in the workplace.

Toxicokinetics

1,2-Dichloroethylene is largely excreted through the lungs. In isolated perfused rat liver systems, cis and trans isomers are metabolized to the same metabolites, dichloroacetic acid and dichloroethanol. In this system, the *cis* isomer is metabolized to a greater extent than the trans isomer. The metabolites are formed by an epoxide intermediate. Studies with rat liver microsomes exposed to 1,2-dichloroethylene cause a fall in microsomal cytochrome P450 content without affecting other microsomal enzymes. The decrease in cytochrome P450 only occurred in the presence of reduced nicotinamide adenine dinucleotide, suggesting that the chloroethylene must be converted to a metabolite that exerts its destructive action. The loss of P450 was attributed to the destruction of heme since the fall in cytochrome P450 was always accompanied by parallel decrease in microsomal heme content.

Mechanism of Toxicity

The acute narcotic effects are due to the physical interaction of the material itself on the cells of the central nervous system (CNS). The long-term effects are most likely due to the production of an unstable reactive intermediate during biotransformation.

Acute and Short-Term Toxicity (or Exposure)

Animal

1,2-Dichloroethylene vapor is a CNS depressant and a mild irritant of the mucous membranes. The acute oral LD_{50} for a 60:40 *cis-trans* mixture in rats is reported as greater than 2000 mg kg⁻¹. Inhalation exposure to 16 000 ppm for 4 h was lethal to rats, but

8-min exposures to the same concentration produced anesthesia.

Human

The major effect of 1,2-dichloroethylene is narcosis; it has been used in a combination with ether (Dichloren) as an anesthetic in at least 2000 cases. No evidence of eye toxicity was seen in these cases. In high concentrations, exposure to 1,2-dichloroethylene causes CNS depression; in milder exposures, it can produce nausea, vomiting, weakness, tremor, epigastric cramps, burning of the eyes and vertigo. One fatality has been reported that was due to inhalation of a very high vapor concentration in a small enclosure. Exposure to the vapor of dichloroethylene may cause burning of the eyes. Other symptoms of acute exposure are nausea, vomiting, and epigastric distress. Symptoms of exposure-related narcosis including drowsiness, tremor, incoordination, dizziness, and weakness; these symptoms clear quickly after exposure is terminated.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure studies have shown that repeated inhalation of up to 1000 ppm 1,2-dichloroethylene resulted in no identified ill effects in rats, rabbits, guinea pigs, and dogs. Dogs narcotized by inhaling 1,2-dichloroethylene vapor developed superficial corneal turbidity that cleared within 48 h and did not disturb vision.

Human

1,2-Dichloroethylene is a defatting agent, and repeated skin exposure may cause irritation and dermatitis. No cancer bioassays or epidemiological studies were available to assess the carcinogenicity of 1,2-dichloroethylene. US Environmental Protection Agency has placed *cis*-1,2-dichloroethene in weight-of-evidence group D, not classifiable as to human carcinogenicity, based on the lack of or negative human or animal cancer data. *trans*-1,2-Dichloroethylene has not undergone a complete evaluation and determination under US EPA's IRIS program for evidence of human carcinogenic potential.

Clinical Management

Persons exposed to 1,2-dichloroethylene should have their vital signs closely monitored; the heart should be monitored by EKG. Epinephrine and other catecholamines should be avoided, especially beta agonists since they may increase the risk of arrhythmias. Pulmonary edema, renal failure, and liver injury should be managed symptomatically. However, based on toxicity similarities to trichloroethylene, data on plasma levels of following 1,2-dichloroethylene overdose/exposure are not clinically very useful. Renal and liver function tests should be monitored in the presence of suspected kidney or liver injury.

Environmental Fate

cis-1,2-Dichloroethylene may be released to the environment in emissions and wastewater during its production and use. Under anaerobic conditions that may exist in landfills or sediment, one is likely to find 1,2-dichloroethylenes that are formed as breakdown products from the reductive dehalogenation of trichloroethylene and tetrachloroethylene. The cis-1,2-dichloroethylene is apparently the more common isomer found although it is mistakenly listed as the trans isomer. The trans isomer, being a priority pollutant, is more commonly analyzed for and the analytical procedures generally used do not distinguish the isomers. If cis-1,2-dichloroethylene is released on soil, it should evaporate and/or leach into the groundwater where very slow biodegradation should occur. If released into water, cis-1,2-dichloroethylene will be lost mainly through volatilization (half-life 3 h in a model river). Biodegradation, adsorption to sediment, and bioconcentration in aquatic organisms should not be significant. In the atmosphere cis-1,2dichloroethylene will be lost by reaction with photochemically produced hydroxyl radicals (half-life 8 days) and scavenged by rain. Because it is relatively long lived in the atmosphere, considerable dispersal from source areas should occur. The general population is exposed to cis-1,2-dichloroethylene in urban air as well as in contaminated drinking water from ground water sources. Occupational exposure will be via dermal contact with the vapor and liquid or via inhalation. 1,2-Dichloroethene evaporates rapidly into air.

Exposure Standards and Guidelines

Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL): The current OSHAPEL for 1,2-dichloroethylene is 200 ppm (790 mg m⁻³ as an 8 h time-weighted average (TWA) concentration.

National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL): The NIOSH has established a REL for 1,2-dichloroethylene of 200 ppm (790 mg m⁻³) as a TWA for up to a 10 h workday and a 40 h workweek.

American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV): The ACGIH has assigned 1,2-dichloroethylene a TLV of 200 ppm (793 mg m⁻³) as a TWA for a normal 8 h workday and a 40 h workweek.

Rationale for Limits: The NIOSH limit is based on the risk of narcotic effects and mucous membrane irritation. The ACGIH limit is based on the no-effect level of 1000 ppm in animals.

See also: Catecholamines; Cytochrome P-450.

Further Reading

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Relevant Website

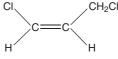
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dichloroethene, 1,2-.

Dichloropropene, 1,3-

Jing Liu

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 542-75-6
- SYNONYMS: 1,3-Dichloro-1-propene; 1,3-Dichloropropylene; Dorlone; Nemex; Telone; Vidden D
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Halogenated hydrocarbon nematocide
- Chemical Formula: $C_3H_4Cl_2$
- Chemical Structure:



Uses

1,3-Dichloropropene is used as a preplant soil fumigant for the control of nematodes.

Exposure Routes and Pathways

Most exposures occur through inhalation due to application techniques and to its volatility. Dermal, ocular, and oral exposures are also possible.

Toxicokinetics

1,3-Dichloropropene can be rapidly absorbed through the skin and via the respiratory and gastrointestinal tracts. Blood levels of the glutathione-conjugate of 1,3dichlororpropene reached steady state within 15 min after oral exposure in rats, indicating rapid absorption. The presence of N-acetyl-cysteine conjugates in the recovered urine of field applicators suggested the chemical is readily absorbed via inhalation. 1,3-Dichloropropene has an elimination half-life of less than 10 min from the bloodstream. Urinary elimination half-lives ranged from 5 to 6 h in rats, from 7 to 10 h in mice, and an average of 9.5 h in humans. Dichloropropene primarily undergoes conjugation with glutathione to form a mercapturic acid and oxidation to carbon dioxide. An additional route of metabolism reported in mice involves stereospecific epoxidation to the corresponding 1,3-dichloropropene oxide. The predominant route of excretion is via the urine ($\sim 50-80\%$ in rat and mouse, respectively) as mercapturic acid conjugates. Lesser amounts are eliminated through feces.

Mechanism of Toxicity

Although there is substantial documentation regarding health effects of 1,3-dichloropropene, little data concerning mechanisms of toxicity are available. 1,3-Dichloropropene acts as an irritant and sensitizer. Macromolecular binding of this compound may also contribute to its toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of 1,3-dichloropropene is about 470 mg kg⁻¹ in rats and 640 mg kg⁻¹ in mice. The dermal LD_{50} in rabbits is 504 mg kg⁻¹. Inhalation LC_{50} in mice is 4650 mg m⁻³ every 2 h.

Lung/tracheal congestion, fluid in the pleural cavity, atelectasis, emphysema, and/or pulmonary edema and lung hemorrhaging were observed in rats exposed to 1,3-dichloropropene through inhalation. Rabbits exhibited stomach and intestinal hemorrhage after dermal exposure. Ocular or dermal exposure may cause conjunctival and corneal irritation, erythema, edema, necrosis, and subcutaneous or skeletal muscle hemorrhage in rats, rabbits, and guinea pigs. 1,3-Dichloropropene was not embryotoxic or teratogenic in inbred rats or rabbits at doses that produced maternal toxicity.

Human

1,3-Dichloropropene has moderate acute toxicity. The health effects of 1,3-dichloropropene may involve many organ systems including liver, kidney, lung, gastrointestinal tract, and mucous membranes. Vapors of 1,3-dichloropropene are irritating to the eyes and respiratory tract, possibly causing delayed pulmonary edema. Individuals may experience eye, nose, and throat irritation, nausea, vomiting, headache, and chest discomfort. Contact dermatitis has been reported in farmers exposed to the compound.

Chronic Toxicity (or Exposure)

Animal

Both male and female dogs ingesting 1,3-dichloropropene (15 mg kg⁻¹ day⁻¹) for either 13 weeks or 1 year exhibited primarily regenerative hypochromic, microcytic anemia. Chronic exposure via the oral route has also caused neoplastic and preneoplastic lesions of the stomach in rats. Hyperplasia and hyperkeratosis of the forestomach and urinary bladder hyperplasia were reported in mice exposed to one formulation of 1,3-dichloropropene (Telone IIb) for 2 years through inhalation.

Human

Chronic dermal exposure may cause skin sensitization. 1,3-Dichloropropene has been classified in Group B2 as a possible human carcinogen through both inhalation and oral exposures.

In Vitro Toxicity Data

Using isolated rat hepatocytes, 1,3-dichloropropene was shown to be cytotoxic as measured by increases in phospholipid hydroperoxides and lactate dehydrogenase. 1,3-Dichloropropene also exhibited nephrotoxicity *in vitro* using rat renal cortical slices, where *p*-aminohippurate uptake was decreased. Genotoxicity of 1,3-dichloropropene was observed as increases in sister-chromatid exchange in human lymphocytes *in vitro*.

Clinical Management

Treatment is symptomatic and supportive.

Environmental Fate

1,3-Dichloropropene is mobile and persistent, particularly in colder climates. When injected into the soil, its mobility is controlled by temperature, soil type, and moisture. Even though volatilization occurs from the soil surface, most 1,3-dichloropropene is degraded through hydrolysis to 3-dichloroallyl alcohol. The overall half-life of the compound in soil ranges from a few days to more than 9 weeks depending on conditions. Adsorption to sediment and bioconcentration in fish are not important processes.

Ecotoxicology

1,3-Dichloropropene is highly toxic to invertebrates and moderately toxic to birds, mammals, and fish.

Exposure Standards and Guidelines

The National Institute for Occupational Safety and Health and American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average for 1,3-dichloropropene is 1 ppm (5 mg m^{-3}) .

The reference concentration (RfC) established by the Environmental Protection Agency (EPA) is 0.02 mg m^{-3} based on hypertrophy/hyperplasia of nasal respiratory epithelium in mice.

The chronic reference dose set by the EPA is $0.0003 \text{ mg kg}^{-1} \text{day}^{-1}$ (oral).

See also: Pollution, Water.

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http://www.epa.gov – US Environmental Protection Agency.

Dichlorvos

Nikita Mirajkar and Carey N Pope

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- SYNONYMS: DDVF; DDVP; Canogard; Dedevap; Estrosol; Herkol; Vapona; Apavap; Benfos; Fly-Bate; Fly-Die; Fly-Fighter
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Synthetic dimethoxy organophosphorus insecticide
- CHEMICAL STRUCTURE:

Uses

Because of its high vapor pressure, dichlorvos is useful in the control of insects in closed spaces (e.g., warehouses, greenhouses, animal shelters, homes, and restaurants). It is available in oil solutions, emulsifiable concentrates, aerosols, and baits. Therapeutically, dichlorvos is used as a broad-spectrum anthelmintic (for destroying or expelling intestinal worms). It is also used as a feed through larvicide to control botfly larvae in the manure. It is primarily used for insect control. Dichlorvos is also a breakdown product of the organophosphorus pesticide trichlorfon (metrifonate).

Exposure Routes and Pathways

Exposure to dichlorvos vapor can result in exposure through not only the respiratory route but also the dermal and oral routes (e.g., through contamination of feed).

Toxicokinetics

Animals exposed to dichlorvos vapor were found to absorb at least 50% of the total material by the respiratory route. Dichlorvos can also be absorbed through the oral and dermal routes. Following oral exposure, dichlorvos is rapidly detoxified in the liver. Metabolites include O,O'-dimethyl phosphate, monomethyl phosphate, O-methyl-O-2,2-dichlorovinyl phosphate (desmethyl dichlorvos), and inorganic phosphate. Detoxification processes for dichlorvos are also found in plasma.

Under most conditions, dichlorvos is not detectable in any tissues. Dichlorvos is not stored in tissues, it does not accumulate in secretions (e.g., milk), and it is below detection levels in the blood of various species at exposure levels in excess of 10 times those effective for insect control. At exceptionally high concentrations (90 mg m⁻³ or about 2000 times normal exposure levels), dichlorvos was detectable in various tissues of the rat.

Dichlorvos is rapidly metabolized and excreted by mammals following any route of exposure.

Desmethyldichlorvos and dimethylphosphate are rapidly excreted or further metabolized. The glucuronide conjugate of dichloroethanol is excreted in the feces. Species differences in elimination are common. For example, following oral administration, the cow eliminates $\sim 50\%$ through the feces, whereas the rat excretes only about 3% via the feces.

Mechanism of Toxicity

The toxicity of dichlorvos is due to inhibition of acetylcholinesterase and the signs of toxicity are generally similar to those caused by other organophosphorus insecticides. Dichlorvos is a direct inhibitor of cholinesterases; thus, toxicity rapidly follows exposure and recovery is also rapid. With inhalation exposures, airway acetylcholinesterase inhibition is possible in the absence of significant blood enzyme inhibition. The fly head acetylcholinesterase appears more sensitive to inhibition by dichlorvos relative to mammalian brain acetylcholinesterase. At high doses, dichlorvos may cause hyperglycemia and abnormal glucose tolerance.

In Vitro Toxicity Data

Dichlorvos has been shown to be positive in the Ames assay and in other bacterial and mammalian cell mutagenesis assays.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dichlorvos is of moderate acute toxicity, with an oral LD_{50} value in rodents from 50 to 150 mg kg^{-1} . While the LC_{50} for inhibiting mammalian brain ace-tylcholinesterase is similar between dichlorvos and paraoxon (i.e., the active metabolite of parathion), the acute LD_{50} values for these agents are considerably different, due in part to the more rapid metabolism and elimination of dichlorvos.

In rabbits, at acute doses, dichlorvos was found to be a mild skin and eye irritant.

Human

Inhalation, dermal, or oral exposure to dichlorvos can result in systemic toxicity through inhibition of acetylcholinesterase. Symptoms of acute exposure to dichlorvos may include blurred vision, nausea, headache, and shortness of breath.

Increased risk from exposure to dichlorvos will occur in persons who have reduced lung function, convulsive disorders, liver disorders, or recent exposure to cholinesterase inhibitors.

Chronic Toxicity (or Exposure)

Human

Symptoms of chronic exposure to dichlorvos are similar to those of acute exposure, in addition to which there could be tension, insomnia, loss of appetite, apathy, trembling, and confusion.

Experimental studies suggest that relatively high exposures may produce delayed neurotoxicity, whereas the dibutyl analog is capable of inducing delayed neurotoxicity at relatively low doses.

In humans, the plasma cholinesterase appears more sensitive than erythrocyte cholinesterase to inhibition by dichlorvos; thus, discrimination between these two activities may be warranted during assessment of exposures.

Dichlorvos is classified as a mutagen and a possible human carcinogen, based on the results of animal studies. It is, however, not classified as a teratogen. It has been shown that dichlorvos may affect the immune system.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered followed by 2 and 4 mg (in adults) or 0.015 and 0.05 mg kg^{-1} (in children) every 10 and 15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Environmental Fate

In soil, dichlorvos is generally not active, and has low persistence, with a half-life of 7 days.

In general, it is not absorbed from the soil by plants. Dichlorvos undergoes hydrolysis and biodegradation. Soil pH influences dichlorvos degradation, with more rapid breakdown under alkaline conditions.

Dichlorvos adsorbs very poorly to soil particles and is soluble in water, in which it degrades primarily by hydrolysis, with a half-life of ~ 4 days in lakes and rivers.

Ecotoxicology

Dichlorvos is only slightly toxic to plants. Dichlorvos is moderately toxic to fish and highly toxic to aquatic invertebrates. However, in the presence of UV light, toxicity of dichlorvos to aquatic organisms can be increased 5–150-fold. It does not bioaccumulate in fish. The toxicity of dichlorvos to birds and mammals ranges from low to moderate. Dichlorvos is highly toxic to bees. Dichlorvos can be hazardous to endangered species.

Exposure Standards and Guidelines

- Reference dose is $0.0005 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Accepted daily intake is $0.001 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Threshold limit value is 0.9 mg m⁻⁻

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides.

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Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dichlorvos. http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Dieldrin

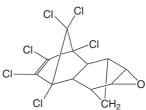
Benny L Blaylock

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- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organochlorine insecticide
- CHEMICAL FORMULA: C₁₂H₈Cl₆O
- CHEMICAL STRUCTURE:



 SYNONYMS: 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-endo,exo-5,8dimethanonaphthalene; Alvit; Dieldrix; Octalox; Quintox; Red Shield



Uses

Dieldrin is used as an insecticide and its use has been significantly restricted in the United States and several other countries. There are currently no Environmental Protection Agency registrations for dieldrin.

Exposure Routes and Pathways

The most important exposure routes for dieldrin are oral and dermal.

Toxicokinetics

Dieldrin is readily adsorbed for the gastrointestinal tract, the respiratory tract, and through the skin. In mammals, two major metabolism routes of dieldrin seem to be predominant: (1) direct oxidation by cyto-chrome oxidases, resulting in 9-hydroxydieldrin; and (2) the opening of the epoxide ring by epoxide hydrases, resulting in 6,7-*trans*-dihydroxydihydroaldrin. Dieldrin is hydroxylated to 9-hydroxydieldrin by liver microsomal monooxygenases in rats. Metabolism of dieldrin is three to four times more rapid in male than in female rats. Excretion in humans is primarily in the feces via the bile. Dieldrin is also excreted via lactation in nursing mothers.

Mechanism of Toxicity

Dieldrin characteristically stimulates the central nervous system (CNS) causing hyperexcitation and generalized seizures. Both *in vitro* experiments using rat brain membranes and intravenous or intraperitoneal administration of aldrin and dieldrin to rats have shown that these agents are capable of blocking the activity of GABA by blocking the influx of chloride through the GABA_A receptor–ionophore complex.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for rats is 46 mg kg⁻¹. Convulsions are the principle CNS effect. In birds, acute symptoms include tail feathers spread and pointed either upward or downward, hyperexcitability, jerkiness in gait, ataxia, dyspnea, myasthenia, fluffed feathers, immobility, terminal wing-beat convulsions, or opisthotonos. Mortalities usually occurred 1–9 days following treatment.

Human

CNS excitation culminating in convulsions was the principal toxic effect noted in occupational studies of

workers employed in either the manufacture or application of aldrin or dieldrin. Patients exposed to dieldrin may also experience other symptoms including headaches, dizziness, hyperirritability, general malaise, nausea and vomiting, anorexia, muscle twitching, and myoclonic jerking. Dieldrin is not classifiable as to its carcinogenicity in humans by International Agency for Research on Cancer.

Chronic Toxicity (or Exposure)

Animal

In addition to its CNS effects, dieldrin increases hepatocarcinogenesis with chronic exposure. Dieldrin has been shown to suppress macrophage function and T-dependent humoral immune functions. Reproductive effects in rats were observed when pregnant females were dosed with 1.0 mg kg^{-1} aldrin subcutaneously. A significant but slight decrease in fertility was observed in female mice exposed to 1.3 or $1.95 \text{ mg kg}^{-1} \text{ day}^{-1}$ of dieldrin from 4 weeks prior to mating through weaning.

Human

Dieldrin has caused numerous cases of chronic poisoning to workers who have sprayed the compound for several months. Characteristically there is headache, dizziness, and involuntary muscular movements. In severe cases there are epileptic convulsions with loss of consciousness. The only ocular disturbance so far noted in human beings has been blurred vision of undetermined cause, and nystagmus accompanying incoordination and tremor. In a study of five male farm workers exposed to a mixture of herbicides and pesticides including dieldrin, four were found to have suffered impotence after chronic exposure; sexual function recovered after termination of exposure.

Clinical Management

Treatment is symptomatic. Activated charcoal as a slurry has been reported to absorb aldrin and increase its rate of excretion after oral exposure. Emesis is not recommended due to potential CNS depression or seizures. Diazepam or phenobarbital is used when anticonvulsant therapy is necessary.

Environmental Fate

In temperate soil, the half-life of dieldrin is ~ 5 years. Most dieldrin and aldrin found in surface water are the result of runoff from contaminated soil. With this level of persistence, combined with high lipid solubility, the necessary conditions for dieldrin to bioconcentrate and biomagnify in organisms are provided. Bioconcentration factors of 12 500 and 13 300 have been reported for guppies and sculpins, respectively. It is likely that dieldrin is bioconcentrated by aquatic organisms rather than bioaccumulated. Dieldrin exhibits low water solubility, high stability, and semivolatility. These characteristics favor its long-range transport. In the air, dieldrin is degraded by ultraviolet light to the more persistent photodieldrin within a few days.

Ecological Effects

Dieldrin has low phytotoxicity. Plants are affected only by application rates much higher than suggested use rates. The acute toxicity of dieldrin is quite variable for aquatic invertebrates, with insects being the most sensitive group (values range from 0.2 to $40 \,\mu g \, l^{-1}$). It is highly toxic to most species of fish tested in the laboratory (values range from 1.1 to $41 \,\mu g \, l^{-1}$). In frogs, the 96 h LC₅₀ of dieldrin ranged from 8.7 $\mu g \, l^{-1}$ for *Rana catesbeiana* tadpoles to 71.3 $\mu g \, l^{-1}$ for the tadpoles of *Rana pipiens*. Spinal deformities in embryo– larval tests were observed at concentrations as low as $1.3 \,\mu g \, l^{-1}$ for *Xenopus laevis* after a 10 day exposure.

There is significant variation in the acute toxicity of dieldrin to avian species. Pigeons have an acute oral LD_{50} values in the range of 26.6 mg kg⁻¹ while, in mallard ducks, the acute oral LD_{50} is 381 mg kg⁻¹. Mallard ducklings were exposed to dieldrin in the diet for 24 days. A 24 day no-observed-adverse-effect level of 0.3 µg dieldrin per gram diet, based on growth impairment, was determined. Reproduction success in birds has not been consistently affected in the absence of maternal toxicity.

Other Hazards

Dieldrin is a noncombustible substance and does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. It is not compatible with strong oxidizers, active metals such as sodium, strong acids, or phenols.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.0001 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Reference dose is $0.005 \text{ mg kg}^{-1} \text{ day}^{-1}$
- Permissible exposure limit is 0.25 mg m^{-3} (8 h).

See also: Aldrin; Diazepam; Organochlorine Insecticides.

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Relevant Websites

http://www.atsdr.cdc.gov - Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dieldrin.

- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Dieldrin.
- http://www.osha-slc.gov US Department of Labor, Occupational Safety and Health Administration.

Diesel Exhaust

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• SYNONYMS: Diesel engine emissions; Diesel exhaust particulate; Diesel particulate

Description

Diesel exhaust is a complex mixture of hundreds of constituents in either a gas or particle form. Gaseous components of diesel exhaust include carbon dioxide, oxygen, nitrogen, water vapor, carbon mono xide, nitrogen compounds, sulfur compounds, and numerous low-molecular-weight hydrocarbons. Among the gaseous hydrocarbon components of diesel exhaust that are individually known to be of toxicologic relevance are the aldehydes (e.g., formaldehyde, acetaldehyde, acrolein), benzene, 1,3-butadiene, and polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs. Diesel engines are used to power heavy machinery, locomotives, ships, buses, heavy-duty trucks, and some light-duty trucks and passenger cars.

Exposure Routes and Pathways

Inhalation is the primary route of exposure to diesel exhaust. Incidental ingestion following deposition on soil or vegetation is also a possible route of exposure.

Toxicokinetics

Clearance of diesel particles from the alveolar region of the lung (area of gas exchange) varies from ~ 2 months in rats to almost 1 year in humans. The slower particle clearance rates in humans may result in greater extraction of organics. High-exposure concentrations reduce the lung clearance in animals, further increasing the lung burden. Biological fluids are relatively ineffective in extracting organics adsorbed to diesel particle surfaces. Phagocytosis by macrophages is much more effective in extracting organics. A fraction of organics was eluted in this manner within hours with the more tightly bound fraction removed with a half-life of ~ 1 month. Elution rates are generally more rapid than particle clearance rates so most of the organic fraction is assumed to be bioavailable even with no clearance inhibition.

Mechanism of Toxicity

Comparison of toxic responses in animals exposed to whole diesel exhaust or filtered diesel exhaust indicates that the principal etiologic agent of noncancerous health effects in animals is diesel particulate. Animal experiments provide strong support for the premise that diesel exhaust toxicity results from a mechanism that is analogous to that of other relatively inert particles in the lung. Tumor induction at high doses may be primarily the result of lung particle overload with associated inflammatory responses. Although tumorigenic responses could not be detected under nonparticle-overload conditions, the animal experiments lack sensitivity to determine if a threshold exists. Some studies do support the existence of a threshold if inflammation is assumed to be a prerequisite for lung tumor induction. Most of the carcinogenicity appears to be associated with the portion containing PAHs with four to seven rings. Carcinogenic effects may be a result of the formation of covalent adducts with DNA and subsequent alteration of cellular genetic information. Another proposed mechanism is based on the carcinogenic potential of the particle itself. The particle may induce increases in DNA adducts in the lungs or induce release of mediators from macrophages, many of which are considered to act via promotion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies of responses associated with acute exposure to diesel exhaust have mainly been associated with high

concentrations of carbon monoxide, nitrogen dioxide, and aliphatic aldehydes. Short-term and chronic exposure studies indicate that toxic effects are related to high concentrations of particulate matter. Minimal effects on pulmonary function have been observed in short-term testing even though histological and cytological changes were noted in the lung.

Human

Human studies are comprised of both occupational and human experimental exposures consisting of exposure to diesel exhaust in the occupational environment and exposure to diluted diesel exhaust or diesel particulate matter under controlled conditions.

Symptoms of acute exposure of humans to concentrations above ambient environmental concentrations are irritation of mucous membranes, eyes, and respiratory tract evidenced by early induction of an inflammatory response in healthy humans. Chest tightness and wheezing may occur. Neurophysiological effects include headache, nausea, heartburn, vomiting, weakness, and tingling of extremities and light-headedness. Diesel exhaust odor may cause nausea, headaches, and loss of appetite. Exposure may also increase allergic response to known allergens in some individuals and induce or exacerbate asthma. Studies conducted over the work shifts of individuals exposed to diesel exhaust indicate that reversible changes in pulmonary function in humans can occur due to diesel exhaust exposure, although it is not possible to relate these changes to specific exposure levels.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure studies have been performed on rats, mice, guinea pigs, hamsters, cats, and monkeys. Changes were similar to those noted in short-term exposure studies (accumulation of particles in the lung, increase in lung weight, increase in macrophages and leukocytes, hyperplasia of alveolar epithelium, and thickening of alveolar septa). Decreased resistance to respiratory tract infections has been noted in mice exposed to diesel exhaust. Limited animal data are available indicating alteration in liver structure and function. The lowest exposure levels resulting in impaired pulmonary function varied by species.

Certain extracts of diesel exhaust have been demonstrated to be both mutagenic and carcinogenic in animals and in humans. Lung tumors were induced in female mice and Fischer 344 rats; however, the doseresponse relationship is unclear. Dermal, skin painting, and subcutaneous injection in mice also elicited tumorigenic responses.

Human

Epidemiologic studies of exposure to diesel exhaust and occurrence of lung cancer provide evidence that is consistent with a causal association. Overall, the human evidence for potential carcinogenicity for diesel exhaust is considered to be strong, but less than sufficient for diesel exhaust to be considered as a human carcinogen because of exposure uncertainties (lack of historical exposure data for exposed workers) and an inability to address all potential confounding factors.

Diesel Fuel

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 68334-30-5 (Diesel oil); CAS 68476-34-6 (Diesel fuel no. 2)
- Synonyms
 - Diesel fuel (general) = auto diesel, automotive diesel oil (ADO), diesel engine road vehicle (DERV), diesel, diesel fuel oil, gas oil
 - Diesel fuel no. 1 = no. 1 diesel, kerosene, arctic diesel, diesel fuel oil no. 1, diesel oil no. 1, dipolar
 - Diesel fuel no. 2 = diesel fuel, diesel fuel oil no.
 2, diesel oil no. 2
 - Diesel fuel no. 4 = marine diesel fuel, distillate marine diesel fuel
- CHEMICAL/PHAMACEUTICAL/OTHER CLASS: Petroleum hydrocarbon mixture of branched-chain alkanes, cycloalkanes, aromatic compounds, and sulfurized esters

Uses

Diesel fuel no. 1 is primarily used in city buses. Diesel fuel no. 2 is used in railcars, trucks, and boats. Diesel fuel no. 4 is used in marine vessels.

Background Information

Diesel oil is a complex mixture produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C9–C20 and boiling points in the range of ~163– 357° C (325–675°F).

Diesel fuel no. 1 is a straight-run middle distillate with a boiling range consistent with that of kerosene. It contains branched-chain alkanes (paraffins), cycloalkanes (naphthenes), aromatics, and mixed aromatic *See also:* Pollution Prevention Act, US; Polycyclic Aromatic Hydrocarbons (PAHs); Respiratory Tract.

Further Reading

- Kagawa J (2002) Health effects of diesel exhaust emission a mixture of air pollutants of worldwide concern. *Toxicology* 27(181–182): 349–353.
- United States Environmental Protection Agency (2000), Health Assessment Document for Diesel Exhaust (EPA/ 600/8-90/057E), July 2000.

cycloalkanes. The boiling point range of diesel no. 1 largely eliminates the presence of benzene and polycyclic aromatic hydrocarbons (PAHs). Kerosene contains less than 0.02% benzene and low levels of PAHs.

Diesel fuel no. 2 is a blend of straight-run and catalytically cracked streams, including straight-run kerosene, straight-run middle distillate, hydrodesulfurized middle distillate, and light catalytically and thermally cracked distillates. The boiling range is generally ~160–360°C (320–680°F). Diesel fuel no. 2 is similar in composition to fuel oil no. 2. Some of the PAHs contained in fuel oil no. 2, and therefore probably present in diesel fuel no. 2, include phenanthrene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, and benzo(*a*)pyrene.

Diesel fuel no. 4 is also called marine diesel fuel. It is the most viscous of the diesel fuels and contains higher levels of ash and sulfur. Diesel fuel no. 4 may contain more than 10% PAHs.

Exposure Routes and Pathways

The most common exposure pathway is dermal exposure from handling during transfer, fueling, and repair of diesel-powered vehicles. Although the constituents of diesel are not sufficiently volatile for inhalation of vapors to be an exposure route of concern, inhalation of diesel aerosols can occur. Ingestion of diesel, often associated with aspiration into the lungs, can occur as a result of accidental poisoning or suicide attempts.

Toxicokinetics

Since diesel fuel is a mixture of numerous individual substances, absorption, metabolism, and excretion are very complicated and have not been completely characterized. Systemic effects following dermal and oral exposure and inhalation of diesel aerosols have been demonstrated, indicating that absorption can occur via all routes of exposure.

The alkanes, cycloalkanes, and aromatic compounds present in diesel are lipophilic and tend to distribute to tissues with higher adipose tissue content. The reversibility and short-term nature of many effects observed during acute exposure indicate that retention of the principal diesel fuel components in body tissues is limited.

The alkanes and cycloalkanes in diesel fuel are generally not readily metabolized, and are mostly excreted unchanged through the lungs, with a very small fraction excreted in the urine. The aromatic constituents of diesel are subject to oxidative metabolism and are typically excreted in the urine as watersoluble metabolites.

Mechanism of Toxicity

The mechanism of action for diesel fuels is not well characterized due to the complexity of its petroleum hydrocarbon mixture. The presence of additives that improve fuel combustion or prevent microbial growth may contribute to toxicity. Based on research conducted with individual components of diesel fuels, the primary mechanism of action for central nervous system (CNS) depression from diesel fuel is the reversible, physical interaction of the aromatic and aliphatic hydrocarbons with cell membranes. Renal toxicity is possibly attributed to oxidative metabolites of some of the aromatic constituents. Eye and skin injury are attributable to direct irritant action and the high lipid solubility that may dissolve protective skin oils and allow penetration into the skin tissue. The dermal carcinogenesis observed in rodents subjected to chronic dermal exposure to diesel may be attributed to the genotoxic activity of PAHs and the promoting activity of repeated dermal injury.

Acute and Short-Term Toxicity (or Exposure)

Animal

The principal toxicities observed in animals acutely or subacutely exposed to diesel are dermal irritation by the dermal route and renal toxicity, liver toxicity, and CNS depression from all routes of exposure. Application of marine diesel fuel to the skin of mice resulted in ulceration and in diesel fuel-induced chromosomal aberrations on bone marrow cells of rats.

Diesel fuel has been demonstrated to have a low toxicity in animals following oral exposure. The LD_{50} in rats ranges from 7.5 to ~9 g kg⁻¹.

Human

Inhalation or ingestion of diesel fuel resulted in acute and persistent lung damage in humans. Kidney toxicity has been observed in dermally exposed individuals using diesel fuel as a skin degreaser or a shampoo. In a suicide attempt, a woman ingested 1.5 l of diesel fuel and developed toxic lung disease and fever, which was resolved over the next 4 months.

Chronic Toxicity (or Exposure)

Animal

In acute irritation tests in rabbits, diesel fuel was only mildly irritating to the eyes but severely irritating to the skin. Male and female mice dosed dermally with 2000–40 000 mg kg⁻¹ of marine diesel fuel for 14 consecutive days demonstrated skin lesions and acanthosis, parakeratosis, hyperkeratosis, and inflammatory infiltrates of the dermis. Mice receiving > 20000 mg kg⁻¹ displayed 100% mortality.

No treatment-related mortality was observed in mice administered $250-4000 \text{ mg kg}^{-1}$ marine diesel fuel by dermal application 5 day week^{-1} for 13 weeks; however, the 4000 mg kg^{-1} dose group exhibited chronic dermatitis at the site of application. Rabbits exposed to diesel fuel no. 2 for 24 h day⁻¹, 5 day week⁻¹, for 2 weeks at doses of 1 and 4 ml kg⁻¹ exhibited no mortality and 67% mortality, respectively. All dying animals exhibited signs of chronic dermal irritation, severe anorexia, and depression as the test progressed. Primary causes of death were depression and anorexia attributed to dermal irritation with infection rather than any systemic toxicity, although some liver necrosis was noted.

Rats dosed dermally with 1000 ml kg^{-1} diesel fuel per day, 5 day week⁻¹, for 2 weeks had demonstrated weight loss, reduced liver weights, serum glucose, serum protein, and serum cholesterol, as well as a reduction in hemoglobin, hematocrit, red cell count, and blood lymphocyte counts. Marine diesel fuel produced lesions in the kidneys of mice treated dermally with 50 µl undiluted fuel three times a week for 60 weeks. Kidney lesions were not observed in a second dermal study in which B6C3F1 mice were treated with up to 500 ml kg⁻¹ of marine diesel fuel diluted in acetone five times per week for 103 weeks.

Rats exposed to an aerosol of diesel fuel no. 2 at 100 mg ml^{-1} demonstrated very mild histological changes in the liver and thyroid. No other biochemical effects, hematological effects, or tissue changes were observed in the exposed animals. Continuous 90 day inhalation exposure to 50 or 300 mg m⁻³ of marine diesel fuels produced hyaline droplet nephropathy and reduced body weight gain in male rats.

Pregnant rats exposed to 100 and 400 ppm diesel fuel no. 2 via inhalation on days 6–15 of gestation did not produce offspring with developmental or fetotoxic effects.

Carcinogenicity responses are primarily dependent on the type of diesel fuel applied. Diesel fuel no. 2 did not induce a significant increase in carcinogenesis in Swiss mice when applied at 0.05 ml three times a week for 62 weeks, even in the presence of extreme skin irritation. In another study, diesel fuel no. 2 did not produce tumors by itself: however, it did promote the development of skin tumors initiated by other chemicals. In contrast, marine diesel fuel induced a significant increase in the incidence of squamous cell papillomas and carcinomas when applied to the skin of 49 or 50 male and B6C3F1 mice at doses of 250 and 500 ml kg^{-1} , 5 day week^{-1} . The 500 ml kg^{-1} group study was terminated at 84 weeks due to development of severe skin ulcerations, and the 250 ml kg⁻¹ group study was carried out for 103 weeks. The chemical composition of the marine diesel fuel tested was not completely chemically characterized but consisted of a greater percentage of aromatics and a lesser percentage of alkanes compared to diesel fuel no. 2. It has been suggested that skin carcinogenesis of diesel fuels is probably promoted by chronic irritation and hyperplasia; however, the lack of carcinogenesis in the more refined diesel fuels even in the presence of marked skin irritation indicates that high concentrations of genotoxic PAHs in marine diesel fuel may be involved in the carcinogenic mechanism.

The International Agency for Research on Cancer (IARC) has judged that there is limited evidence for the carcinogenicity in experimental animals of marine diesel fuel. There is limited evidence for the carcinogenicity in experimental animals of straightrun kerosene and sufficient evidence for the carcinogenicity in experimental animals of light vacuum distillates, and of light catalytically cracked distillates and of cracked residues derived from the refining of crude oil.

Human

The predominant effect reported is skin changes from chronic dermal exposure, including cutaneous hyperkeratosis. In a case–control study of cancer at many sites, there was evidence of an increased risk of squamous cell carcinoma of the lung in men estimated to have had substantial exposure to diesel fuel. There was also an indication of an increased risk of cancer of the prostate. No attempt was made to separate the effects of combustion products from those of exposure to diesel fuel itself. Overall, the IARC Working Group has judged that there is inadequate evidence for the carcinogenicity of diesel fuels in humans. Marine diesel fuel is possibly carcinogenic to humans (group 2B). Distillate (light) diesel fuels are not classifiable as to their carcinogenicity to humans (group 3).

In Vitro Toxicity Data

Fuel oil no. 2 gave borderline positive results for mutagenicity in the Ames *Salmonella typhimurium* assay. It was mutagenic to mouse lymphoma cells in forward mutation assays. Another sample did not induce mutation in bacteria or algae; a sample of marine diesel fuel and aliphatic and aromatic fractions of an unspecified diesel fuel were also nonmutagenic to bacteria.

Clinical Management

Skin exposed to diesel fuel should be washed thoroughly with soap and water to minimize local irritation and prevent further absorption. Exposed eyes should be rinsed with large quantities of water for at least 15 min.

Diesel fuel may be aspirated into the lungs following vomiting, causing aspiration pneumonia. Therefore, inducing vomiting following ingestion is not indicated unless the threat of severe renal, liver, or CNS toxicity outweighs potential development of aspiration pneumonia. Inducing vomiting may be indicated if large quantities were ingested or the fuel is suspected to contain highly toxic additives. Vomiting may be induced by administering syrup of ipecac (30 ml for adults and 5 ml for children 1–12 years of age). Activated charcoal may be considered for patients who have ingested another toxic substance.

Environmental Fate

Fuel oil no. 2 is released to the environment during its production, formulation, and use. Direct release to aquatic environments occurs during its use in mosquito control as a coating on breeding waters. If released into soil, fuel oil no. 2 will strongly adsorb. It may biodegrade in water and soil or volatilize from water (half-life of 4.4–4.8 h from a model river) and moist soil surfaces, but adsorption may attenuate the rate of these processes. In water, adsorption to sediment is important. Bioconcentration in aquatic organisms may be limited for the chief components due to metabolism. If released to the atmosphere, degradation of vapor phase components of fuel oil no. 2 by reaction with photochemically produced hydroxyl radicals (estimated half-life on the order of 1 day or less) will be significant.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

International Agency for Research on Cancer (IARC) (1989) Monographs on the evaluation of the car-

Dietary Restriction

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Introduction

Diet restriction (DR), also referred to as caloric restriction, is defined as limitation in the amount of calories consumed by an individual. DR refers to reduction in caloric intake without any reduction in the daily requirement of vitamins and minerals. Diet restriction has been traditionally defined in terms of percent reduction in calories as compared to ad libitum caloric intake. Extensive research in the past century has shown that reduction in food consumption (and in turn, reduction in calories) is associated with a number of beneficial effects such as increase in life span, delay in aging processes, decrease in diseases such as cancer, inflammatory disorders and type-2 diabetes, and protection from toxic effects of various toxicants. In recent years, DR has been recognized as the only lifestyle modification that can be used as a potential cure against 'Syndrome X', a combination of various obesity-related disorders such as type-2 diabetes, and cardiovascular problems. Although the most common experimental DR paradigm in rodents and primates is to restrict calories by 30–40%, in humans, reduction even as low as 10% of normal caloric intake has been found to be beneficial. An important finding of human and animal studies is that DR has to be adopted as a lifestyle rather than a 'one time' treatment in order to derive full benefit. Most of the positive changes brought about by DR are lost once the individual starts consuming ad libitum calories.

Effect of DR has been studied in a range of models including yeast (*S. cereveacae*), rotifers, worms (*C. elegans*), flies (*Drosophila*), spiders, fish, rodents (many strains of rats and mice), guinea pigs, primates cinogenic risk of chemicals to humans. *Diesel Fuels* 45: 219–237.

Scheepers PT and Bos RP (1992) Combustion of diesel fuel from a toxicological perspective. II. Toxicity. *International Archives of Occupational and Environmental Health* 64: 163–177.

Relevant Website

http://www.oehha.ca.gov – (US) State of California. Health Effects of Diesel Exhaust.

(rhesus monkeys and chimpanzees), and humans. The beneficial effects of DR have been observed in all the species that have been studied. While most of the mechanistic information regarding the decrease in cancer incidences, delay in aging, and protection against chemical-induced toxicities comes from rodent models, human studies have demonstrated that DR can bring about improvement in general health of an individual and decrease the risk of 'syndrome X' and related disorders such as heart disease.

The beneficial effects of DR can be broadly divided into three categories: antiaging effects, prevention of disease incidence including cancer, and protection from toxicant-induced injury.

Antiaging Effects of DR

Effect on Animals

Most striking effect of DR is increase in lifespan of the diet-restricted individuals. Longevity has been observed in all the species studied from yeast to rodents and long-term studies on primates are currently underway.

The antiaging effects of DR are best understood in rodent species (**Table 1**). It is known that rats and especially the mice subjected to DR either starting from early age or from middle age accrue the benefits of increased life span. In one such study the C3B10F1 mice subjected to 40% DR had a 20% increase in life span. The experiments with rats and mice indicate that the increase in life span is pronounced in mice as compared to rats. The mechanisms proposed for the greater increase in life expectancy in mice include greater decrease in the energy expenditure by mice on DR. On the other hand, the genetic predisposition of rats to obesity makes them less sensitive to physiological changes induced by DR.

There is substantial evidence suggesting that moderate diet restriction, lifelong or started in the middle

 Table 1
 Antiaging effects of diet restriction

Effect	Model
Prolongation of life span	Male/female white rats
	C3B10RF1 mice
	S. cereveacae (yeast)
	C. elegans
	F344 \times Brown Norway F1 rats
Attenuation of	Sprague–Dawley, F344 rats,
inflammatory disorders	and Balb/c mice
Prevention of dysregulation of cytokines	C57BL/6 mice
Decreased accumulation of protein carbonyls (markers of oxidative stress) in brain	C57BL/6 mice
Decreased accumulation of protein carbonyls in skeletal muscles	C57BL/6 mice
DR maintains 'young' gene expression profile in aged mice livers	C3B10RF1 mice

age, prevents various age-associated diseases. DR maintains the overall physiology in 'young' state and prevents age-associated diseases in cardiovascular system, brain, liver, muscles, and kidney's. The most prominent effect of DR is the attenuation of inflammation that leads to a variety of disorders. Moderate and long-term DR prevents dysregulation of cytokine levels and cytokine related signaling, especially that of TNF- α , IL-6 and NF- κ B. Decrease in oxidative stress has been postulated as a mechanism behind many of the antiaging effects of DR. Decrease in oxidative stress results in lower age-associated dysregulation in inflammation-related signaling and thus delays aging process. This notion is supported by the observation that DR decreases the 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative stress, in brain and other tissues such as liver, heart and skeletal muscles.

It has been recognized that neurodegenerative lesions of brain are one of the major complications in aging. A plethora of studies have shown that DR modulates neurophysiology at the molecular, cellular, and behavioral level thereby preventing loss of nervous function associated with aging. DR prevents aging-induced protein oxidative damage. This is evident from the decreased accumulation of protein carbonyl and sulfhydryl groups, which increasingly accumulate in aging animals. Decrease in accumulation of these protein carbonyls has been correlated to retention of sensorimotor coordination and improvement in learning process in the aged DR mice. DR rats and mice have decreased deposition of lipofuschin granules, another marker of aging in the brain. Similar results have been obtained in skeletal muscles where DR mice did not exhibit the fourfold

increase in the levels of mitochondrial carbonyls and lipid peroxides observed in aged *ad libitum* fed mice. DR leads to induction of heat shock proteins (HSPSs) and stress proteins in the brain, which have been viewed as a potential mechanism behind the decreased oxidative stress in aged DR brains. Apart from oxidative stress other relatively less investigated mechanisms have been put forth to explain longevity due to DR. These include changes in the neuropeptide Y levels, decrease in glycation and glycoxidation, decrease in body temperature, and altered gene expression and protein degradation.

With the advent of cDNA based microarray technology during the last decade, understanding gene expression profile in aging and calorie restricted (antiaging) rodents has gained the center stage. The transcriptome of the DR rodents offers evidence and explanations for many of the anti-aging physiological modifications observed in these animals. The gene expression profile of the aged mice indicated marked inflammatory response, oxidative stress, and reduced neuronal plasticity and neurotrophic support. DR selectively attenuated the age-associated induction of the genes encoding inflammatory and stress responses. These data also indicate a metabolic shift towards lowered accrual of macromolecular damage due to increased protein turnover. Aging was accompanied by changes in gene expression of genes associated with increased inflammation, cellular stress, fibrosis, reduced apoptosis, xenobiotic metabolism, normal cell cycle, and DNA replication. DR reversed majority of these gene changes associated with aging and shifted the 'normoaging' genomic profile towards the 'slow-aging' profiles associated in DR mice.

It can be seen that decreased oxidative stress in DR may be a combination of two events. First, a decreased production of reactive oxygen species (ROS), especially at complex I in the mitochondria. The second mechanism seems to be induction of antioxidant mechanisms such as in increase in glutathione and glutathione-s-transferase activity. Similarly, increased expression of HSPs has been observed in DR and postulated to play a role in decrease oxidative stress. Thus the two opposing forces, decreased production of ROS and increased scavenging the ROS, both are induced in DR leading to decreased oxidative stress. The antiaging effects of DR are strongly linked to this decreased in oxidative stress. The prevention of inflammatory disorders by DR may also be directly linked to decreased oxidative stress since it is known that ROS mediate the production of proinflammatory cytokines such as TNF- α and produce inflammation. Thus, the literature evidence supports the antioxidation theory in the light of anti-aging effects of DR.

Effects on Humans

The limited but striking human evidence, from the biosphere studies, indicates that diet restriction improves human physiology that may eventually lead to increase in longevity. In these biosphere studies, eight human volunteers (four male and four female) stayed in a materially closed but energetically open (sunlight, electric power, and heat) environment and sustained on food material grown inside the biosphere. These subjects underwent an $\sim 30\%$ calorie restriction during the 6 months of stay in the biosphere. The clinical data obtained on the body mass index, serum glucose, triglycerides, serum cholesterol, blood pressure, and leukocyte counts indicated striking improvement after the 6 month period. Although these data from short-term experiments cannot predict the antiaging effects of DR in humans, they indicate that DR of humans is certainly possible and the beneficial effects that were observed in animals under experimental conditions can be achieved in humans.

Anticarcinogenic Effects of DR

Decrease in tumor incidence was one of the first observed favorable effects of reduced food intake observed in experiments conducted in early twentieth century. Since then substantial evidence has shown that DR results in inhibition of tumor promotion and decreases in both spontaneous and chemical-induced cancer incidence.

Effect on Animals

DR has been shown to delay onset of a number of other spontaneous tumors in rodents. This includes hepatoms, breast tumors, pancreatic islet cell tumors, renal tumors, mammary gland cancers, pituitary tumors, and pheochromocytomas. Decrease in spontaneous appearance of preneoplastic foci in the liver was observed in the SPF Wistar rats. Similarly, marked reduction in spontaneous hepatoma was observed in B6C3F1 mice diet restricted for 12 months. In rodents, DR has been shown to decrease colon cancer incidence. Recently it has been shown that DR prevents spontaneous sarcomas and lymphomas in $p53^{-/-}$ mice, which are genetically susceptible to a number of neoplasms. The decrease in tumor incidence is linked to the increase in life expectancy of DR animals, especially rodents, where the incidence of spontaneous tumors is the leading cause of death in rodents.

The hallmark of the anticarcinogenic effect of DR is the ability of diet restriction to prevent chemical-induced tumors. The first reports of inhibition of chemical carcinogenesis came in the 1940s. Benzo[*a*]pyrene-induced skin tumors were decreased in diet restricted ABC, C57, and Swiss strains of mice. Interestingly, it was demonstrated that the decrease in tumor incidence is independent of any particular source of calories. Contrary to the popular belief of 'low-fat' or 'low-carb' diets, it was demonstrated that decrease in cancers following DR depends mainly upon reduction in the total number of calories rather than decrease in calories coming from either only fat or carbohydrates. Since the first reports in the 1940s to 1950s, the anticarcinogenic effect of DR on chemical-induced tumors has been extensively studied in various tissues and after exposure to a variety of chemicals.

There are a plethora of reports indicating that DR can prevent chemical carcinogen in a number of tissues. It has been shown that 40% DR completely inhibits growth of mammary tumors in female rats treated with 7,12-dimethylbenz(a)anthracene. Colon tumors induced by 1,2-dimethylhadrazine are also inhibited by 40% DR in rats. Short-term fasting inhibits appearance of altered hepatic foci induced by the initiation-promotion model of diethylnitrosamine and phenobarbital. In a classic initiation-promotion model, 40% DR during and following the promotion phase (promoted by 12-O-tetradecanoylphorbol-13acetate) of skin tumors initiated by 7,12-dimethylbenz(*a*)anthracene lead to significant reduction in skin papillomas. DR inhibited mammary tumors induced by 1-methyl-1-nitrosourea in a dose-dependent manner. Other cancers prevented by DR include 6nitrochrysene-induced liver tumors in B6C3F1 mice, preneoplastic foci and pancreatic tumors initiated by azaserine in Lewis rats, decrease in azoxymethaneinduced colonic cell proliferation, a prerequisite to colon cancer, in F344 rats, decreased tumorigenicity of 4-aminobiphenyl and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in CD1 mouse bioassay by DR, and inhibition of whole-body radiation-induced myeloid leukemia. Recently, it has been demonstrated that 30-40% calorie restriction inhibits growth of syngenic CT-2A malignant mouse astrocytoma by almost 80%.

The mechanism(s) by which DR inhibits cancer incidence is not completely understood and is currently under investigation in several laboratories. Three hypotheses have gathered substantial experimental evidence (Figure 1): (1) changes in drug metabolizing enzymes leads to decreased bioactivation of carcinogens thereby decreasing DNA adduct formation and tumor formation; (2) decreased DNA damage due to lower oxyradical stress owing to DR leads to lower cancer incidence; and (3) selective removal of initiated and transformed cells by DR animals. Decreased bioactivation of carcinogens

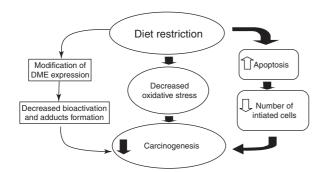


Figure 1 Proposed mechanisms of anticancer effects of DR. Three main mechanisms proposed to explain the anticarcinogenic effects of DR include decreased oxidative stress, increased selective apoptosis of initiated cells, and decreased metabolism of carcinogens.

pertains only to inhibition of chemical-induced carcinogenesis. According to this theory the DR animals have decreased bioactivation of carcinogens. DR decreases expression of sex-specific drug metabolizing enzymes (DMEs) CYP 2C11 (in males) and corticosterone sulfotransferase, which are closely associated with bioactivation and tumor production by carcinogens such as 2-acetylaminofluorene or aflatoxin-B₁. Similarly, decrease in CYP 2A1 in the testicular Leydig cells by DR correlates with the decreased Leydig cell hyperplasia and tumors. Despite the limited experimental evidence, the decreased tumor incidence correlates well with decrease in DMEs by DR.

It has been postulated that increase in DNA damage due to ROS contributes heavily to carcinogenesis. The dividing cells such as hepatocytes have a greater chance of fixing and multiplying the number of the mutations caused by DNA damage leading to transformation of cells. DR is known to decrease the oxidative stress leading to less number of mutations. Decreased oxidative stress in the DR animals is known to occur via decrease in mitochondrial leakage of free radicals. This decrease is not due to lower oxygen consumption but is due to decreased leakage of the reactive species at Complex I in the mitochondrial electron transport chain.

The third hypothesis put forth to explain the anticarcinogenic effects of DR holds that selective removal of initiated cells takes place in DR. It has been shown in the liver tumor model that increased apoptosis and decrease in the rate of cell proliferation in DR animals play an important role in decreasing spontaneous as well as chemical-induced tumors. Similar enhancement in apoptosis has been shown in brain tumor model. The increase in apoptosis is a mechanism devised by the DR animals to cope up with the decreased availability of food. The damaged and weak cells are efficiently removed in the DR animal, which also helps in efficient management of a tight energy budget. Moreover, upregulated apoptosis also removes the cells containing mutated DNA and transformed cells, which are the precursors of tumorigenesis. The successful inhibition of tumors by DR in the $p53^{-/-}$ mice, which are predisposed to tumorigenesis due to deletion of pro-apoptotic gene p53, suggests that the increased rates of apoptosis in DR animals are independent of p53 activation.

The hunt for the mechanisms behind inhibition of chemically induced or spontaneous tumors by DR is still on. Certain other mechanisms such as decreased signaling via the IGF pathway have been postulated specifically in the colon cancer inhibition by DR. It can be seen from the collective evidence that decrease in cancer incidence in DR may be a result of multiple mechanisms. DR induces decrease in oxidative stress mediated by decreased production of ROS, which, in turn, leads to decreased DNA damage. Decrease in DNA damage and lowered mutagenesis is a key for decreased tumor production. On the other hand, increased apoptosis removes those small numbers of cells that inherit the mutated DNA thereby providing a second line of defense. In the mitotic tissues such as liver, kidney, and gastrointestinal tract, DR has been shown to decrease the rate of cell proliferation through more efficient management of energy budget. Decrease in proliferation leads to decreased propagation of mutations as higher cell division provides an opportunity to fix and propagate mutations. A combination of decreased oxidative stress and rate of cell division and increased apoptosis play significant role in inhibition of cancer incidences by DR.

Effect on Humans

The data on anticancer effects of DR on humans are limited. It has been shown in humans that obese persons subjected to DR have decreased rectal cell proliferation, a biomarker related to colon cancer. In a recent retrospective study in Swedish women, it was noted that women with less caloric intake had substantially lower incidence of breast cancer. These reports indicate that the anticancer effects of DR observed in the experimental models are reproducible in humans.

Protection against Acute Toxicity by Diet Restriction

In spite of extensive research on the beneficial effects of DR, the protection offered by DR against acute chemical toxicity has been hardly studied. Nevertheless, there are a few substantial reports indicating that DR can offer protection against acute exposure

Protection from	Species	Proposed mechanism
Ozone-induced lung damage	Male F344 rats	Decreased inflammatory response combined with increased antioxidant status
Cardiotoxicity of isoproterenol (IPR)	Male Sprague–Dawley rats	Decreased core body temperature of DR rats after IPR treatment
Aspirin and acidified ethanol-induced gastric toxicity	Male F344	DR prevents decline in GSH and ATP in the gastric tissue
Gancyclovir-induced gastrointestinal tract damage	B6C3F1 mice	Higher tissue repair and regeneration in DR rats
LPS-induced inflammation and liver toxicity	Balb/c mice	Increased levels of corticosterone leading to decrease in inflammation
Thioacetamide-induced lethal liver injury	Male Sprague–Dawley rats	Enhanced compensatory liver tissue repair due to prompt upregulation of promitogenic signaling

to lethal doses of chemicals. These include protection against ozone-induced lung toxicity, lipopolysaccharide (LPS)-induced inflammatory liver damage, cardiotoxicity of isoproterenol, acute toxicity of gancyclovir, aspirin (nonsteroidal anti-inflammatory drug)-induced gastric damage and thioacetamide-induced liver injury (**Table 2**). In all these studies shortterm DR (up to 3 months) had been employed. The mechanisms involved in protection from acute toxicities are as diverse as the chemicals inducing the toxicity and the organs in which they produce damage. These studies indicate that long-term DR not only prevents age-associated diseases but shortterm DR also protects from acute doses of chemicals.

Effects on Animals

It has been observed that three weeks of DR in F344 rats protects the DR rats from ozone-induced (2 ppm) lung injury. Markers of lung injury such as polymorphonuclear neutrophils (PMNs), IL-6, ascorbate, total glutathione (GSH), α -tocopherol, and fibronectin content, estimated 24 h of ozone exposure in the bronchoalveolar lavage (BAL) fluid indicated lower tissue damage in DR rats. This was accompanied by lower secretion of IL-6, fibronectin, and lower increase in infiltration of PMN. It was concluded that the protection offered by DR is mediated by lower inflammatory response and higher activation of anti-inflammatory mechanisms. This notion is supported by the improved anti-oxidant status of DR lungs illustrated by higher α -tocopherol, urate, and ascorbate in the BAL. These results were confirmed by an independent study in male Sprague-Dawley rats where 20% DR protected the rats from acute exposure to ozone.

Moderate DR also protects from toxicant-induced liver injury. Male Sprague–Dawley rats subjected to 35% DR for three weeks survived a lethal dose of a model hepatotoxicant, thioacetamide. This protection

was in spite of a 2.5-fold higher thioacetamideinduced bioactivation-mediated liver injury experienced by the DR rats. This increase in the initial liver injury was due to induction of hepatic CYP2E1, an enzyme involved in the bioactivation of thioacetamide. Further studies indicated that the mechanism behind the protection offered by DR against thioacetamide toxicity is timely stimulation of compensatory liver tissue repair in the DR rats. It has been demonstrated that when toxicants produce injury in liver (and other organs such as kidney) a concomitant stimulation of tissue repair occurs initiated by intricate signaling via cytokines, growth factors, and nuclear receptors. The compensatory liver cell division starts much earlier in DR rats after thioacetamide treatment due to timely expression of signaling molecules such as IL-6, TGF- α , HGF, and PPAR- α . This prompt increase in cell division results in steady decline in liver injury in the DR rats. In the ad libitumfed controls, a delay in initiating cell division was observed. These observations have shed light on a new dimension of DR-mediated control of cell division, where the signaling involved in compensatory cell division is stimulated but mitogenic signaling, which can lead to cancer, is inhibited. During carcinogenesis, a decrease in cell cycle genes is observed in DR animals while a prompt upregulation of these cell division genes is observed during toxicant-induced acute injury when a timely compensatory tissue repair is necessary for survival.

In a recent study, DR mice exhibited lower inflammatory damage induced by LPS. Balb/c mice were subjected to 40% DR and challenged with $25 \,\mu g$ of LPS. The DR mice had attenuated increases in the proinflammatory cytokines consistent with the lower liver damage characterized by lower plasma alanine aminotranferase (ALT), aspartate amino-transferase (AST) and histopathological analysis. The DR mice also had higher circulating corticosterone, a mediator of antiinflammatory action in DR,

after LPS treatment. The authors concluded that the protection from LPS challenge in DR mice is mediated by increased corticosterone, which leads to decrease in inflammatory response. Although data from other DR experiments support the author's notion of increased anti-inflammatory action, a sharp increase in liver cell division and repair that may play role in long-term survival of the LPS-treated mice was not evaluated in this study.

There are other examples of DR-mediated protection from toxicant-induced injury. Forty percent DR for 12 weeks in male Sprague-Dawley rats protected from that cardiotoxicity of a widely used β -adrenergic agonist, isoproterenol (IPR). It took a large dose $(300 \text{ mg kg}^{-1} \text{ killed } < 10\% \text{ of DR rats versus}$ $15 \text{ mg kg}^{-1} \text{ killed } 50\% \text{ of } ad \text{ libitum fed rats}) \text{ of}$ IPR to kill DR rats. The underlying mechanisms are not clear. However, the authors observed a rapid decrease in core body temperature in the DR rats after IPR treatment. The decreased body temperature may have led to decreased uptake of the parent compound and its metabolites by heart which may explain the protection offered by DR. DR also protects from gancyclovir-induced gastrointestinal tract (GIT) necrosis. Similar to protection from thioacetamideinduced liver injury, the mechanism behind protection against gancyclovir-induced GIT injury models is the enhanced tissue regeneration and repair in the DR animals. Female B6C3F1 mice diet-restricted for 4 weeks (40% restriction) are resistant to gancyclovir.

Effects on Humans

No data are available on the effects of DR on acute toxicity in humans.

DR and Pharmaceutical Drug Safety Testing

Preclinical toxicology testing of pharmaceutical agents is a very important component of the drug development process. Rodent bioassays (using species such as Sprague-Dawley rats) recommended by the Food and Drug Administration have been traditionally used by the drug industry to monitor toxicological effects and carcinogenic potential of the candidate drug molecules. During such long-term cancer bioassay studies, it was observed that control animals suffer from spontaneous tumors towards the end of the assays. This caused serious problems in the final analysis of the data due to animal variation resulting in decreased statistical significance. To overcome this problem, the rodents used in the assay were moderately diet-restricted (25-30% DR) for the duration of the assays. Moderate DR of rodents has a number of advantages such as reduction in animal-to-animal variation, decrease in occurrence of spontaneous tumors, and reduction in diet-related endocrine, renal, and cardiac diseases. Interestingly, moderate DR did not affect the activities of phase I and phase II drug metabolizing enzymes and the toxicokinetics of the candidate drugs. However, larger doses of the candidate drugs have to be used to produce significant toxicological effects. These observations have led to believe that moderate DR of rodents used in bioassays will significantly improve the quality and accuracy of longterm bioassays.

DR and Human Health

Although extensive evidence has been generated supporting its beneficial effects, DR is far from being adopted as a lifestyle choice by people. Obesity remains a major health risk in the United States at this time. Unlike experimental animals, DR in humans is completely voluntary and it has to be adopted as a lifestyle and done consistently throughout the life to accrue its beneficial effects. Both these conditions pose formidable roadblocks in adoption of DR as a lifestyle choice. Recently a concept called 'caloric restriction mimetics' has been put forth by some investigators. It is argued that since people are less likely to adopt DR as a lifestyle by their own choice, pharmacological agents that induce similar type of effects can be developed, which can help people to accrue the same benefits that long-term DR can provide. Some initial experiments have indicated that 2-deoxy-glucose (2-DG), a glucose analog, can be used as a caloric restriction mimetic. Data obtained from subchronic treatment of rats indicate that 2-DG may be a promising candidate since it reproduced some of the beneficial effects of DR namely, decrease in body weight gain, increased insulin sensitivity, and decrease in mean body temperature. However, other beneficial effects of DR such as increased life span, decreased disease incidence (including cancer), and protection from toxicity of chemicals still remain to be studied following treatment with the caloric restriction mimetics.

Summary

Overall, evidence gathered over last 90 years indicates that DR is the most effective modulation that may result in substantial improvement in quality of health via a number of mechanisms including overall decrease in oxidative stress, prompt tissue repair following injury, decreased disease incidence including cancer and increased life span. The importance of DR as a lifestyle modification is more than ever today, since obesity and obesity-related disorders such as 'syndrome X' are number one public health concerns in the United States.

See also: Carcinogenesis; Toxicity, Acute.

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Dietary Supplements

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Dietary supplements have traditionally been products made of one or more of the essential nutrients, such as vitamins, minerals, and protein. Now, a dietary supplement in the United States is a product taken by mouth that contains a 'dietary ingredient' intended to supplement the diet, as defined by Congress in the Dietary Supplement Health and Education Act (DSHEA) of 1994. As defined in the DSHEA, the 'dietary ingredients' in these products may include: vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites. The dietary supplements may make certain claims of function or purpose such as: building strong bones (calcium), maintaining cell integrity (antioxidant), or maintaining bowel regularity (fiber). Some are used to supplement the diet by increasing the total dietary intake.

The DSHEA set up a new framework for the US Food and Drug Administration (FDA) regulation of dietary supplements. It also created an office in the National Institutes of Health to coordinate research on dietary supplements, and it called on then President Clinton to set up an independent dietary supplement commission to report on the use of claims in dietary supplement labeling. It is now easy to spot a supplement because DSHEA requires manufacturers to include the words 'dietary supplement' on product labels. Also, starting in March 1999, a 'Supplement Facts' panel will be required on the labels of most dietary supplements. The DSHEA mandated information that will be required on the labels of dietary supplements includes:

- statement of identity (e.g., 'ginseng');
- net quantity of contents (e.g., '60 capsules');
- structure–function claim and the statement 'This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease';
- directions for use (e.g., 'Take one capsule daily');
- supplement Facts panel (lists serving size, amount, and active ingredient);
- other ingredients in descending order of predominance and by common name or proprietary blend; and
- name and place of business of manufacturer, packer, or distributor. This is the address to write for more product information.

Besides FDA, individual states can take steps to restrict or stop the sale of potentially harmful dietary supplements within their jurisdictions.

Approximately 29000 dietary supplements are currently available to American consumers, and annual sales of dietary supplements in the United States are approaching \$16 billion, and an average of 1000 new products are developed each year. Although manufacturers are restricted from claiming that using their products leads to therapeutic benefits, surveys show that many people take supplements for purposes such as treating colds or alleviating depression. According to other survey data, the majority of consumers believe these products to be either reasonably or completely safe. Some dietary supplements are extracts or concentrates; however, they may also be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders. Further, they can be in other forms, such as a bar; however, if they are, information on their label must not represent the product as a conventional food or a sole item of a meal or diet. They must be intended for ingestion through the alimentary canal and cannot have been previously subject of an Investigational New Drug Application (IND), New Drug Application (NDA), or Biological License Application (BLA) filing. Regardless of how the dietary supplements are produced, DSHEA places them all in a class of 'foods', not drugs, and requires that they all be labeled as a dietary supplement (**Table 1**).

Issues

Providing the public with truthful and nonmisleading information about products for one's health is the responsibility of the FDA. Due to the thousands of individual dietary supplements produced, FDA cannot monitor or regulate the products enough to ensure protection of unsafe products. Unless the supplement is an NDA, the FDA does not require the manufacturers to submit any safety information on dietary ingredients in the dietary supplement before marketing. Therefore, the agency must depend upon adverse event reports, product sampling, consumer complaints, inspections, market-place surveys, information in scientific literature, and other sources to identify danger. The FDA can restrict certain substances that pose a 'significant and unreasonable risk', however, they cannot act upon this until substantial harm occurs to a consumer. And even though harm may occur, banning a product may take years. For example, products containing ephedra have caused harm to thousands of consumers for several years, but FDA has not been able to restrict its use.

As the dietary supplement industry drastically continues to grow, so does the risk. Possible fraudulent products as well as consumer injury are both widely reported. It is very simple for a consumer to obtain such products of fraud due to advertisements on the internet, TV, and magazines. These articles promote a new product claiming to be a 'miracle cure', 'magical', or 'new discovery', however, if the product was a 'cure' it would already be reported and used by health care professionals. Some promotions claim that the product can cure a wide range of unrelated diseases, but no product can do this. There are even some that claim to be backed by scientific studies, but the references may be inadequate or nonexistent. For instance, if a list of references is provided, the citations cannot be traced, or if they are traceable, the studies are out of date, irrelevant, or poorly designed.

Advertisement and easily available products have caused an increase in the problem of fraudulent products and consumer injury. Certain products such as GHB (gamma hydroxybutyrate) and GLB (gamma butyrolactone), were both sold as 'sleep aids' or 'relaxers', better known as date rape drugs. Both of these products have caused death in some cases, but are still on the market as a dietary supplement.

Another issue with safety of dietary supplements is the purity of the ingredients. The supplements are not required to go through any control measures before they are put on the market unlike drugs. Cases such as the L-tryptophan one in 1989 are therefore subject to be repeated. The impurities of this product led to an epidemic of eosinophilia–myalgia syndrome, with 1500 reported cases and 37 deaths.

Some other examples of problem products are given in Table 2 and may vary according to the individual consumer.

Great stress, to ensure efficacy, is put on those products containing stimulants, such as caffeine or ephedra, to those on a diet, workout often, or under the age of 18. These products can cause long-term stress to the body if used for extensive amounts of time. Ephedra arises in the concern of the FDA because of the mechanism of ephedra in the human body. The adrenaline-like stimulant can cause dangerous effects to the nervous system and heart. Some of these effects include heart attack, seizure, stroke, and even death. There must be caution because the risk can increase with the dose, and with strenuous exercise. It specifies certain groups (such as women who are pregnant or breast feeding) who should never use these products and lists other conditions, such as diseases and the use of certain medications that rule out the use of ephedrine alkaloids.

Other reported dangers are the kava-containing supplements. Supplements containing the herbal ingredient kava are promoted for relaxation. For instance, kava is known to relieve stress, anxiety, and tension for sleeplessness, and menopausal symptoms. There has not yet been a determination from FDA that kava-containing products have the ability to perform such benefits. Kava-containing products have been associated with liver-related injuries, including hepatitis, cirrhosis, and liver failure.

PC-Specs is a dietary supplement most commonly used to lower blood pressure as well as in trials to treat prostate cancer. PC-Specs is a mixture of eight herbs – seven of them Chinese. However, the danger is that the supplement contains warfarin and alprazolam. It is commonly prescribed as a blood thinner for

 Table 1
 Commonly used dietary supplements

Herb	Suggested use	Potential toxicity	Potential drug interactions	Comments
Black cohosh (<i>Cimicifuga</i> racemosa)	Menopausal symptoms	Gastrointestinal discomfort	None known	No long-term studies showing efficacy or safety
Chast tree berries (<i>Vitex</i> agnus-castus)	Premenstrual syndrome, mastodynia	Pruritus	May have dopaminergic activity; therefore, avoid with use of dopamine- receptor antagonist (e.g., neuroleptics)	Small, short-term studies suggest efficacy
Cranberry (Vaccinium macrocarpon)	Urinary tract infections	Nephrolithiasis (with cranberry concentrate tablets)	None known	Treatment efficacy not proven; small studies show possible efficacy for prevention
Dong quai (Angelica sinensis)	Menopausal symptoms	Rash	Increased international normalized ratio in patients taking warfarin	No clinical evidence of efficacy
Echinacea (<i>E. purpurea,</i> <i>E. pallida, E. angustifolia</i>)	Upper respiratory infections	Hypersensitivity reactions	Theoretically, may antagonize the effect of immunosuppressive medications	Variations in plant species studied, part of plant used, and extraction methods make conclusion regarding efficacy difficult
Ephedra (<i>E. sinica,</i> mahuang)	Asthma, congestion, weight loss	Hypertension, arrhythmia, myocardial infarction, stroke	Avoid use with monoamine oxidase inhibitors and cardiac glycosides; potential for serious toxicity when combined with out stimulants	Probably effective for short-term weight loss when combined with caffeine; long-term data lacking
Evening primrose (<i>Oenothera biennis</i>)	Eczema, irritable bowel syndrome, mastalgia, premenstrual syndrome, rheumatoid arthritis	Nausea, vomiting, diarrhea, flatulence	Possible lowering of seizure threshold in patients taking antiepileptic medications	Conflicting efficacy data for number of conditions
Feverfew (Tanacetum parthenium)	Migraine prophylaxis	Hypersensitivity reactions	Theoretical risk of increased bleeding when combined with anticoagulants	Few studies support efficacy
Garlic (Allium sativum)	Cardiovascular protection	Gastrointestinal upset, bleeding	Theoretical risk of increased bleeding when combined with anticoagulants	Beneficial effects unproven
Ginger (<i>Zingiberis rhizoma</i>)	Motion sickness, dyspepsia	None known	Theoretical risk of increased bleeding when combined with anticoagulants	Has also been used for nausea and vomiting of pregnancy and osteoarthritis

Ginkgo biloba	Dementia, claudication, tinnitus	Gastrointestinal upset, headache, dizziness, bleeding, seizure	Theoretical risk of increased bleeding when combined with anticoagulants	May have modest effects on cognitive performance and functioning in patients with Alzheimer disease or multi-infarct dementia; no evidence to support prevention of memory loss or dementia
Ginseng (<i>Panax</i> species; Asian ginseng, Korean ginseng, American ginseng)	Fatigue, diabetes	Generally considered safe; rare reports of hypertension, insomnia, headache, and mastalgia	May interact with monoamine oxidase inhibitors and warfarin (decreased prothrombin time)	Currently, little data to support its use
Kava kava (Piper methysticum)	Anxiety	Rash, sedation, liver toxicity	May potentiate effects of benzodiazepines; best to avoid with other antioxidants or alcohol because of risk of excess sedation	Studies suggest efficacy; no data on addition potential
Kola nut (<i>Cola nitida</i>) Saw palmetto (<i>Serenoa</i> <i>repens</i>)	Fatigue Prostatic hyperplasia	Irritability, insomnia Mild gastrointestinal effects	Caution when used with other stimulants None known	Contains caffeine Short-term studies show improvement in symptoms; no evidence for prevention of BPH or prostate cancer
St. John's wort (Hypericum perforatum)	Depression, anxiety	Headache, insomnia, dizziness, gastrointestinal irritation	Can decrease levels of cyclosporine, digoxin, oral contraceptives, theophylline, and indinavir; serotonin syndrome can occur when combined with prescription SSRIs	May be effective for mild to moderate depression
Valerian (Valeriana officinalis)	Insomnia	Headaches	Avoid use with benzodiazepines because of sedation	Theoretical risk of addiction with prolonged use

Agent	Use	Issues
Ayem	USE	155065
Ephedra	Weight loss	Through 2001, there were more than 13 000 health complaints and 100 deaths
Kava	Stress relief	Liver damage – 11 cases of liver failure as of March, 2002
PC-Specs	Lowers blood pressure	Product contaminated with warfarin, leading to bleeding. Also use represents a drug claim
St. John's wort	Antidepressant	Interacts with drugs such as indinavir

Table 2 Example of problem products

patients who are prone to, or recovering from, strokes and other blood clotting disorders. Warfarin can interact with other drugs and cause uncontrollable bleeding and susceptibility to bleeding.

St. John's wort is a herb that has been used for centuries for medicinal purposes, including treating depression. St. John's wort interacts with certain drugs, and these interactions can be dangerous. Some patients who take antidepressant drugs such as St. John's wort, do not experience relief from their depression. Other patients have reported unpleasant side effects from their prescription medication, such as a dry mouth, nausea, headache, or effects on sexual function or sleep. St. John's wort has the potential to interact with other drugs that an individual might be taking and cause adverse side effects including lowering effectiveness of the other drugs. Since St. John's wort is not a proven therapy for depression, it is also not regulated by the FDA. There have been no reported deaths due to the intake of St. John's wort.

Many companies and programs are trying to advance the idea of more regulation of ingredients in the dietary supplements to help reduce risks and injury. However, no action has taken place to appease the issues at hand.

The purpose of a dietary supplement is to better one's health when in actuality it rarely does and the person remains in the average state of poor health. They are meant to enhance a healthy diet, not substitute it. With the use of several dietary supplements one could experience several side effects without gaining the possible benefits.

To bolster the FDA's ability to evaluate the safety of dietary supplements, a 2004 report ('Framework for Evaluating the Safety of the Dietary Supplements') from the Institute of Medicine and the National Research Council of the (US) National Academies outlines a science-based process for assessing supplement ingredients, even when data about a substance's safety in humans is scarce. This approach to safety evaluation works within the regulatory parameters set by the Dietary Supplement Health and Education Act (DSHEA), which does not require manufacturers to provide safety data on their products. The report stated that supplement makers, the public, and others need to increase their reporting of health problems related to supplement use in order to further improve the agency's ability to protect consumers.

Further, a 2003 guidance document ('Guidance for the safety assessment of botanicals and botanical preparations for use with food and food supplements') was developed and published by an expert group of the Natural Toxin Task Force of the European Branch of the International Life Sciences Institute (ILSI Europe) and discussed with a wider audience of scientists at a workshop held in May 2002 in Marseille, France.

Finally, supplement users who suffer a serious harmful effect or illness that they think is related to supplement use should call a doctor or other healthcare provider. He or she in turn can report it to FDA MedWatch by calling 1-800-FDA-1088 or going to www.fda.gov/medwatch/report/hcp.htm on the Med-Watch Website. Patients' names are kept confidential. To file a report, consumers will be asked to provide:

- name, address, and telephone number of the person who became ill;
- name and address of the doctor or hospital providing medical treatment;
- description of the problem; and
- name of the product and store where it was bought.

Consumers should also report the problem to the manufacturer or distributor listed on the product's label and to the store where the product was bought.

See also: Saint John's Wort.

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Diethyl Ether

Angelica Becaria

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 60-29-7
- SYNONYMS: Ether; Ethyl ether; Diethyl oxide; Ethyl oxide; Ethoxyethane; Sulfuric ether; Anesthetic ether
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Ether
- CHEMICAL FORMULA: C₄H₁₀O
- Chemical Structure: CH₃-CH₂-O-CH₂-CH₃

Uses

Diethyl ether is used in the production of rubber, plastics, paints, coatings, perfumes, and cosmetics. It is used as a solvent or extractant for fats, waxes, oils, resins, dyes, and alkaloids. It is also used as a fuel additive, alcohol denaturant, and as a medical anesthetic.

Exposure Routes and Pathways

Inhalation is the main route of exposure to diethyl ether. Occupational exposure to diethyl ether may occur through inhalation and dermal contact with this compound at workplaces where diethyl ether is used. Exposure to this chemical may also occur via inhalation of ambient air and ingestion of contaminated drinking water.

Toxicokinetics

Diethyl ether is immediately absorbed from inhaled air into the bloodstream and passes rapidly into the brain. More than 80% will be eliminated through the lungs and another 1–2% excreted in the urine. The remainder may deposit in fatty tissue. Radiotracer

Relevant Websites

- http://www.fda.gov (US) Food and Drug Administration (FDA), An FDA Guide to Dietary Supplements.
- http://www.nap.edu (US) National Academy of Sciences (2004), the Institute of Medicine and the National Research Council, Committee on the Framework for Evaluating the Safety of the Dietary Supplements, National Research Council, Dietary Supplements: A Framework for Evaluating Safety.

studies in rats have shown that diethyl ether can be degraded to carbon dioxide. 90% of diethyl ether applied on skin is absorbed after 20 min.

Mechanism of Toxicity

The mechanism and site of action of diethyl ether are unknown. In the past, most solvents were thought to interfere with the bulk properties of membranes such as membrane fluidity and permeability, thus causing a generalized perturbation to neuronal membranes. In recent years, it has emerged that specific sites such as ion channels and other receptors are the more likely targets.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of high concentrations of ether produces central nervous system (CNS) changes, such as behavioral effects, excitation, depression, and unconsciousness. Male mice exposed by inhalation to 13 300–30 000 ppm of diethyl ether for 20 min had decreased excitability, reduced muscle tone, and reduced sensorimotor activity. Diethyl ether is a mild eye irritant. The reported toxic doses for mice include the following: LC₅₀ (inhalation), 31 000 ppm per 30 min; LD₅₀ (intraperitoneal), 2.4 g kg⁻¹; and LD₅₀ (intravenous) 996 mg kg⁻¹.

Human

The target organ of ether is the CNS. Inhalation of high concentrations may cause CNS effects including headache, dizziness, unconsciousness, and coma. It is, however, rare to find death due to an inhalation exposure. Ingestion poisonings are of rapid onset, short duration and clinically similar to ethanol overdose. Diethyl ether is an irritant to the eye, skin, and mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Rats exposed orally to $3500 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 13 weeks to diethyl ether presented signs of toxicity characterized by decrease in appetite, weight loss, and death.

Human

Repeated dermal exposure may cause the skin to become dry and cracked due to oil extraction. Several reports have suggested that long-term exposure to diethyl ether may have health effects, but there is not enough information available to draw firm conclusions.

In Vitro Toxicity Data

Mutagenicity studies in cultured mammalian cells are ambiguous. Positive and negative results have been reported. Bacterial mutagenicity tests have been primarily negative. Aged ether (containing peroxides) has been shown to be mutagenic.

Clinical Management

Contact with the skin should be minimized by thoroughly washing affected areas for at least 15 min. Symptoms of dermatitis should be treated if necessary. If ingested, vomiting should not be induced since ether poses an aspiration hazard and chemical pneumonitis may occur. CNS depression may result from ingestion. Treatment should be symptomatic. There are no known antidotes to diethyl ether.

Environmental Fate

The industrial use of diethyl ether may result in its release to the environment through various waste streams. In air, diethyl ether will exist as a vapor and will be degraded in the atmosphere after reacting

Diethylamine

Janice McKee

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with hydroxyl and nitrate radicals. Half-lives of these reactions in air are estimated to be 1.2 and 5.8 days, respectively. In soil and water, diethyl ether is expected to volatilize and biodegradation is likely to be a slow process. Bioconcentration of diethyl ether in aquatic organisms is low.

Ecotoxicology

The LC₅₀ for *Poecilia reticulate* (guppy) is shown to be 2138 ppm for 14 days. The LC₅₀ for *Pimephales promelas* (fathead minnow) is 2560 mg l⁻¹ for 96 h.

Other Hazards

Diethyl ether is extremely flammable. Its volatility and low ignition temperature make it one of the most dangerous fire hazards in the laboratory. Ether vapor forms explosive mixtures with air due to the formation of unstable peroxides. Diethyl ether may react violently with halogens or strong oxidizing agents.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is set at 8 h time-weighted average of 400 ppm, which is equivalent to 1200 mg m^{-3} . Fifteen minutes short term exposure limit is 500 ppm. The 'immediately dangerous to life or health' concentration is 1900 ppm and is based on 10% of the lower explosive limit for safety considerations.

See also: Anesthetic Agents; Volatile Organic Compounds (VOC).

Further Reading

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• CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Amine

- Chemical Formula: $C_4H_{11}N$
 - Chemical Structure: $(C_2H_5)_2NH$
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 109-89-7
- SYNONYMS: *n*-Ethylethanamine; *n*,*n*-Diethylamine; Diethamine

Uses

Diethylamine is used in the manufacture of rubberprocessing chemicals, adhesives, pharmaceuticals, insect repellents, resins, flotation agents, and dyes. It is used as a corrosion inhibitor in the metal industries and is used in electroplating. It is also used as a solvent for removing impurities from oils, fats, and waxes, and is used as a polymerization inhibitor.

Exposure Routes and Pathways

Occupational exposure may occur through inhalation and dermal contact. The general population may be exposed through the ingestion of food and the use of tobacco products.

Toxicokinetics

Diethylamine is rapidly absorbed, with the rate of skin absorption dependent on the size of the area involved and the duration of contact. Diethylamine is primarily excreted unchanged in the urine.

Mechanism of Toxicity

The toxic effects of diethylamine are due primarily to its corrosive action on tissues.

Acute and Short-Term Toxicity (or Exposure)

Diethylamine is a corrosive skin and eye irritant and severe respiratory tract irritant.

Animal

Diethylamine is a primary skin irritant and is an irritant to the eyes and mucous membranes. The dermal LD_{50} in rabbits was 580 mg kg^{-1} . An inhalation LC_{50} of 4000 ppm in rats was reported for a 4 h exposure. The oral LD_{50} in rats has been reported to be 540 mg kg^{-1} . Intraperitoneal injection in rats resulted in a moderate inhibitory effect with respect to liver function and monoamine oxidase activity.

Human

Diethylamine is a severe skin and eye irritant. Eye exposure to diethylamine can cause edema of the corneal epithelium, generally without pain and causing colored halos around lights. This effect generally clears within 24 h. Intense eye exposures cause blurring, photophobia, and discomfort from the roughness of the corneal epithelium. Direct contact with skin has a corrosive effect, causing erythema and blistering. Respiratory tract irritation is expected from inhalation exposures. Ingestion of diethylamine causes severe burns to the oral tissues, with emesis, abdominal pain, and diarrhea.

Chronic Toxicity (or Exposure)

Animal

Rabbits exposed by inhalation to 100 ppm for 6 weeks experienced irritation of the lung tissue and cornea, moderate peribronchitis, nephritis, a slight thickening of the vascular walls, and multiple punctate erosions and edema of the cornea. Changes in the liver were also noted, including parenchymatous degeneration. Parenchymatous degeneration of the heart muscle has been observed in rabbits at these concentrations but has not been confirmed in other species.

Human

Chronic diethylamine exposure could aggravate existing respiratory diseases.

In Vitro Toxicity Data

Diethylamine was evaluated for mutagenicity in the *Salmonella*/microsome preincubation assay. It was negative in these tests up to $3333 \,\mu g$ per plate in the presence and absence of Aroclor-induced rat or hamster liver S9.

Clinical Management

Exposed skin and eyes should be irrigated with copious amounts of water. After inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. Humidified supplemental oxygen (100%) should be administered with assisted ventilation as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gasses. If pulmonary edema is present, positive end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, the use of diluents is controversial. Emesis or lavage should be avoided. A fall in blood pressure may indicate a delayed gastric or esophageal perforation.

Environmental Fate

Diethylamine will exist solely as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with hydroxyl radicals; the half-life for this reaction in air is estimated to be 5 h. In soil, diethylamine is expected to have high mobility. Volatilization from moist soil surfaces is not expected; however, it may volatilize from dry soil surfaces. Biodegradation may be rapid in water. The potential for bioconcentration in aquatic organisms is low.

Ecotoxicology

An LC₅₀ for the fathead minnow has been reported to be $855 \text{ mg}l^{-1}$ for 96 h.

Other Hazards

Diethylamine is highly flammable. It is incompatible with strong oxidizing agents.

Exposure Standards and Guidelines

Diethylamine occupational exposure standards and guidelines include the following:

- USA: Occupational Safety and Health Administration (OSHA) permissible exposure limit (Table Z-1) is 25 ppm (75 mg m⁻³).
- USA: The vacated 1989 OSHA permissible exposure limit of 10 ppm (30 mg m⁻³) and short-term exposure limit (STEL) of 25 ppm (75 mg m⁻³) is still enforced in some states.
- USA: National Institute for Occupational Safety and Health values include a recommended exposure limit of 10 ppm (30 mg m⁻³), a 15 min STEL of 25 ppm (75 mg m⁻³), and an 'immediately dangerous to life or health' value of 200 ppm.

- USA: American Conference of Governmental Industrial Hygienists recommended exposure limit is 5 ppm, with a 15 min STEL of 15 ppm, with a skin notation.
- Australia: 10 ppm, STEL 25 ppm.
- *Germany*: 10 ppm, short-term level 20 ppm for 10 min, four times per shift.
- *Sweden*: 10 ppm, short-term value 15 ppm for 15 min, with a skin notation.
- United Kingdom: 10 ppm, and 10 min STEL of 25 ppm.

See also: Corrosives; Respiratory Tract.

Further Reading

- American Conference of Governmental Industrial Hygienists, Inc. (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th edn., vols. I– III. Cincinnati, OH: ACGIH.
- Clayton GD and Clayton FE (eds.) (1993–1994) Patty's Industrial Hygiene and Toxicology, 4th edn., vols. 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. New York: Wiley.

Relevant Websites

http://www.bibra.co.uk – Diethylamine and its Hydrochloride (BIBRA Toxicity Profile).

http://www.osha-slc.gov – Diethylamine (Occupational Safety and Health Guideline).

Diethylene Glycol

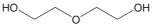
Lu Yu

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 111-46-6
- SYNONYMS: Ethanol, 2,2'-oxybis-; β,β'-Dihydroxydiethyl ether; Bis(β-hydroxyethyl) ether; Bis(2hydroxyethyl) ether; Brecolane NDG; Deactivator E; Deactivator H; Dicol; Digenos; Diglycol; Digol; Dissolvent APV; DEG; Ethylene diglycol; TL4N; 2,2'-Oxybis[ethanol]; 2,2'-Oxydiethanol; 2,2'-Oxyethanol; 3-Oxapentane-1,5-diol; Glycol hydroxyethyl ether; 3-Oxa-1,5-pentanediol; 2-Hydroxyethyl ether; Ethanol, 2,2'-oxydi-; Glycol ether; 2,2-Di(hydroxyethyl) ether; Carbitol; Diethylenglykol; Dihydroxydiethyl ether; 2,2'-

Dihydroxyethyl ether; 2-(2-Hydroxyethoxy)ethanol; Iethyleneglycol; HADB 69; NSC 36391

- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Glycol
- CHEMICAL FORMULA: C₄H₁₀O₃
- CHEMICAL STRUCTURE:



Uses

Diethylene glycol is used as dehydrating agent for natural gas processing; as a humectant for tobacco, casein, synthetic sponges; as a lubricating and finishing agent for textiles; as a constituent of brake fluids, lubricants, mold release agents, antifreeze formulations, and inks; as a plasticizer for cork, adhesives, paper, packaging materials, and coatings; as a solvent for printing inks and textile dyes; as an intermediate in the production of diethylene glycol dinitrate; dioxane; and as an intermediate in the production of some resins, morpholine, polyurethane, triethylene glycol, surfactants, and diethylene glycol esters and ethers. It is also used in lacquer industry and in cosmetics. Diethylene glycol is produced commercially as a by-product of ethylene glycol production.

Exposure Routes and Pathways

Occupational exposure occurs through inhalation, dermal contact, and digestion. Accidental ingestion has been reported, such as the 1985 scandal of Austrian wine diluted with diethylene glycol. If diethylene glycol is released to the atmosphere, it may expose to general population through inhalation of vapor.

Toxicokinetics

After oral administration, diethylene glycol will be absorbed rapidly and almost completely through the gastrointestinal tract. It was reported that 10% of dermal applied diethylene glycol is absorbed by rats. Diethylene glycol was rapidly distributed throughout all organs and tissues following administration with maximum levels in kidney and minimum levels in the adipose tissue, and excreted mainly unchanged through urine. A portion of diethylene glycol is metabolized to oxylate. The half-life for diethylene glycol following a single oral dose of 1, 5, or 10 ml was reported to be between 6 and 10 h in rats in a study.

Mechanism of Toxicity

In large doses, diethylene glycol is a central nervous system (CNS) depressant; and lethality, which occurs within 24 h of a large single dose, is considered to be the result of this effect. Smaller doses, which produce acute toxicity with injury or delayed lethality, primarily affect the kidneys and the liver and are associated with renal insufficiency due to swelling of the convoluted tubules and plugging of the tubules with debris.

Acute and Short-Term Toxicity (or Exposure)

Animal

Diethylene glycol is not irritating to the skin or eyes of the experimental animals. LD_{50} ranged from 7.7 g kg⁻¹ in rat i.p. study to 26.9 g kg⁻¹ in rabbit oral study. Symptoms of acute toxicity were similar for rabbits, dogs, mice, and guinea pigs and consisted

of thirst, diuresis, and refusal of food, followed several days later by low urine volume, proteinuria, prostration, dyspnea, bloating, coma, low body temperature, and death.

Human

Diethylene glycol is not highly irritating to mucous membranes or by application to skin. Relatively low hazard has been associated with industrial use of diethylene glycol due to its low vapor pressure and low dermal penetration. The average fatal dose in people who drank sulfanileamide elixir with diethylene glycol was $\sim 1 \text{ ml kg}^{-1}$. Probable lethal dose to human is $0.5-5 \text{ g kg}^{-1}$. Median diethylene glycol dose that was fatal in 85 of 87 children was estimated to be 1.3 ml kg^{-1} (range $0.22-4.42 \text{ ml kg}^{-1}$). Effects from accidental ingestion or ingestion of contaminated medications include CNS depression, nausea, vomiting, headache, diarrhea, abdominal pain, polyruia, oliguria, anuria, leukocytosis, ascites, hydrothorax, hydropericardium, hypotension, hemorrhages, congestion in the stomach and intestines, distention of the leptomeningeal veins, pulmonary edema, pericardial hemorrhage, centrilobular necrosis with slight jaundice and liver enlargement, enlarged kidneys, cortical necrosis of the kidneys, and acute renal failure. The results of a case-control, cohort study and toxicological evaluation of acute kidney failure among 109 Haitian children (18 years or younger) attributed to diethylene glycol contamination of glycerin used in the local manufacture of acetaminophen oral syrup are reported. Another case control study concluded that paracetamol elixirs with diethylene glycol were responsible for a large outbreak of fatal renal failure in Bangladesh.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to diethylene glycol produces renal and liver lesions. In repeated dose studies, rats given a diet containing 0.25% for 30 days were not affected. Degenerative kidney lesions occurred at dietary concentrations of 1000 ppm (1%) for 30 days. Rats administered diethylene glycol via drinking water exhibited narcosis, weight depression, and mortality at concentrations of 5% and 20%. Diethylene glycol was administered in the diet at concentrations of 2000 ppm (2%) and 4000 ppm (4%) for ~2 years. Males administered the 4000 ppm diet had a higher incidence of bladder stones, and one male had a bladder tumor. These data suggest that diethylene glycol is not a primary carcinogen; however, high dietary concentrations can result in formation of bladder stones, which can lead to development of bladder tumors due to irritation. A dose level without effect was assumed to be between 10 000 and 40 000 ppm in drinking water for rats in a 4 week study. A no-adverse-effect level in rat of 1.25% in the drinking water has been derived from a 2 year study. Diethylene glycol at 3.5% was reproductive toxic in Swiss mice. There was no maternal or developmental toxicity at 1250 mg kg⁻¹ day⁻¹ of diethylene glycol in Swiss mice; 5000 mg kg⁻¹ day⁻¹ produced significant maternal toxicity, but no evidence of developmental toxicity; 10,000 mg kg⁻¹ day⁻¹ caused maternal toxicity and developmental toxicity.

In Vitro Toxicity Data

Diethylene glycol was not shown to be mutagenic in *Salmonella* microsome in tests reviewed. Negative results were also obtained in tests for SCE and chromosome aberrations in Chinese hamster ovary cells, reverse mutation tests in *Saccharomyces cerevisiae* D7, SOS chromotest in *Escherichia coli* PQ37. Diethylene glycol is not genotoxic.

Clinical Management

Usual measures for decontamination (ipecac/lavage, activated charcoal, cathartics) are recommended within 2 h of ingestion. Renal and hepatic function should be monitored and supported. If acidosis occurs, treatment should begin with 1 or 2 mEq kg⁻¹ (for children 1 mEq kg^{-1}) of sodium bicarbonate intravenously, repeated every 1 or 2 h as needed. Hemodialysis may be necessary for severe acid/base disturbances or renal failure. Treatment with ethanol has been effective in animals, but efficacy data for humans are not available.

Diethylstilbestrol

Xuannga Mahini

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 56-53-1
- SYNONYMS: 4,4'-(1,2-Diethyl-1,2-ethene-diyl)bisphenol; 4,4'-Dihydroxy-α,β-diethylstilbene;

Environmental Fate

Diethylene glycol has been detected in drinking water, ground water, surface water, and indoor air resulting from its release to the environment from its production and use. When released to soil or water, diethylene glycol is highly mobile; adsorption to soil and sediment is very low; volatilization is not expected to be important. It is not susceptible to photolysis on soil surface, but is expected to biodegrade quickly in both soil and water. Bioaccumulation of diethylene glycol in aquatic organism is expected to be low due to an estimated bioconcentration factor of 0.05.

When released to the atmosphere, vapor-phase diethylene glycol is degraded by reaction with photochemically produced hydroxyl radicals with an estimated half-life of 13 h. Diethylene glycol in air in particulate-phase may be removed by wet deposition.

Other Hazards

Flammable, must be preheated before ignition will occur.

Exposure Standards and Guidelines

Workplace environmental exposure level 8 h timeweighted average is 10 mg m^{-3} .

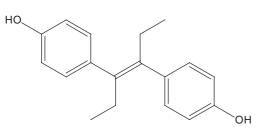
See also: Ethylene Glycol.

Further Reading

- Diethylenglykol (1995) *Toxikologische Bewertung*, vol. 11, 114pp. Heidelberg: Berufsgenossenschaft der chemischen Industrie.
- Woolf AD (1998) The Haitian diethylene glycol poisoning tragedy. *Journal of the American Medical Association* 279: 1215–1216.

3,4-bis(*p*-Hydroxyphenyl)-3-hexene; α, α' -Diethylstilbenediol; α, α' -Diethyl-4,4'-stilbenediol; Antigestil; Bio-des; Bufon; Comestrol; Cyren A; Dawe's Destrol; DEB; DES; Dibestrol '2' premix; Di-estryl; Distilbene; Domestrol; Estilbin 'MCO'; Estrobene; Estrosyn; Fonatol; Grafestrol; Hi-bestrol; Iscovesco; Makarol; Micrest; Microest; Mislestrol; Neo-oestranol I; Oestrogenine; Oestromenin; Oestromensyl; Oestromon; Palestrol; Phenol-4,4'(1,2-diethyl-1,2-ethenediyl)bis-(E); Serral; Sexocretin; Sibol; Stil; Stilbestrol; Stilboestrol; Stilbetin; Stilboffral; Stilboestroform; Stilkap; Stilrol; Synestrin; Synthoestrin; Synthofolin; *trans-* α , α' -Diethyl-4,4'-stilbenediol; *trans*-Diethylstilbesterol; *trans*-Diethylstilbestrol

- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: A nonsteroidal, synthetic stilbene derivative with estrogenic activity. It is an odorless, white crystalline powder, with a molecular weight of 268.36. Its *cis*-isomer tends to revert to the *trans*-form
- Chemical Formula: C₁₈H₂₀O₂
- CHEMICAL STRUCTURE:



Uses

Diethylstilbestrol is an effective estrogenic agent that was formerly used in estrogenic hormone therapy (for menstrual disorders, postpartum breast engorgement, postcoital contraceptive, prevention of spontaneous abortion), and in chemotherapy of various cancers including postmenopausal breast cancer and prostate cancer. It was also used in biomedical research and veterinary medicine (growth promoter for cattle and sheep; veterinary drug to treat estrogen deficiency disorders). As diethylstilbestrol is a drug once prescribed during pregnancy to prevent miscarriage or premature deliveries, an estimated 5-10 million persons in the United States were exposed to diethylstilbestrol from 1938 to 1971, including pregnant women prescribed diethylstilbestrol and their children. In 1953, published research showed that diethylstilbestrol did not prevent miscarriages or premature births. However, diethylstilbestrol continued to be prescribed until 1971. In 1971, the US Food and Drug Administration (FDA) issued a Drug Bulletin advising physicians to stop prescribing diethylstilbestrol to pregnant women. The FDA warning was based on a study published in 1971 that identified diethylstilbestrol as a cause of a rare vaginal cancer in girls and young women who had been exposed to diethylstilbestrol before birth (in the womb). The US Department of Health and Human Services (HHS) and FDA banned the use of diethylstilbestrol in food animal production in the United States in 1979. Also, FDA implementing regulations (21 CFR Part 530.41) of the Animal Medicinal Drug Use Clarification Act of 1996 prohibit the extra-label use of diethylstilbestrol in food-producing animals.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to diethylstilbestrol. It is available in an oral dosage form. All oral and parenteral drug products contain 25 mg or more of diethylstilbestrol per unit dose. Occupational exposure to diethylstilbestrol may occur through inhalation and dermal contact with this compound at workplaces where diethylstilbestrol is produced or used.

Toxicokinetics

Diethylstilbestrol is readily absorbed through the gastrointestinal tract. It is metabolized in the liver by oxidation and conjugation with sulfuric and glucuronic acids. A certain proportion undergoes enterohepatic circulation. The major metabolites of diethylstilbestrol are the oxides, sulfuric conjugates, and the glucuronic conjugates. Diethylstilbestrol is widely distributed throughout most body tissues with major concentrations in fat tissue. Protein binding is 50–80%. The glucuronides and sulfates of diethylstilbestrol are excreted in the urine. A portion is excreted in the bile but is mostly reabsorbed via the enterohepatic circulation.

Mechanism of Toxicity

Diethylstilbestrol stimulates estrogen receptorcontaining tissue. In the 1950s and early 1960s, diethylstilbestrol was an accepted treatment for threatened miscarriages. In 1970, it was first reported that young women whose mothers had been given diethylstilbestrol during the first trimester of pregnancy had an increased incidence of vaginal dysplasia or vaginal adenocarcinoma. Approximately 25% of males exposed to diethylstilbestrol *in utero* exhibit genital lesions and low sperm counts.

Acute and Short-Term Toxicity (or Exposure)

Animal

In cases of diethylstilbestrol intoxication, treated pigs had thickened bladder, with gross distention and acute inflammation, enlarged pelvic urethra, hemorrhages, thickening of mucosa, enlarged prostate gland, and seminal vesicles. Large doses of diethylstilbestrol administered to mice obliterated medullary cavities on long bones, and extramedullary hematopoiesis occurred in the liver, spleen, and adrenal glands.

Human

Toxicity other than gastrointestinal effects is most common following acute ingestion: nausea, vomiting, abdominal cramps, bloating, and diarrhea. Fullness and tenderness of the breast and edema were also observed. Severe migraine was reported in some. Endometriosis and its attendant pain were also seen.

Chronic Toxicity (or Exposure)

Animal

As in humans, exposure to diethylstilbestrol was shown to cause cancer in animals. Breast tumors were observed in animal administered diethylstilbestrol in the diet. Male and female rats fed with diethylstilbestrol in the diet were also shown to have liver and pituitary cancer. Cancer of the cervix and vagina occurred in female mice injected subcutaneously with diethylstilbestrol. Although male mice did not show unusual tumors in any organ, they developed single or multiple epididymal cysts. Male golden hamsters injected with diethylstilbestrol developed kidney tumors. Ovarian lesions and tumors were found in female dogs that received diethylstilbestrol subcutaneously. Diethylstilbestrol was shown to be teratogenic to Rhesus monkeys. Cattle that had been implanted with diethylstilbestrol failed to conceive or had dead fetuses. Following an oral administration of diethylstilbestrol, abortion was observed in pregnant cattle; following subcutaneous implantation, changes in pelvic morphology was observed.

Ovarian tumors were found significantly in the female offspring of treated mice. A decrease in reproductivity was observed in the female offspring of treated mice, where alteration of reproductive tracts was observed in the male offspring. Results from rodent studies also indicate that potent estrogenic chemicals such as diethylstilbestrol can adversely affect sperm counts and quality.

In laboratory animal studies of elderly third-generation diethylstilbestrol-exposed, female mice, an increased risk of uterine cancers, benign ovarian tumors, and lymphomas was found. Elderly thirdgeneration diethylstilbestrol-exposed male mice were at an increased risk of certain reproductive tract tumors. Both the female and male mice studied were the offspring of female mice exposed to diethylstilbestrol before birth (in the womb).

Human

According to the US Environmental Protection Agency's Office of Health and Environmental Assessment diethylstilbestrol is considered to be a group A human carcinogen, which is based on sufficient evidence in humans and sufficient evidence in animals.

More than 30 years of research has confirmed that health risks are associated with diethylstilbestrol exposure. However, not all exposed persons will experience the following diethylstilbestrol-related health problems.

The known health effect for women who are prescribed diethylstilbestrol while pregnant is a modestly increased risk (30%) for

• Breast cancer (Which means when considering breast cancer risks across a lifetime, one in six women prescribed diethylstilbestrol during pregnancy will get breast cancer. In comparison, only one in eight unexposed women will get breast cancer across their lifetime.).

The known health effects for women exposed to diethylstilbestrol before birth (in the womb), known as diethylstilbestrol daughters, are an increased risk for

- Clear cell adenocarcinoma (40 times more likely than unexposed women), a rare kind of vaginal and cervical cancer.
- Increased risk for clear cell cancer appears to be highest for diethylstilbestrol daughters in their teens and early 20s. However, cases have been reported for diethylstilbestrol daughters in their 30s and 40s.
- Reproductive tract structural differences in the uterus and fallopian tube (T-shaped uterus, small uterine cavity, and/or uterine constrictions).
- Pregnancy complications, including ectopic (tubal) pregnancy and preterm (early) delivery.
- Infertility.

The known health effect for men exposed to diethylstilbestrol before birth (in the womb), known as diethylstilbestrol sons, is an increased risk for

• Noncancerous epididymal cysts (growths on the testicles), hypotropic testes, capsular induration of the testes, and cryptorchidism.

Although laboratory animal studies of mice exposed to diethylstilbestrol before birth (in the womb) suggested an increased risk of autoimmune disease in female mice, studies among humans have reported mixed results. One study indicated that autoimmune diseases occurred more often in women exposed to diethylstilbestrol before birth (in the womb), known as diethylstilbestrol daughters, than in the general population. However, no one autoimmune disease (such as rheumatoid arthritis or lupus) occurred more often than others. Researchers will continue to explore this issue.

Third-generation children (the offspring of diethylstilbestrol daughters and sons) are just beginning to reach the age when relevant health problems (such as reproductive tract problems), have been studied.

A study of the health risks for the grand-daughters of women prescribed diethylstilbestrol while pregnant or third-generation daughters was published in 2002. The researchers compared findings of pelvic examinations of 28 diethylstilbestrol grand-daughters with findings noted in their mothers (diethylstilbestrol daughters). Even though abnormalities were present in more than 60% of diethylstilbestrol daughters, no abnormalities were found in the diethylstilbestrol grand-daughters. Diethylstilbestrol grand-sons are being studied at the Netherlands Cancer Institute. Early research reported that hypospadias, misplaced opening of the penis, occurred 20 times more frequently among sons of diethylstilbestrol daughters.

Purpura, edema, leg cramps, gynecomastia, porphyria cutanea tarda, and chloasma may be associated with the chronic use of diethylstilbestrol. Various cancers in premenopausal women have been shown to occur. Other chronic toxic effects include thrombocytopenia, gynecomastia, and fluid retention.

In Vitro Toxicity Data

Clastogenic activity was evaluated in three cultures of rat liver cells. Neither chromatid aberrations nor chromosome aberrations were increased significantly.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as deemed appropriate to the patient's level of consciousness and the history of the ingestion. Activated charcoal may be used to adsorb diethylstilbestrol or concomitant ingestants.

Environmental Fate

Diethylstilbestrol's production and use in biochemical research, medicine, and also in veterinary medicine

may result in its release to the environment through various waste streams. It may also be released to the environment during transport, storage, or disposal. If released to soil, diethylstilbestrol is predicted to strongly adsorb to the soil. Volatilization from the dry or wet soil surface would probably be unlikely. The extent of biodegradation in soil is not known, although diethylstilbestrol has been shown to be resistant to degradation in activated sludge. If released to water, diethylstilbestrol may bioconcentrate in aquatic organisms and strongly adsorb to suspended solids and sediments. Diethylstilbestrol is expected to be essentially nonvolatile on water surfaces. Diethylstilbestrol would not be susceptible to hydrolysis. The extent of biodegradation in natural waters is not certain, although diethylstilbestrol has been shown to be resistant to degradation in activated sludge. If released to the atmosphere, diethylstilbestrol vapors should rapidly oxidize, primarily by reaction with ozone. It is expected to exist solely in the particulate phase in an ambient atmosphere. Particulate-phase diethylstilbestrol may be removed from the air by wet and dry deposition.

Other Hazards

Side effects noted after clinical administration were headache, nausea, vomiting, and, sometimes, vaginal bleeding. Prominent gynecomastia and other feminizing effects were produced in males occupationally exposed to estrogens. Changes in secondary sexual characteristics are fully reversible on cessation of exposure.

Exposure Standards and Guidelines

The Comprehensive Environmental Response, Compensation, and Liability Act reportable quantity for diethylstilbestrol is 1 lb or 0.454 kg when there is a release of this designated hazardous substance. As stipulated in 40 CFR 261.33, when diethylstilbestrol, as a commercial chemical product or manufacturing chemical intermediate or an offspecification commercial chemical product or a manufacturing chemical intermediate, becomes a waste, it must be managed according to Federal and/or State hazardous waste regulations (Resource Conservation and Recovery Act). The state of Florida drinking guideline for diethylstilbestrol is $100 \,\mu g l^{-1}$.

See also: Developmental Toxicology; Environmental Hormone Disruptors; Reproductive System, Female.

Further Reading

- IARC (1972 to present) Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva: World Health Organization, International Agency for Research on Cancer (multivolume work), vol. 21, p. 205.
- Kaiser J (2000) Endocrine disrupters. Panel cautiously confirms low-dose effects. *Science* 290(5492): 695-697.

Relevant Websites

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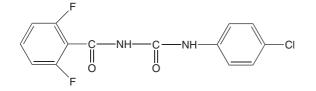
- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Diethylstilbestrol.
- http://www.epa.gov NIH/NIEHS. EDRI Federal Project Inventory: 06995-02 Toxicity of Environmental Estrogens.

Diflubenzuron

Nili Jin

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 35367-38-5; CAS 51026-04-1; CAS 104790-81-0
- SYNONYMS: Dimilin; Difluron; 1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea; Micromite; Vigilante; DFB; N-[[(4-chlorophenyl)amino]carbonyl]-2,6difluorobenzamide
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Benzoylphenylurea insect growth regulator
- CHEMICAL FORMULA: C₁₄H₉ClF₂N₂O₂
- CHEMICAL STRUCTURE:



Uses

Diflubenzuron is used as an insecticide, larvicide, ovicide, and insect growth regulator.

Exposure Routes and Pathways

Ingestion is the primary route for intentional or accidental exposure. Dermal and inhalation exposure are also possible.

Toxicokinetics

Diflubenzuron is well absorbed from the digestive tract and has widespread distribution in the tissues. The major metabolic route in mammals is via hydroxylation. The major route of elimination is via feces; however, urinary elimination is equally important in some species. After a single intravenous dose of diflubenzuron, tissue levels peaked within 15 min in most tissues. Elimination rate after oral administration differs from species to species but is generally completed within 3 days. No literature is available on toxicokinetics in human.

Mechanism of Toxicity

Diflubenzuron inhibits the enzyme chitin synthase, which is required in the final step of chitin synthesis. Chitin is a polysaccharide and a major constituent of the exoskeleton of insects. In insects, the trachea is held open by rings of chitin. The exoskeleton and waxy covering also prevent water loss. Inhibiting chitin synthesis therefore can provide an effective means of pest control. Moreover, vertebrates and most plants do not utilize chitin, thus making diflubenzuron a target-selective pesticide.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of diflubenzuron is low by any route of exposure. The main effects are methemoglobinemia and liver and spleen lesions. The acute oral LD_{50} in rat is >4.64 g kg⁻¹ body weight; acute dermal LD_{50} in rat is >10 g kg⁻¹ body weight; acute derinhalation LD_{50} is >2.49 mgl⁻¹. The no-observedadverse-effect level based on methemoglobinemia is 2 mg kg⁻¹ body weight per day in rats and dogs and 2.4 mg kg⁻¹ body weight per day in mice.

Human

No study has reported on the acute toxicity of diflubenzuron in humans.

Chronic Toxicity (or Exposure)

Animal

Long-time exposure of lab animals to diflubenzuron resulted in higher methemoglobin levels and spleen and liver damage. There was no evidence of treatment-related carcinogenicity.

In Vitro Toxicity Data

Diflubenzuron is not mutagenic.

Clinical Management

Diflubenzuron has very low systemic side effects, if absorbed through the skin. The exposed area should be thoroughly washed with soap and water. Eyes should be washed with copious amounts of roomtemperature water for 15 min in cases of eye contamination. If small amounts are ingested, no treatment is needed. Low toxicity is seen in nontargeted species. Symptomatic treatment is recommended.

Environmental Fate

Diflubenzuron adsorbs to and remains relatively immobile in soil. Degradation of diflubenzuron is largely through microbial hydrolysis and photolysis with a half-life range of 0.5–1 week. Uptake of diflubenzuron through leaves does not occur. Residual diflubenzuron in soil may be absorbed by plants.

Ecotoxicology

There is no significant acute or chronic toxicity of diflubenzuron on small mammals, birds, fish, or mollusks. All chitin-synthesizing organisms, such as shrimp, lobster, and crabs, are susceptible to diflubenzuron toxicity.

Other Hazards

Diflubenzuron is not flammable. If diflubenzuron is involved in a small fire, the fire should be extinguished with carbon dioxide, dry powder, or alcoholresistant foam.

Exposure Standards and Guidelines

On the basis of the no-observed-adverse-effect level (NOAEL) of 2 mg kg^{-1} body weight per day derived in long-term toxicity studies on mice, rats, and dogs and applying a 100-fold uncertainty factor, $0.02 \text{ mg kg}^{-1} \text{ day}^{-1}$ exposures are expected to be safe.

See also: Pesticides.

Further Reading

- Fischer SA and Hall LW Jr. (1992) Environmental concentrations and aquatic toxicity data on diflubenzuron (dimilin). *Critical Reviews in Toxicology* 22(1): 45–79.
- Ishaaya I (1993) Insect detoxifying enzymes: Their importance in pesticide synergism and resistance. *Archives of Insect Biochemistry and Physiology* 22(1–2): 263–276.
- US Department of Agriculture (1977) Common Name for the Pest Control Chemical N-[[(4-Chlorophenyl)amino]carbonyl]-2,6-Difluorobenzamide 'diflubenzuron'. New York: American National Standards Institute.
- WHO (1995) *Diflubenzuron: Health and Safety Guide*. Geneva: World Health Organization.
- WHO (1996) Diflubenzuron: Environmental Health Criteria, 184. Geneva: World Health Organization.

Relevant Website

http://www.inchem.org – Chemical Safety Information from Intergovernmental Organizations.

Difluoroethylene, 1,1-

Kashyap N Thakore and Harihara M Mehendale

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 75-38-7
- SYNONYMS: 1,1-Difluoroethene; NCI-C60208; Vinylidene fluoride
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Flammable gas; Strong oxidizing agent
- Chemical Formula: $C_2H_2F_2$





Uses

Difluoroethylene is used in the manufacture of polyvinylidene fluoride, which is used as a thermal, chemical, and ultraviolet light-resistant agent, and as an anticorrosive agent. The monofilament form is used as filter cloth in the pulp and paper industry. It is used as an insulator due to its high melting temperature. Elastomeric copolymers are used for their heat- and moisture-resistant properties, primarily in industrial, aerospace, and automotive applications.

Exposure Routes and Pathways

The primary exposure route is inhalation.

Toxicokinetics

Absorption is very rapid after inhalation and reaches a steady state within minutes of exposure and blood levels decline rapidly at the end of exposure. Biotransformation is very slow; difluoroethylene may produce alkylating intermediate and some acetone. The tissue/air partition coefficients were determined to be 0.07, 0.18, 0.8, 1.0, and 0.29 for water, blood, liver, fat, and muscle, respectively. Difluoroethylene is eliminated as fluoride ions in urine.

Mechanism of Toxicity

Difluoroethylene may interact with the hepatic microsomal monooxygenase to form epoxide. It inhibits microsomal mixed function oxidase *in vitro*.

Acute and Short-Term Toxicity (or Exposure)

Animal

Difluoroethylene is not acutely hepatotoxic at dose levels up to 82 000 ppm by inhalation for 3.5 h in normal rats, whether fed or fasted.

Human

No data are available for occupational exposure. Acute exposure causes nausea, dizziness, and headache. Difluoroethylene is harmful if swallowed, inhaled, or absorbed through skin.

In occupational settings, technical measures should prevent any contact with the skin and mucous membranes. Workers potentially exposed to this compound should wear personal protective equipment and their work should be carried out only in restricted and ventilated areas. Clothing and equipment after use should be placed in an impervious container for decontamination or disposal. Difluoroethylene is listed as a hazardous air pollutant.

Chronic Toxicity (or Exposure)

Animal

It is carcinogenic in long-term bioassays in Sprague– Dawley rats by oral administration.

Human

It may cause heritable genetic damage and may cause tumors of sense organs, skin, and appendages. No data are available for the evaluation of carcinogenicity.

Clinical Management

In case of dermal or ocular contact, the eyes and skin should be flushed with water for 15–20 min. For inhalation exposure, the patient should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be administered. Life-support measures should be continued until medical assistance has arrived. Do not administer liquids to or induce vomiting in an unconscious or convulsing person.

Environmental Fate

It has medium to high mobility in soil and may degrade in soil. Volatilization is a major fate process for 1,1-difluoroethylene in water. Aquatic bioconcentration and adsorption to sediment are not expected to be important fate processes. It exists as a gas in the ambient atmosphere and degrades by reaction with photochemically produced hydroxyl radicals.

Other Hazards

1,1-Difluoroethylene is extremely flammable and explosive. It may polymerize violently under hightemperature conditions or upon contamination with other products.

See also: Pollution, Air.

Relevant Websites

- http://www.cdc.gov/niosh NIOSH (2003) *Pocket Guide to Chemical Hazards*. Cincinnati, OH: National Institute for Occupational Safety and Health.
- http://www.iarc.fr IARC (1999): Monographs on the Evaluations of Carcinogenic Risks to Humans: Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide, vol. 71, p.1551.

Digitalis Glycosides

Michael Wahl

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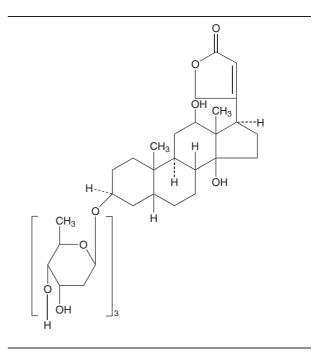
- REPRESENTATIVE CHEMICALS: Digoxin; Digitoxin
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 20830-75-5 (Lanoxicaps); CAS 71-63-6 (Crystodigin)
- SYNONYMS: Lanoxin; Foxglove
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Digoxin is a cardiac glycoside congener of digitalis with a hydroxyl group at the C12 position. Digitoxin is a cardiac glycoside congener of digitalis that does not contain a C12 hydroxyl group
- CHEMICAL STRUCTURES:

Digoxin is also available for parenteral administration and parenteral toxic exposures can occur.

Toxicokinetics

Digoxin

Oral administration of digoxin tablets and liquid results in 60–85% absorption from the small intestine. Liquid-filled digoxin capsules are 90–100% absorbed. The presence of food or other medications may delay oral absorption. Approximately 80% of digoxin is absorbed following intramuscular administration. Minimal metabolism occurs. Cleavage of the sugar moieties occurs in the liver and via bacteria in the large intestine. Protein binding is 20–30%. Volume of distribution approximates 41 kg⁻¹ in adults. Digoxin is excreted in the urine primarily as



Uses

Digitalis glycosides are positive inotropic agents used in the management of patients with congestive heart failure. They control ventricular rate in supraventricular arrhythmias including atrial fibrillation and atrial flutter.

Exposure Routes and Pathways

Ingestion is the most common exposure pathway following accidental and intentional ingestions.

unchanged drug. In healthy patients, the half-life ranges from 34 to 44 h. The half-life can be prolonged in renal failure.

Digitoxin

Oral absorption of digitoxin is rapid and complete. It is extensively metabolized in the liver to several active metabolites including digoxin. Protein binding is 97%. The volume of distribution approximates 0.47-0.761 kg⁻¹. Renal, biliary, and fecal elimination occur. The half-life can range from 4 to 14 days.

Mechanism of Toxicity

The digitalis glycosides interfere with the Na^+,K^+ -ATPase pump with a resultant intracellular loss of potassium and intracellular increases in sodium and calcium. The net effects of this are increased myocardial contractility and decreased cardiac conduction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals manifest toxic effects similar to those seen in humans. Cases of cows ingesting large quantities of plants that contain cardiac glycosides demonstrated anorexia, weakness, diarrhea, and arrhythmias.

Human

Nausea and vomiting are frequently seen. Changes in level of consciousness may be observed. Rhythm disturbances are common signs of toxicity. The most common arrhythmias include bradycardia, heart block, and paroxysmal atrial tachycardia. Premature ventricular contractions and ventricular tachycardia are less common. Severe hyperkalemia can also occur following acute ingestion of a digitalis glycoside. Serum potassium levels as high as $13.5 \text{ mEq } l^{-1}$ have been reported after acute digitalis ingestion. Digoxin serum concentrations can be extremely high immediately following an acute ingestion. Normal digoxin serum concentration is $0.5-2 \text{ ng ml}^{-1}$. Normal digitoxin serum concentrations are $18-22 \text{ ng ml}^{-1}$. These may decrease over 8–12 h as distribution of the drug occurs.

Chronic Toxicity (or Exposure)

Animal

Digoxin has been used in animals to treat congestive heart failure as in humans. Some concern has been raised about potential effects of drugs like digoxin causing toxicity in aquatic animals from sewer effluent or landfill leachate. In a study using a *Hydra vulgaris* model, digoxin at concentrations of 1 mg l^{-1} for 17 days and did not demonstrate adverse effects in feeding or bud formation.

Human

Anorexia, nausea, vomiting, and diarrhea occur after chronic exposure. Decreases in level of consciousness and delirium may be observed. Visual changes including color changes and snowy vision have been described frequently. Common arrhythmias that occur during chronic toxicity include premature ventricular contractions, ventricular tachycardia, and ventricular fibrillation. Hypokalemia is often present in chronic toxicity and actually precipitates toxicity. Serum digoxin/digitoxin concentrations will be elevated but not as high as those seen in acute toxic exposures.

In Vitro Toxicity Data

Studies have demonstrated that Tween 80, a common nonionic surfactant, increases permeability of digoxin in Caco-2 cells.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. A baseline 12-lead electrocardiogram should be obtained and continuous cardiac monitoring should be utilized. A digoxin/ digitoxin serum concentration should be obtained as well as a serum potassium level. Gastrointestinal decontamination procedures should be used as necessary based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used for substantial recent ingestions. In patients with severe dysrhythmias, serum potassium concentrations $> 5 \text{ mEq l}^{-1}$, and elevated digoxin/digitoxin serum concentrations, digoxin immune Fab should be given. This sheep antibody, which binds digitalis glycosides, is effective in reversing both acute and chronic toxicities. Each 40 mg vial binds 0.6 mg of digoxin/digitoxin. Dosage should be based on serum concentration of digoxin/digitoxin or amount ingested. If these are unavailable, 10 vials can be administered.

Digoxin immune Fab can be administered over 30 min or it can be administered intravenous push to patients in cardiac arrest. Since it will pull digoxin/ digitoxin out of tissue sites, serum concentrations of digoxin/digitoxin will rise. These will represent bound digitalis and should not be reacted to clinically. Adverse reactions to digoxin immune Fab include exacerbation of heart failure and atrial arrhythmias as well as hypokalemia. Allergic reactions have not been commonly reported. The antigen/antibody complex should be eliminated within 5 days of administration. This may be delayed beyond 7 days in patients with renal failure. In chronic toxicity, hypokalemia should be treated cautiously with potassium replacement since rapid increases in serum potassium can exacerbate conduction disturbances. Ventricular arrhythmias can he treated with phenytoin or lidocaine. Overdrive pacing should also be considered. Class IA antiarrhythmics such as quinidine should be avoided since they can cause conduction disturbances. Conduction disturbances should be managed with a transvenous pacemaker.

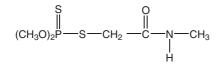
See also: Cardiovascular System; Foxglove.

Dimethoate

Nikita Mirajkar and Carey N Pope

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- CHEMICAL NAME: O,O-Dimethyl-S-2-(methylamino)-2-oxoethyl phosphorodithioate
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 60-51-5
- SYNONYMS: Phosphamide; Cygon; De-fend; Rogor; Rogodial; Roxion; Dimetate; Devigon; Dicap; Dimet; Rogodan
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Synthetic organophosphorus pesticide of the phosphoroth-ionate class
- CHEMICAL STRUCTURE:



Uses

Dimethoate is a systemic and contact insecticideacaricide used on a range of insects including mites, flies, aphids, and planthoppers. Dimethoate is used very commonly in livestock for the control of botflies and mites. Formulations include aerosols, dusts, granules, and emulsifiable concentrates.

Exposure Routes and Pathways

Dimethoate can be absorbed by the oral, dermal, or inhalation route.

Toxicokinetics

Dimethoate is rapidly absorbed after any route of administration in mammals. It is rapidly metabolized in the liver. Like other phosphorothionate pesticides, the parent compound is activated by cyp450 to

Further Reading

- Shumaik GM, Wu AW, and Ping AC (1988) Oleander poisoning: treatment with digoxin-specific Fab antibody fragments. Annals of Emergency Medicine 17: 732–735.
- Smolarz A, Roesch E, Lenz E, et al. (1985) Digoxin specific antibody (Fab) fragments in 34 cases of severe digitalis intoxication. Journal of Toxicology. Clinical Toxicology 23: 327–340.

the active metabolite, omethoate. A major route of detoxification of the parent compound is hydrolysis of the C–N bond. Pretreatment with phenobarbital increases sensitivity to dimethoate in mice. In male rats, $\sim 60-80\%$ of an orally administered dose of dimethoate is eliminated via the kidneys within 24 h of exposure. Elimination was almost complete within 48 h of exposure. Female rats appear to eliminate dimethoate at a slower rate. The rate of metabolism and elimination of dimethoate varies in different species. Dimethoate produces less toxicity in animals with high rates of dimethoate metabolism and animals with high liver-to-body weight ratios.

Mechanism of Toxicity

Dimethoate exerts toxicity through inhibition of acetylcholinesterase. The oxidative metabolite (i.e., omethoate) is two to three orders of magnitude more potent in inhibiting acetylcholinesterase than the parent compound. The *N*-demethylated omethoate may be the most potent inhibitor of cholinesterases. The enzyme in red blood cells may be more sensitive to inhibition than plasma enzyme following dimethoate exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD₅₀ for pure dimethoate in rodents is around 500 mg kg⁻¹, but reported values using technical products range from 28 to 400 mg kg⁻¹. Early formulations contained the solvent methyl Cellosolve, which appears to have participated in chemical changes upon storage that increased mammalian toxicity. In studies comparing dermal and oral exposures, the dermal LD₅₀ values were generally reported to be about twice as high. In a reproductive toxicity test, dimethoate exposure in the drinking water (~10 mg kg⁻¹ day⁻¹) was associated with 60% plasma cholinesterase inhibition in adult mice and altered pup survival and growth but was without teratogenic effects. Teratogenic effects (e.g., fused sternebrae) were reported in rats receiving 12 mg kg⁻¹ day⁻¹ dimethoate but were absent in animals treated with lower doses (i.e., 3 and 6 mg kg⁻¹ day⁻¹). Dimethoate was mutagenic in mice. Mutagenic effects were reported to be more prominent in mice given a single high dose of dimethoate than in mice given 1/12 of the same dose daily for 30 days.

Human

Dimethoate shows moderate toxicity after absorption through oral, dermal and inhalation routes.

Characteristic signs of acetylcholinesterase inhibition (e.g., diarrhea, nausea, and abdominal cramps, sweating, blurred vision, difficulty in breathing or respiratory depression, and slow heartbeat) have been reported following dimethoate exposure. High exposures to dimethoate may be associated with a relapse, where the patient stabilizes and then suddenly gets much worse. Dimethoate does not cause delayed neurotoxicity but has been associated with the intermediate syndrome of organophosphate poisoning.

Respiratory ailments, recent exposure to cholinesterase inhibitors, impaired cholinesterase production, or liver malfunction may potentiate the toxicity of dimethoate. Also, high environmental temperatures or exposure of dimethoate to light (visible or UV) may enhance its toxicity.

Chronic Toxicity (or Exposure)

Animal

Rats given oral doses of dimethoate at 5, 15, or $30 \text{ mg kg}^{-1} \text{ day}^{-1}$ for over a year showed an increase in malignant tumor formation. Increased tumor incidence was not dose dependent, however. Hence, there is inconclusive evidence of carcinogenicity.

Human

Chronic exposure to dimethoate in man can produce disorientation, irritability, confusion, impaired memory and concentration, severe depression, speech difficulties, delayed reaction times, headache, nightmares, sleepwalking, and drowsiness or insomnia. It may also produce nausea, weakness, malaise, and loss of appetite.

Under normal conditions, there is little likelihood of impaired reproductive function, teratogenic, mutagenic, or carcinogenic effects in humans.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2-4 mg (in adults) or $0.015-0.05 \text{ mg kg}^{-1}$ (in children) every 10-15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

After effective intervention, patients should be closely monitored for the possibility of sudden relapse.

Environmental Fate

Dimethoate has low persistence in soil with a halflife of ~ 20 days. It evaporates from dry soil surfaces and is biodegradable. Since dimethoate is broken down rapidly by soil micro organisms, its breakdown is much faster in moist soils. In alkaline soils, it is degraded by hydrolysis. Since it is highly soluble in water and adsorbs very poorly to soil particles, it may leach into groundwater.

The half-life of dimethoate in river water is ~ 8 days. It does not bioaccumulate in aquatic organisms, nor does it adsorb to suspended particles in water. Dimethoate undergoes significant hydrolysis, especially under alkaline conditions. However, losses by photolysis and evaporation from open waters are not expected to be significant. Dimethoate is not toxic to plants.

Ecotoxicology

In birds, dimethoate is moderately to very highly toxic. Acute oral LD_{50} values of 41.7 mg kg⁻¹ in mallards and 20 mg kg⁻¹ in pheasants have been reported. Since birds are unable to metabolize dimethoate as rapidly as mammals, it shows greater toxicity in these species.

Dimethoate produces moderate toxicity in fish, with reported LC_{50} values of 6.2 mg l^{-1} in rainbow trout and 6.0 mg l^{-1} in bluegill sunfish. However, it is much more toxic to aquatic invertebrate species like stoneflies and scuds. Dimethoate is highly toxic to honeybees. The 24 h topical LD_{50} for dimethoate in bees is 0.12 µg per bee.

Dimethyl Ether

Gerald L Kennedy

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 115-10-6
- SYNONYMS: Methyl ether; Dymel
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Ether
- CHEMICAL FORMULA: C₂OH₆
- CHEMICAL STRUCTURE: CH₃-O-CH₃

Uses

Dimethyl ether is used as a refrigerant, as a propellant in aerosol products, as a starter for gasoline engines in cold weather, as a rocket propellant, and as a specialty solvent in chemical synthesis.

Exposure Routes and Pathways

Inhalation is the main exposure route of dimethyl ether; dermal is possible and ingestion is less likely.

Toxicokinetics

Dimethyl ether is rapidly absorbed by the respiratory tracts with steady-state levels attained within 30 min. The material is cleared rapidly with the mean biological half-life being ~ 90 min. Absorption was proportional to the dimethyl ether concentration breathed. No tissue storage, particularly in adipose tissue, was seen.

Exposure Standards and Guidelines

- Reference dose is $0.0005 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Acceptable daily intake is $0.002 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Threshold limit value is 0.7 mg m⁻³

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://www.epa.gov - US Environmental Protection Agency.

Mechanism of Toxicity

Higher concentrations of dimethyl ether act on the central nervous system to produce narcosis. The effects are rapidly reversible which is consistent with the very rapid bioelimination of the molecule. Dimethyl ether has in the past been considered for use as a human anesthetic. It should be noted that this chemical can produce cardiac sensitization similar to the effects of epinephrine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic effects on animals from inhalation exposure include anesthetic effects and decreased blood pressure. The 4 h inhalation lethal concentration 50% in rats is 164 000 ppm (16.4%). Cardiac sensitization occurred in dogs exposed to concentrations of 20% or greater.

Human

The main effects of dimethyl ether in humans have been related to its depressant activity on the central nervous system (CNS). Depression of the CNS has been reported at concentrations ranging from 6% to 10% in air. Again, the rapid uptake and clearance of the chemical limits the possibility of other systemic damage in man. However, inhalation of high concentrations of vapor may cause heart irregularities, unconsciousness, and death. Vapor reduces oxygen available for breathing as it is heavier than air. Direct contact with liquid dimethyl ether may produce frostbite. Vapors of dimethyl ether may produce eye irritation with discomfort, tearing, or blurring of vision. Inhalation exposure may be associated with nonspecific discomfort such as nausea, headache, or weakness. Signs of CNS depression may include dizziness, headache, confusion, incoordination, and loss of consciousness. Abusers of this product could show increased susceptibility to the cardiac arrhythmia effects of epinephrine (cardiac sensitization).

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation exposure to rats and mice caused changes in white blood cell counts, anesthesia, and reduced weight gain. Chronic exposure of rats to 20 000 ppm (2%) caused liver weight reduction and alteration in enzymes associated with liver damage. In another study, observations included decreased red blood cell counts, spleen changes, and decreased survival of males at 10 000 and 25 000 ppm. Hemolysis and red blood cell destruction occurred at 25 000 ppm.

Tests in rats have shown no carcinogenic response at concentrations up to 25 000 ppm. Developmental toxicity including careful evaluations of fetal structural integrity was unaffected in two rat studies, one employing inhalation exposures up to 40 000 ppm, the other to 28 000 ppm. A host-mediated assay in which bacterial indicator organisms incubated in the peritoneal cavity of mice being treated with dimethyl ether showed no increase in mutations.

Human

No information regarding the long-term effects of dimethyl ether in humans has been reported. Again, the first sign of response to the chemical would be expected from the CNS and repeated exposures would most likely behave like a series of acute exposures.

In Vitro Toxicity Data

Dimethyl ether is inactive in genetic tests including the *Salmonella* assay (with and without metabolic activation in at least four strains), HPRT reversion in CHO cells, DNA repair/synthesis in rat liver cells, and the sex-linked recessive lethal test in the fruit fly.

Clinical Management

If high concentrations are inhaled, immediate removal to fresh air should be done. The person should be kept calm. Artificial respiration should be employed on a nonbreathing individual. If breathing is difficult, support oxygen should be given and a physician called. For skin contact, the exposed area should be flushed with water for at least 15 min. The skin should be treated for frostbite if necessary by gently warming the affected area. If irritation is present, it is recommended that a physician be called. For eye contact, flushing should be done with plenty of water for 15 min and a physician called. Ingestion is not considered a potential route of exposure.

It is important to note that because of possible disturbances of cardiac rhythm, catecholamine drugs such as epinephrine should be used with special caution only in situations of emergency life support.

Environmental Fate

Dimethyl ether released to the atmosphere would be expected to exist almost entirely in the vapor phase since the vapor pressure is 4450 mmHg at 25°C. It is susceptible to photooxidation via vapor phase reaction with photochemically produced hydroxyl radicals. An atmospheric half-life of 5.4 days has been calculated. It will also exhibit very high mobility in soil and, therefore, it may leach to groundwater. If dimethyl ether is released to water, it will not be expected either to significantly absorb to sediment or suspended particulate matter, bioconcentrate in aquatic organisms, or directly photolyze. No data concerning the biodegradation of dimethyl ether in environmental media were located but on many ethers are known to be resistant to biodegradation. Dimethyl ether would not be expected to bioconcentrate in aquatic organisms.

Ecotoxicology

Acute toxicity to aquatic organisms is not particularly high with 48 h no-observed-effect concentrations greater than 4000 ppm in both daphnids and guppies.

Exposure Standards and Guidelines

There are no exposure standards but one of the producers for dimethyl ether has an internal guideline that suggests airborne exposure of 1000 ppm both 8 and 12 h time-weighted averages would not be xpected to result in adverse health effects.

See also: Anesthetic Agents.

Further Reading

American Industrial Hygiene Association (1996) Workplace Environmental Exposure: Dimethyl Ether. Bohnenn LJM (1979) Dimethyl ether: Alternative aerosol propellant. Drug and Cosmetic Industry 125: 58-74.

Caprinol TG (1975) Toxicological aspects of dimethyl ether. European Journal of Toxicology And Environmental Hygiene 8: 287–290.

Dimethyl Sulfoxide

Samantha E Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 67-68-5
- SYNONYMS: Methyl sulfoxide, DMSO
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Sulfoxides
- CHEMICAL FORMULA: C₂H₆OS
- CHEMICAL STRUCTURE:

0 || H₃C СН₃

Uses

Dimethyl sulfoxide (DMSO) has excellent solvent properties and acts as a skin penetration enhancer for drugs and other substances by increasing the permeability of the barrier layer of the skin. It is used in the topical administration of drugs, the production of synthetic fibers, the application of pesticides, as an antifreeze, hydraulic fluid, and in the manufacturing of industrial cleaners and paint strippers. Its antiinflammatory and analgesic effects, and the ability to quench free radicals have been by physicians and others for various therapeutic uses.

Exposure Routes and Pathways

Exposure via dermal absorption and inhalation for hospital and veterinary personnel is common due to the use of DMSO in drugs. Industrial applications may lead to dermal and eye contact, and inhalation, with oral exposure a less likely route. The public may also be exposed via the use of DMSO in drugs, and to low levels via the environment (e.g., air and drinking water) via human uses and from natural productions (see below). Kirwin CJ and Galvin JB (1993) Ethers. In: Clayton GO, Claytin FE, and Allan RE (eds.) *Patty's Industrial Hygiene and Toxicology*, 4th edn., vol. II, PartA, pp. 445–525. New York: Wiley Interscience.

Toxicokinetics

DMSO is readily absorbed by animals and humans by dermal and oral routes and enhances absorption of many other chemicals by those routes. Higher concentrations of DMSO are more readily absorbed than more dilute solutions of DMSO in water. After dermal application, radiolabeled DMSO has been detected in blood within five minutes along with halitosis resulting from a metabolite, dimethyl sulfide. Distribution to other organs has been reported to occur within 20 min. Radiolabeled DMSO was detected in bones and teeth of animals within 1h. DMSO can affect ionic balance due to its facilitation of the absorption of many other substances through biological membranes. The major metabolites of DMSO in humans are dimethyl sulfone and dimethyl sulfide. Following oral administration, about twothirds of the dose is excreted in urine as unchanged DMSO, $\sim 20\%$ as dimethyl sulfone, and < 5% is exhaled as dimethyl sulfide. These metabolites have also been identified in monkeys and rats. In the eyes, the highest levels appear to accumulate in the cornea, and the lowest in the lens. DMSO has a calculated half-life of 16 h in blood; the corresponding value for dimethyl sulfone is 38 h. About 3% of oral doses is exhaled as dimethyl sulfide in humans; the percentage appears to be slightly higher in animals. DMSO can persist in serum for more than 2 weeks after a single exposure.

Mechanism of Toxicity

Most physiological properties of DMSO appear to be related to its penetration properties, its potential to inhibit or stimulate enzymes and to act as a free radical scavenger, and its ability to cause histamine release from mast cells. These properties are largely based on DMSO's chemical characteristics, including its hydrogen bonding behavior, water affinity, ability to interchange with water in membranes, and ability to react with organic molecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of DMSO is generally quite low in animals. DMSO causes mild skin and eye irritation in rabbits at as low as 100 mg. Rat oral LD_{50} values range from 14.5 to 28 gkg⁻¹ and dermal LD_{50} values range up to 40 gkg⁻¹. The intraperitoneal and intravenous LD_{50} in mice, rats and dogs exceeds 15 gkg⁻¹. Acute lethal doses in experimental animals have been shown to produce rapid breathing, restlessness, coma, hyperthermia, and rapid death, or death after several days caused by renal failure. DMSO is an experimental teratogen and also causes other reproductive effects in experimental animals.

Human

DMSO is an irritant of the eyes, skin, and respiratory system. Absorption rapidly results in a garlic-like taste and odor. The odor has also been described as an oyster – or onion – like breath and body odor. Dermal application can produce erythema, scaling, contact urticaria, and stinging and burning sensations. Nausea, vomiting, abdominal cramps, chills, chest pains, and drowsiness have been reported, along with erythema, and itching, and transient hemolysis with hemoglobinuria. Anaphylaxis has been said to be a rare occurrence, and transient photophobia and color vision disturbances have been reported.

Chronic Toxicity (or Exposure)

Animal

Repeated exposures lead to renal and hepatic lesions. Prolonged eye contact causes corneal injury (opacities), and repeated dermal application results in irritation and urticaria. DMSO has been reported to cause adverse reproductive effects in animals. It is a questionable carcinogen with some experimental tumorigenic data.

Human

Overexposure may result in urticaria, headache, lethargy, nausea, and dizziness. In a few cases, eosinophilia and sulfhemoglobinemia have been reported following intravenous administration of DMSO. The human TD_{Lo} is 1800 mg kg⁻¹ for skin, and 606 mg kg⁻¹ for intravenous administration. The lenticular changes causing myopia seen in animals following chronic use have not yet been reported in humans.

Clinical Management

Eye exposure should be followed by irrigation with water for at least 15 min; exposed skin should be washed thoroughly with soap and water. Resulting burns or skin irritation should be treated with standard therapy. Cases of dermal sensitization reactions may require topical antiinflammatory agents. If DMSO is swallowed, vomiting should not be induced. Charcoal in water, or with a cathartic should be administered to prevent absorption. Liver and kidney function and blood parameters should be monitored.

Environmental Fate

DMSO is naturally released in the environment, primarily by the oxidation of dimethyl sulfide that is biologically produced in soil, water, and vegetation. It is produced by phytoplankton, and may be released during its production, transport, disposal, and use as a solvent, medicinal analgesic, and other uses. It is fairly resistant to biodegradation based upon screening tests. DMSO may be reduced by some reducing agents that may occur in soil. If released in water, it should disproportionate to dimethyl sulfide and dimethyl sulfone, and may be reduced by reducing agents that may occur in natural waters. In the atmosphere, DMSO will exist primarily in the vapor phase, and will react with photochemically produced hydroxyl radicals with a half-life of \sim 7 h. It also may be released during its production, transport, disposal, and use as a solvent and medical analgesic.

See also: Sensory Organs.

Further Reading

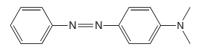
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Dimethylaminoazobenzene

Heriberto Robles

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 60-11-7
- SYNONYMS: 4-Dimethylaminoazobenzene (preferred name); DAB; DMAB; 4-Dimethylaminoazobenzol; 4-Dimethylaminophenylazobenzene; Dimethyl yellow; Brilliant fast oil yellow; Fast yellow; Fat yellow
- Chemical Formula: C₁₄H₁₅N₃
- CHEMICAL STRUCTURE:



Uses

Dimethylaminoazobenzene was once used as a coloring agent for butter and margarine. Dimethylaminoazobenzene was also used as an intermediate in the production of dyes, photosensitive polymers, and reusable films. Dimethylaminoazobenzene is no longer used as a dye and coloring agent.

Toxicokinetics

In laboratory animals, dimethylaminoazobenzene is metabolized in the liver to 4-aminoazobenzene and *N*-hydroxy-4-aminoazobenzene and excreted in bile.

Mechanism of Toxicity

Metabolites of dimethylaminoazobenzene can bind to liver cell macromolecules. Binding to liver cell macromolecules can then lead to liver carcinomas. Diets high in riboflavin have been shown to reduce the binding of dimethylaminoazobenzene to liver macromolecules. The reduced binding was shown to result in a reduced incidence of liver carcinomas.

Acute and Short-Term Toxicity (or Toxicity)

Animal

The oral LD_{50} has been reported as 200 mg kg^{-1} in rats and 300 mg kg^{-1} in mice.

Human

Overexposure to dimethylaminoazobenzene has been reported to produce skin, lung, and blood damage.

Signs and symptoms of overexposure include contact dermatitis, difficulty in breathing, coughing, bloody sputum, bronchial secretions, methemoglobinemia, bloody urine, and frequent and painful urination.

Chronic Toxicity (or Exposure)

Animal

Oral administration of dimethylaminoazobenzene has been shown to produce liver cancer in rats and bladder tumors in dogs. Carcinogenic effects have also been produced following dermal and subcutaneous applications in rats and mice. Dimethylaminoazobenzene is also a teratogen.

Human

The only long-term effect reported for workers exposed to dimethylaminoazobenzene was contact dermatitis.

In Vitro Toxicity Data

Dimethylaminoazobenzene is mutagenic in genotoxicity studies.

Clinical Management

There is no special clinical treatment for overexposures to dimethylaminoazobenzene. Basic life support measures should be implemented and further chemical exposure and absorption should be prevented by removing contaminated clothing and washing the affected area. If ingested, the esophagus and digestive tract may be irritated and may be burned. Therefore, a careful examination should be performed and gastric lavage instituted only if the esophagus is not damaged. It is believed that lavage may be effective at removing the ingested material. Medical examination should look for signs of irritation, abnormalities, and hypersensitivity.

Environmental Fate

Dimethylaminoazobenzene may be released to the environment as a waste industrial product or from unintentional accidental releases. If released to soil it is expected to adsorb strongly to soil particles and not percolate down to groundwater. However, the chemical exists mostly in its ionized form in soils with neural and basic pHs. Therefore, the degree of water solubility and percolation will be influenced by soil and water pHs. The chemical is soluble in organic solvents (alcohol, ether, oil) and essentially insoluble in water (its solubility is in the parts per million range). Therefore, if released to water, it may bioconcentrate in aquatic organisms and/or adsorb to organic matter in sediment. No information is available regarding biodegradation rates for the chemical in environmental media.

Exposure Standards and Guidelines

Dimethylaminoazobenzene is listed as a hazardous air pollutant under the Clean Air Act and, when released to the environment, is a hazardous waste. The US Environmental Protection Agency has classified dimethylaminoazobenzene as a probable human carcinogen. This classification is based on the fact that, although there is no epidemiological evidence that links dimethylaminoazobenzene exposure to the development of human cancer, there is sufficient evidence from laboratory animal studies.

National Institute for Occupational Safety and Health considers 4-dimethylaminoazobenzene to be a potential occupational carcinogen. Special precautions must be taken when working with dimethylaminoazobenzene. Personnel handling dimethylaminoazobenzene must follow industrial hygiene and health

Dimethylmercury

Diem HaMai and Stephen C Bondy

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 593-74-8
- SYNONYMS: Mercury dimethyl; Methylmercury; Methyl mercury
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alkyl mercuries
- CHEMICAL FORMULA: (CH₃)₂Hg
- CHEMICAL STRUCTURE: CH₃-Hg-CH₃

Uses

The primary use of dimethylmercury (DMM) is to calibrate research equipment, as in its application as a standard reference material for ¹⁹⁹Hg NMR measurements.

Background Information

History

In 1997, Karen Wetterhahn, an internationally renowned researcher of the carcinogenic effects of protection requirements for handing potentially carcinogenic substances. At a minimum dimethylaminoazobenzene exposure should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Dyes; Food, Drug, and Cosmetic Act, US; Polymers.

Further Reading

Klaassen CD (ed.) (2001) Casarett & Doull's Toxicology, The Basic Science of Poisons, 6th edn. New York: McGraw-Hill.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology*. Boca Raton: The Parthenon Publishing Group.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Dimethylaminoazobenzene.

heavy metals on DNA repair proteins, was killed within a few months after a single exposure of less than a milliliter of DMM on her latex-covered hand.

Interest

Monomethylmercury (MMM) is the most toxicologically prominent of organic mercury compounds due to its environmental ubiquity and high potential for bioconcentration. Although DMM is less frequently encountered, it exhibits a far more toxic profile. Based on the lethality of only a few drops of the substance, DMM has been classified as a 'supertoxic' chemical. Absorption of $\sim 100 \,\mu$ l of the colorless liquid is equivalent to a severely toxic dose of 100–200 mg of mercury per 100 ml of whole blood. Its synthesis, transportation, and use should be minimized and exercised with only extreme care.

DMM is much more lethal to humans and other mammals than is MMM, yet studies in isolated cell systems reveal that MMM is more toxic than DMM. This paradox arises from the interaction of several factors:

1. DMM is highly lipophilic, and is thus sequestered longer in tissue depots than is MMM. This also allows more rapid access to the nervous system.

- 2. DMM exists at room temperature as a liquid with high volatility. This is in contrast to the solid state of pure MMM. Together with its lipophilicity, these physical qualities enable high concentrations of DMM to be rapidly absorbed by the skin and lungs. Effectively, these routes circumvent firstpass elimination, thereby prolonging the systemic circulation of DMM and extending its residence time in the body.
- 3. The catabolism of DMM to MMM is required in order to enable its neurotoxicity.

Exposure Routes and Pathways

The primary routes of exposure to DMM are dermal contact and inhalation.

This substance has extensive lipid solubility and is absorbed immediately by the skin. Additionally, DMM is able to penetrate many materials including plastic and rubber compounds such as latex, polyvinyl chloride, and neoprene in a matter of seconds. In permeability tests, a Silver Shield glove of a flexible, plastic-laminate, offered skin protection from DMM for ~4 h. This chemically resistant glove, when worn under an outer glove that is resistant to abrasion and tears, may provide limited protection for direct handling of DMM.

The inhalation route of entry is also toxicologically significant. Because of its high vapor pressure (50– 82 mmHg at 20°C), a toxic fume with a slightly sweet odor forms at room temperature. To avoid inhalation of mercury vapors, all work with DMM must be conducted under a hood with the handler wearing a face shield.

Exposure to DMM may also occur via ingestion with absorption occurring at an order of 90–95%.

Toxicokinetics

DMM differs significantly from its inorganic counterparts in its toxicokinetics and health effects. The absorption, distribution, metabolism, and excretion of DMM resemble that of other organic mercuries, particularly the alkylated ones.

DMM is rapidly absorbed and distributed, yet its metabolism and excretion is relatively slow. The difference between its rate of uptake and elimination could in part account for the severity of the toxic effects of DMM, its long latency period of several months, and its pronounced effects on the brain.

Absorption and Deposition

The extensive lipophilicity of DMM allows the substance to pass readily through biological membranes. This chemical property facilitates its near instantaneous absorption by the skin, lungs, and gastrointestinal tract, and results in its accumulation in depots of adipose tissue, plasma proteins, and brain. Approximately 10% of the body burden of organic mercury is localized in the brain.

Metabolism and Transport

During an initial lag period, DMM undergoes conversion to the monomethylated form, and distribution from blood to tissues (half-life of uptake into hair, 6 days). This biotransformation may occur at sites rich in metabolic enzymes such as the skin, intestinal flora, the liver, and macrophages. Free-radical mechanisms are proposed as another possible means of dealkylation.

In tissues, its monomethylated metabolite may undergo further biotransformation. Ultimately, conversion into inorganic mercury enables the metal to bind to glutathione for biliary excretion. However, much of this complex can also be reabsorbed by the gastrointestinal tract. Such bile-hepatic recycling permits redistribution of mercury.

In contrast to inorganic mercury, alkyl mercuries do not induce metallothionein synthesis in renal or liver cells.

Elimination

In humans and mice, the excretion of organic mercury occurs largely by the fecal route, and follows first-order kinetics. The whole body clearance times and blood clearance periods are longer than those for inorganic mercury with the half-life of DMM being \sim 78 days in humans. Other excretory routes are urine, sweat, and hair.

Mechanism of Toxicity

In contrast to the white crystalline solids of the pure forms of MMM and phenylmercury, DMM exists as a colorless liquid at room temperature with high volatility. These physical qualities enable high concentrations of the substance to be absorbed by exposure pathways of the skin and lungs that circumvent first-pass elimination. Effectively, this prolongs the systemic circulation of DMM, and extends its residence time in the body.

The additional alkyl group flanking the mercury imparts DMM with lipophilicity that exceeds its monoaklyated counterpart, and allows DMM to be sequestered in lipid-rich depots. The metabolic delay allows the neurotoxicity of DMM to remain latent for months.

The gradual conversion into MMM results in the release of DMM from depots such as lipid-rich tissues and plasma proteins, and permits its movement through barriers such as the blood-brain and placenta. A cysteine complex of the monomethylated metabolite penetrates the endotheilial cells of the blood-brain barrier by mimicking methionine and using the large neutral amino acid transporter.

Thus, the toxicity of DMM is mediated by its dealkylation. Cleavage of the carbon-mercury bond generates MMM metabolites, which can form covalent bonds with cellular ligands with amphiphilic properties. The mercury center reacts with sulfur and sulfur-containing thiol groups of enzymes and thereby inhibits them. The metal center of DMM acts as a soft acid, and binds tightly to polarizable donor atoms in soft bases. Within cells, mercury may interact with a variety of proteins, particularly microsomal and mitochondrial enzymes. This can severely impair cell function.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicokinetics and health effects of DMM closely resemble that of MMM. Reports of toxicity due to organic mercury compounds are largely based on the administration of the monomethylated form.

Human

DMM is primarily a neurotoxin, and lethality can result from a single exposure to a few drops. Immediate adverse effects may include irritation to the eyes, respiratory tract, and skin. However, symptoms of intoxication may remain latent for months after the exposure.

The earliest clinical deficits include numbness and tingling sensation of the lips, hands and feet, joint pain, narrowing of vision, hearing difficulties, a widely based gait, and emotional disturbances. These symptoms arise from whole-blood concentrations of mercury that exceed 200 μ g Hgl⁻¹ of whole blood (normal concentration, 1–8 μ g Hgl⁻¹). The progression of symptoms includes incoordination, difficulty in pronouncing words, deafness, emotional disturbances, and ultimately, death.

The small granule cells of the cerebellum are selectively vulnerable to DMM. The cortex is also profoundly affected. Extensive neuronal loss and gliosis occur bilaterally within the primary visual cortex (especially at the calcarine fissure) and auditory cortex, with milder loss in the motor and sensory cortices.

Chronic Toxicity (or Exposure)

Animal

Behavioral, developmental, and systemic effects have been reported for a various rodent species in studies of MMM. In rodents, neuronal degeneration with wide-spread calcium deposition has been found in the brain following chronic daily exposure to DMM.

Human

No reports could be found on the effects of chronic toxicity of DMM in humans.

In Vitro Toxicity Data

Few studies have compared the toxicity of DMM with MMM. In cell culture, MMM is the more toxic of the two compounds. MMM has been shown to inhibit nucleic acid synthesis and decrease cell viability in cultured cells whereas exposure to DMM has resulted in no significant effects on these parameters. DMM induced chromosomal aberrations and mitotic spindle disturbances to a far lesser extent than the monomethylated form. This underscores the significance of its biotransformation to DMM toxicity.

Clinical Management

Chelating agents for mercury, such as cysteine and penicillamine, have been used as intervention measures to reduce the concentration of inorganic mercury. However, chelation therapy has yielded variable success in cases of alkyl mercury poisoning. Studies of MMM suggest that chelators may reduce brain and blood mercury levels if started within a few days after exposure. Surgical gallbladder drains and oral administration of a nonabsorbable thiol resin have been applied in order to interrupt biliary excretion and reabsorption of mercury by the intestine.

In the recent Wetterhahn case, the use of oral succimer (2,3-dimercaptosuccinic acid) and exchange transfusion at the initial onset of symptoms was successful in increasing urinary excretion of mercury and decreasing whole-blood mercury concentration. However, despite repeatedly normal CT and MRI scans of the brain, the patient quickly became unresponsive to all visual, verbal, and light-touch stimuli, 22 days after the first neurological symptoms developed (and 176 days after exposure). This suggests that chelators have little clinical benefit if begun after the onset of neurological symptoms.

Ecotoxicology

Most data is confined to MMM which can be generated from inorganic mercurial wastes by plankton and then progressively bioaccumulated. The final concentration in animals higher up the food chain can be several orders of magnitude higher than those present in the original water. Bacterial synthesis of DMM also takes place in marine and estuarine environments. It is present there at very low levels since it is rapidly hydrolyzed to MMM or vented into the atmosphere.

Other Hazards

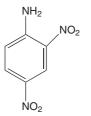
DMM is highly flammable in the presence of strong oxidizing agents. Elevated temperatures cause decomposition into explosive hydrocarbon gases.

Dinitroanilines

Robert A Young

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- CHEMICAL ABSTRACTS SERVICES REGISTRY NUMBERS: There are four isomeric forms of dinitroaniline – 2,3-Dinitroaniline (CAS 602-03-9); 2,4-Dinitroaniline (CAS 97-02-9); 2,6-Dinitroaniline (CAS 606-22-4); 3,5-Dinitroaniline (CAS 618-87-1)
- SYNONYMS:
 - 2,3-Dinitroaniline; 2,3-Dinitrobenzenamine; 2,3-Dinitrophenylamine
 - 2,4-Dinitroaniline; 2,4-Dinitrobenzenamine;
 2,4-Dinitrophenylamine
 - 2,6-Dinitroaniline; 2,6-Dinitrobenzenamine; 2,6-Dinitrophenylamine
 - 3,5-Dinitroaniline; 3,5-dinitrobenzenamine; 3,5dinitrophenylamine
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aromatic amine
- Chemical Formula: $C_6H_5N_3O_4/C_6H_3(NH_2)$ (NO₂)₂
- CHEMICAL STRUCTURE: The structure of 2,4-dinitroaniline, the most common dinitroaniline, is shown below



See also: Methylmercury; Minamata.

Further Reading

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- Ostlund K (1969) Studies on the metabolism of methyl mercury and dimethyl mercury in mice. *Acta Pharma-cologicaet et Toxicologica* 27(Suppl 1): 1–136.
- Weiss B, Clarkson TW, and Simon W (2002) Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environmental Health Perspectives* 110(Suppl 5): 851–854.

Uses

2,4-Dinitroaniline is used in the production of azo dyes.

Exposure Routes and Pathways

Exposure to dinitroanilines is most likely to occur in occupational settings. Inhalation and dermal exposure are the primary routes of exposure.

Toxicokinetics

Most of the information on the toxicokinetics of dinitroanilines pertains to 2,4-dinitroaniline. Dinitroanilines are highly toxic to humans and are well absorbed from all routes of exposure. Nine metabolites were detected in rats administered [¹⁴C]2,4-dinitroaniline orally or intravenously. 2,4-Dinitrophenylhydroxylamine was the main metabolite and was excreted in the urine as the sulfate conjugate and in bile as the glucuronide. Amine hydroxylation and sulfation of 2,4-dinitroaniline are probable detoxification processes that occur rapidly and facilitate clearance.

In rats administered $[^{14}C]^2$,4-dinitroaniline per kilogram orally or intravenously, there was rapid distribution of the compound to all major tissues. Muscle, skin, and adipose tissue contained 65–70% of the ^{14}C activity in the body during the 45 min after dosing. Approximately 70–85% of the aforementioned doses were cleared from most tissues within 6 h after administration. Three days after administration, only residual levels were detected in the major tissues. Urinary excretion of ¹⁴C activity at 6 and 24 h after dosing accounted for 30% and 63%, respectively, of the administered dose. Fecal excretion over 3 days accounted for 23% of the dose. Elimination of 2,4dinitroaniline-derived ¹⁴C activity in the bile amounted to 12.5% of the dose after 5 h.

Mechanism of Toxicity

Much of the toxicity associated with dinitroaniline exposure is the result of methemoglobin formation in which the iron of the hemoglobin molecule is oxidized causing a deficiency in the oxygen carrying capacity of the blood. This produces the cyanosis and other signs of dinitroaniline-induced toxicity.

Acute and Short-Term Toxicity (or Exposure)

Human

Little definitive information is available regarding the toxic effects of dinitroanilines. Dermal and ocular exposure may result in irritation and pain. Inhalation exposure to dinitroanilines may cause irritation, coughing, and throat soreness. Aniline, a structurally similar compound, is a skin and eye irritant and a mild dermal sensitizer. It is rapidly absorbed by all routes of exposure and induces methemoglobinemia. Signs and symptoms of methemoglobinemia include blue skin, headache, dizziness, weakness, lethargy, loss of coordination, coma, and death. Headache and confusion occur early following poisoning, and restlessness, seizures, and coma may occur following severe poisoning. Acute exposure to $3-5 \text{ mg kg}^{-1}$ is associated with signs and symptoms of toxicity that develop within a few hours following exposure. Liver and kidney damage may ensue within 12-72 h postexposure and are probably secondary, hemolysismediated effects. As little as 1 g of aniline has caused human fatalities. The mean lethal dose for humans of the structurally related aniline has been estimated to be in the range of 15–30 g.

Chronic Toxicity (or Exposure)

Animal

The oral LD_{50} values for laboratory species range from 418 mg kg^{-1} (rat) to 1050 mg kg^{-1} (guinea pig). A 4 h inhalation LC_{Lo} of 17 mg m⁻³ is reported

for the laboratory rat. No signs of toxicity were observed in male Fischer 344 rats administered up to $90 \,\mu\text{mol} \, [^{14}\text{C}]_2,4$ -dinitroaniline per kilogram orally or $10 \,\mu\text{mol} \,\text{kg}^{-1}$ intravenously. Animal studies have also shown varying effects on thyroid function.

Human

Human data on the effects of chronic exposure to dinitroanilines are lacking. An increase in the severity of damage to the organs affected by acute exposure would be expected. Additionally, the adverse health effects resulting from prolonged methemoglobinemia are likely to be significant.

Clinical Management

For inhalation exposures, the victim should be removed from the exposure environment and 100% humidified supplemental oxygen should be administered with assisted ventilation as required. Exposed skin and eyes should be copiously flushed with water and thoroughly decontaminated to prevent further absorption. For oral exposure, clinical management should focus on decreasing absorption. Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become comatose or convulsive. Emesis is most effective if initiated within 30 min. Gastric lavage may be indicated if performed soon after ingestion or in patients who are comatose or at risk of convulsing. If the patient is cyanotic and symptomatic, or the methemoglobin level is greater than 30% in an asymptomatic patient, measures should be taken to correct the methemoglobinemia.

Exposure Standards and Guidelines

There are currently no regulatory or health-based guidance values for dinitroanilines.

See also: Aniline.

Further Reading

rand Rienhold.

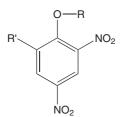
Grayson M and Eckroth D (eds.) (1978) Kirk-Othmer Encyclopedia of Chemical Technology. New York: Wiley. Sax NI and Lewis RJ Sr. (eds.) (1989) Dangerous Properties of Industrial Materials, 7th edn. New York: Van Nost-

Dinitrophenols

David Janz

© 2005 Elsevier Inc. All rights reserved. This article is a revision of the previous print edition article by Tamal Kumar Chakraborti, volume 1, pp. 485–487, © 1998, Elsevier Inc.

- REPRESENTATIVE CHEMICALS: Dinitrophenol (DNP) occurs in six different isomers – 2,3-DNP, 2,4-DNP, 2,5-DNP, 2,6-DNP, 3,4-DNP, and 3,5-DNP
- SYNONYMS: A number of substituted 2,4-DNPs are sold under different trade names; analogs include DNOC, 2,4-Dinitro-6-methylphenol; Binapacryl, 2-*s*-Butyl-4,6-dinitrophenol-3-methylcrotonate; Dinocap, 2,4-Dinitro-6-(1-methy-*n*-heptyl)-phenyl-crotonate; Dinoseb, 2,4-Dinitro-6-*s*-butylphenol
- CHEMICAL STRUCTURE:



Uses

Dinitrophenols are used as fungicides, herbicides, or insecticides. The fungicidal, herbicidal, or insecticidal properties depend on minor differences in the chemical structures of the different dinitrophenol compounds. Several dinitrophenol compounds have more than one pesticidal use. The pesticidal use of one dinitrophenol, dinoseb, was eliminated in the United States in 1986. There has recently been a voluntary cancellation of all US product registrations for the fungicide/miticide Dinocap.

Exposure Routes and Pathways

Dinitrophenol compounds can enter the body through inhalation, oral, or dermal routes of exposure.

Toxicokinetics

Dinitrophenols are rapidly absorbed from the gastrointestinal tract, respiratory tract, and intact skin. They can bind to plasma proteins. After absorption, they are transported through the blood to different organs and distributed in the liver, the kidneys, and the eyes.

Dinitrophenols undergo reduction in the presence of NADPH and nitroreductase and conjugation takes

place at the phenolic site. Humans can slowly detoxify 2,4-DNP to 2-amino-4-nitrophenol, 2-nitro-4-aminophenol, and 2,4-diaminophenol and their glucuronic acid conjugates. The metabolism of DNP is temperature dependent (i.e., DNP metabolism is greatly diminished at low temperatures). In mice, a reduced LD_{50} and increased toxicity for dinitrophenols were observed with an increase in ambient temperature.

In humans, dogs, and rats, 2-amino-4-nitrophenol was found to be the major excretory product. Humans can slowly eliminate both the unchanged compound and the previously mentioned metabolites. Urinary excretion is considered to be the main route of elimination of dinitrophenols. The half-life in the serum of a severely poisoned farmer was calculated to be 13.5 days. The residence half-life in humans is estimated to be 5–14 days. The elimination half-life for dinitrophenols in mice was ~ 6 h.

Mechanism of Toxicity

Dinitrophenols act as uncouplers of oxidative phosphorylation. Oxygen consumption, body temperature, breathing rate, and heart rate are increased following exposure to toxic levels of dinitrophenols. The permeability of mitochondrial membranes to hydrogen ions was found to be increased with the failure of conversion of ADP to ATP. Dinitrophenols reduce the electrochemical (proton) gradient necessary for oxidative phosphorylation by releasing phenolic protons in the mitochondrial matrix. The energy produced due to oxidation is not utilized for the synthesis of ATP but elevates body temperature, which can lead to fatal hyperpyrexia. Inefficient circulation and respiration cannot meet the body's increased metabolic demand, resulting in anoxia and acidosis. Fat serves as an alternative fuel for metabolism. Weight loss occurs as a result of inhibition of lipogenesis from pyruvate and lactate following exposure to dinitrophenols.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dinitrophenols exhibit high acute toxicity in animals. The oral $LD_{50}s$ in rats, mice, guinea pigs, and dogs were reported to be 30, 20–40, 65, and 30 mg kg⁻¹, respectively.

Human

Dinitrophenols are extremely toxic to humans and are well absorbed from all routes of exposure. Fatal

cases of poisonings have been reported as a result of dermal exposure to dinitrophenols. Fever is a very early sign of dinitrophenol toxicity. Hepatic and renal damage were reported within 12–72 h following acute exposure to dinitrophenols. Typical signs of dinitrophenol toxicity were reported to occur within a few hours following acute exposure to 3–5 mg kg⁻¹ of dinitrophenol. Acute signs of toxicity include elevation of blood pressure, heart rate, and body temperature; headache; and mental confusion. Severe poisoning may cause restlessness, seizures, and coma. Cerebral edema was reported in two cases of fatal poisoning. Typical gastrointestinal symptoms may include nausea, vomiting, and abdominal cramps.

Chronic Toxicity (or Exposure)

Animal

Signs seen with acute exposures can also be exhibited following repeated oral exposures to as little as $1 \text{ mg kg}^{-1} \text{ day}^{-1}$ of dinitrophenol. Repeated low-level exposures $(2 \text{ mg kg}^{-1} \text{ day}^{-1})$ can cause peripheral nerve damage. Dinitrophenols have been reported to cause cataracts (after repeated exposure).

Human

The concurrent use of sulfonamide drugs, which are able to bind preferentially to serum albumin, may greatly enhance the acute toxicity of dinitrophenols.

In Vitro Toxicity Data

Dinitrophenols can suppress germ cell viability in vitro.

Clinical Management

Only symptomatic treatment is available. Adequate measures should be taken to maintain fluid and electrolyte balance and keep the body temperature within tolerable limits. Measures should be taken to remove the poison from the body through gastric lavage and saline cathartic. Gastrointestinal absorption may be prevented by administering activated charcoal. Salicylates, which contain a phenolic group, must not be used as antipyretic agents during treatment of DNP poisoning. Therefore, control of temperature should be restricted to physical measures.

Environmental Fate

Dinitrophenols are expected to have high mobility in soil, particularly in moist soils since they will exist primarily as anions. If released into water, dinitrophenols will largely remain in solution and not adsorb significantly to particulate matter or sediment. Volatilization of dinitrophenols from soil or water is not expected to be a significant fate process. Dinitrophenols are not known to undergo hydrolysis in the environment, although photolysis may be an important abiotic degradation process. A bioconcentration factor of <1 in carp suggests that accumulation in aquatic organisms is very low.

Ecotoxicology

Dinitrophenols are very highly toxic to birds and highly toxic to fish. Toxicity is enhanced under acidic conditions. Dinitrophenols do not pose problems with bioaccumulation. Bees are sensitive to these toxicants.

See also: Dinoseb; Pesticides.

Further Reading

Hollingworth RM (2001) Inhibitors and uncouplers of mitochondrial oxidative phosphorylation. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn. San Diego, CA: Academic Press.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Dinitrotoluene

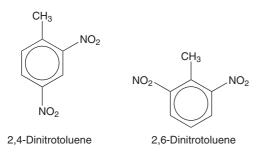
Robert A Young

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• CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Dinitrotoluene (DNT) occurs in six isomeric forms: 2,3-DNT (CAS 602-01-7); 2,4-DNT (CAS 121-142); 2,5-DNT (CAS 619-15-8); 2,6-DNT (CAS 606-20-2); 3,4-DNT (CAS 610-39-9); 3,5-DNT (CAS 618-85-9)

- SYNONYMS:
 - 2,3-DNT: 1-Methyl-2,3-dinitrotoluol; 2,3-Dinitrotoluol

- 2,4-DNT: 1-Methyl-2,4-dinitrotoluol; 2,4-dinitrotoluol
- 2,5-DNT: 1-Methyl-2,5-dinitrotoluol; 2,5-Dinitrotoluol
- 2,6-DNT: 2-Methyl-1,3-dinitrotoluol; 2,6-Dinitrotoluol
- 3,4-DNT: 1-Methyl-3,4-dinitrotoluol; 3,4-Dinitrotoluol
- 3,5-DNT: 1-Methyl-3,5-dinitrotoluol; 3,5-Dinitrotoluol; Binitrotoloene
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aromatic hydrocarbon
- Chemical Formula: C₇H₆N₂O₄
- CHEMICAL STRUCTURE: The chemical structures of the most prevalent and toxicologically important dinitrotoluenes, 2,4-DNT and 2,6-DNT, are shown below



Uses

Dinitrotoluenes are intermediates in the production of toluene diisocyanate but are also used as gelatinizing and waterproofing agents in commercial and military explosives and in the production of polyurethane foams.

Exposure Routes and Pathways

Dinitrotoluenes (DNTs) may occur as a contaminant of soil, surface water, and groundwater. Because DNTs are of low volatility, exposure via the air is inconsequential. The primary route of exposure to DNTs is through contaminated groundwater and, due to their mobility, in surface water as well.

Toxicokinetics

Most of the toxicokinetic data for DNTs are for the 2,4- and 2,6-isomers. Data regarding the absorption of DNT following inhalation exposure are not available, but absorption may be inferred from data on urinary metabolites in workers exposed via inhalation. Efficient absorption of various DNT isomers following oral exposure has been verified in several

animal species. In animals, ingested DNT appears to be readily absorbed (55–90%) within 24 h. Limited human data suggest that dermal exposure may result in significant absorption.

Urine from workers exposed to dinitrotoluene contained 2,4- and 2,6-DNT, 2,4- and 2,6-dinitrobenzoic acid, 2,4- and 2,6-dinitrobenzyl glucuronide, 2-amino-4-nitrobenzoic acid, and N-(acetyl)amino-4nitrobenzoic acid. The most prevalent metabolites were 2,4-dinitrobenzoic acid and 2-amino-4-nitrobenzoic acid, collectively accounting for 74-86% of the dinitrotoluene metabolites detected. Bioactivation of dinitrotoluene in the rat is thought to occur by oxidation of the methyl group to an alcohol by a cytochrome P450-dependent pathway. The benzyl alcohol is then conjugated with glucuronic acid and excreted in the bile. Intestinal microflora hydrolyze the glucuronide and reduce one nitro group, forming an aminonitrobenzyl alcohol, which can be reabsorbed from the intestine. The amino group is oxidized to a hydroxylamine by hepatic enzymes and conjugated with sulfate. Decomposition of the sulfate ester yields a highly electrophilic nitrenium (or carbonium) ion that can react with DNA and other biological nucleophiles. Urinary excretion of these metabolites peaked near the end of the work shift but declined to low or undetectable concentrations by the start of work the following day. The calculated elimination half-lives of total dinitrotoluene-related material detected in urine ranged from 1.0 to 2.7 h and those of individual metabolites from 0.8 to 4.5 h.

Data regarding the distribution of DNT are limited to 2,4-DNT studies in animals. Following oral administration of 2,4-DNT to various laboratory species, the greatest concentrations of the chemical occurred in the liver, kidneys, and blood. Only small amounts were found in the brain, heart, and spleen. A biphasic increase in hepatic levels of 2,4-DNT in rats suggested that the chemical undergoes enterohepatic circulation.

Urinary excretion of these metabolites peaked near the end of the work shift but declined to low or undetectable concentrations by the start of work the following day. The calculated elimination half-lives of total dinitrotoluene-related material detected in urine ranged from 1.0 to 2.7 h and those of individual metabolites from 0.8 to 4.5 h. Urinary excretion in Fischer 344 rats given 2,6-DNT accounted for half of the dose (10 mg kg^{-1}) 72 h after administration of [¹⁴C]-2,6-DNT. 2,6-Dinitrobenzoic acid, 2,6-dinitrobenzyl alcohol glucuronide, and 2-amino-6-nitrobenzoic acid accounted for 95% of the urinary ¹⁴C. Fecal excretion accounted for one-fifth of the dose in 72 h.

Mechanism of Toxicity

The most prominent toxicologic effect of DNT is the formation of methemoglobin and the subsequent effects of reduced oxygen carrying capacity of the blood, which produce the cyanosis and fatigue characteristic of DNT poisoning. DNT and/or its metabolites produce this effect by oxidizing the iron in the hemoglobin molecule. This process also leads to the formation of Heinz bodies, granule-like aggregates of precipitated hemoglobin, which serve as sensitive indicators of toxic insult to the blood. Hepatotoxic effects are due, in part, to cellular damage resulting in altered hepatocytes and deficiencies in biliary excretion. DNT has also been shown to disrupt Sertoli cell function, which may explain, in part, DNT's effect on the male reproductive system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Most of the toxicity data are for the 2,4- and 2,6-DNT isomers. Oral LD₅₀ values for 2,4-DNT are extremely variable ranging from 177 to 609 mg $kg^{-1} day^{-1}$ for rats and from 390 to 1647 mg kg⁻¹ for mice. Rat and mouse oral LD₅₀ values of 216 and $607 \,\mathrm{mg \, kg^{-1}}$, respectively, have been reported for 3,5-DNT. In addition to lethality, acute oral exposures of laboratory animals to 2,4-DNT have resulted in hematologic disorders (methemoglobinemia) and toxic effects in the male reproductive system. Longer-term oral exposures also induce hematologic and reproductive effects in addition to renal and neurologic disorders. For 2,6-DNT, oral LD₅₀ values of 665 and 714 mgkg⁻¹ day⁻¹ have been reported for rats and mice, respectively. The toxicologic effects of 2,6-DNT in animals are similar to those of 2,4-DNT.

Human

Most reports of human toxicity involve exposure to technical-grade DNT. Commercial-grade DNT is usually a combination of 2,4-DNT (\sim 76%) and 2,6-DNT (\sim 19%), with the remaining composition containing various other isomers.

The primary signs of toxicity regardless of the route of exposure are headache, fatigue, nausea, vomiting, and cyanosis resulting from methemoglobin formation. General signs and symptoms may be similar to those of alcohol intoxication. When methemoglobin levels approach 15%, cyanosis appears; and when the methemoglobin levels exceed 40%, weakness and dizziness occur. Methemoglobin levels above 70% may produce muscle tremors, cardiovascular effects, and death.

Chronic Toxicity (or Exposure)

Animal

2,4-DNT at an oral dose of $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 2 years produced liver tumors in rats and at a dose of 97 mg kg⁻¹ day⁻¹ for 2 years produced renal tumors in male mice. 2,6-DNT at doses as low as 7 mg kg⁻¹ day⁻¹ produced hepatocellular carcinomas in male rats following a 1 year oral exposure. The available data also indicate strain differences in the carcinogenic response for several of the DNT isomers.

Human

Long-term exposure to low levels of DNT will result in methemoglobinemia, the severity of which depends on the magnitude of the exposure. Although carcinogenic effects of DNT have been demonstrated in animals, there is currently no evidence of DNT carcinogenicity in humans.

The chronic oral reference doses for 2,4- and 2,6-DNT are 0.002 and $0.001 \text{ mg kg}^{-1} \text{ day}^{-1}$, respectively. The oral slope factor for both 2,4- and 2,6-DNT is 6.8×10^{-1} (mg kg⁻¹ day⁻¹)⁻¹. The US Environmental Protection Agency (EPA) classifies the isomer mixture as a B2 carcinogen (probable human carcinogen; sufficient evidence in animals but inadequate or no evidence from epidemiologic studies). The 3,4-DNT isomer is not classifiable as to its carcinogenicity to humans. The American Conference of Governmental Industrial Hygienists threshold limit value for 2,4-DNT is $0.2 \,\mathrm{mg \, m^{-3}}$, the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit for 2,4-DNT is 1.5 mg m^{-3} , and the NIOSH immediately dangerous to life or health (IDLH) is $50 \,\mathrm{mg\,m^{-3}}$. Both organizations categorize the chemical is a suspected human carcinogen.

In Vitro Toxicity Data

In mammalian cells *in vitro*, it induced DNA strand breaks, gene mutations in mouse lymphoma cells (without activation) but not in Chinese hamster ovary cells, and a low frequency of sister chromatid exchange but not of chromosomal aberrations in Chinese hamster ovary cells. It inhibited intercellular communication but did not induce cell transformation.

Clinical Management

For most cases of DNT poisoning, clinical management involves correction of the methemoglobinemia and associated support therapy.

Environmental Fate

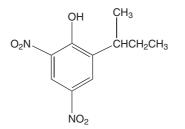
Dinitrotoluenes may enter the environment in wastewater from the processes in which it is made and used. In soil, 2,4-DNT will be slightly mobile (estimated $K_{oc} = 282$). Based on aqueous biodegradation tests, 2,4-DNT may biodegrade in both aerobic and anaerobic zones of soil. 2,4-DNT in water will not bioconcentrate significantly (experimental BCF = 204) and will have a slight tendency to partition to suspended and sediment organic matter $(\log K_{ow} = 1.98)$. Volatilization of 2,4-DNT from water will not be significant. Photolysis will probably be the most important removal process for 2,4-DNT in water. Photolytic half-lives for 2,4-DNT in river, bay, and pond waters were 2.7, 9.6, and 3.7 h, respectively, and the reaction was found to be accelerated in the presence of humic material. The importance of biodegradation in natural waters is unknown. In the atmosphere, 2,4-DNT is estimated to have a half-life of 71 days. 2,4-DNT has been detected in drinking water, seawater, river water, and in wastewater from 2,4,6-trinitrotoluene production.

Dinoseb

Priya Raman

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 88-85-7
- SYNONYMS: Dinitrobutylphenol (DNBP); 2-sec-Butyl-4,6-dinitrophenol; Basanite; Caldon; Chemox; Dynamyte; Elgetol; Gebutox; Hel-Fire; Kiloseb; Nitropone; Premerge; Sinox General; Subitex; Vertac Weed Killer
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Dinitrophenol
- CHEMICAL STRUCTURE:



Exposure Standards and Guidelines

The US EPA has calculated a reference dose (RfD) of $0.002 \text{ mg kg}^{-1} \text{ day}^{-1}$ based on neurotoxicity in dogs and estimates that consumption of this dose or less, over a lifetime, would not likely result in the occurrence of chronic, noncancer effects.

See also: Pollution, Soil.

Further Reading

- Grayson M and Eckroth D (eds.) (1978) Kirk–Othmer Encyclopedia of Chemical Technology. New York: Wiley.
- Tchounwou PB, Newsome C, Glass K, *et al.* (2003) Environmental toxicology and health effects associated with dinitrotoluene exposure. *Reviews in Environmental Health* 18(3): 203–229.
- US Air Force (1989) *The Installation Restoration Program Toxicology Guide*. OH: Wright-Patterson AFB Air Force Systems Command, Aerospace Medical Division.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dinitrotoluene.

Uses

Dinoseb is used as an herbicide, corn yield enhancer, insecticide, and miticide. It is used as a herbicide in soybeans, a variety of vegetables, fruits, nuts, on citrus trees, and with other field crops for control of grasses and broadleaf weeds. Dinoseb is used as an insecticide in grapes. It is produced in emulsifiable concentrates or as water-soluble ammonium or amine salts.

Exposure Routes and Pathways

Oral and dermal routes are the most common routes of exposure to dinoseb. Inhalation of dinoseb can also lead to serious complications.

Toxicokinetics

Dinoseb is rapidly absorbed from the gastrointestinal tract, respiratory tract, and intact skin. It undergoes oxidation of either of the two methyl groups on the *sec*-butyl chain, conjugation of the phenolic products,

and formation of many uncharacterized metabolites. Microsomal enzymes of rat liver reduce the *o*-nitro group of dinoseb. The compound is highly bound to plasma proteins. Hepatic and urinary excretions are the primary routes of elimination. Breakdown products are found in liver, kidney, spleen, blood, and urine. Dinoseb can pass through the placenta and into the fetus of experimental animals.

Mechanism of Toxicity

Dinoseb uncouples oxidative phosphorylation from electron transport by carrying protons across the inner mitochondrial membrane, thereby dissipating the pH gradient and membrane electrochemical potential and preventing the formation of adenosine triphosphate. Following exposure to this chemical, metabolism in all body cells is stimulated, resulting in an increase in oxygen consumption, body temperature, breathing rate, and heart rate. Dinoseb-induced weight loss may occur due to inhibition of lipogenesis from pyruvate and lactate. The body fat serves as the major fuel for the extra metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values in rats and guinea pigs were 25– 58 mg kg⁻¹. The dermal LD_{50} in rabbits was 80– 200 mg kg⁻¹ and 200–300 mg kg⁻¹ in guinea pigs. Dinoseb is not a skin irritant. Neurological and skeletal malformations have been observed in laboratory animals exposed to dinoseb.

Human

Acute exposure to dinoseb is associated with signs and symptoms of toxicity that develop rapidly within a few hours following exposure. Hyperthermia and profuse sweating are the early manifestations of toxicity. Liver and kidney damage may ensue within 12-72 h postexposure. Early symptoms of dinoseb toxicity include headache and confusion followed by restlessness, hyperactivity, seizures, and coma following severe poisoning. The respiratory rate is usually markedly increased. Sinus tachycardia, ventricular tachycardia, and ventricular fibrillation may occur. Following ocular exposure to dinoseb, cataracts, secondary glaucoma, paresis of accommodation, and nystagmus have been reported. Other signs and symptoms following exposure to dinoseb include fatigue, thirst, insomnia, weight loss, flushing of the face, nausea, vomiting, abdominal pain, occasional diarrhea, methemoglobinemia, and hemolytic anemia.

Dinoseb has the potential to cause damage to the immune system, liver, kidneys, and spleen. Direct skin contact with dinoseb results in irritation, yellow stains, burns, and dermatitis.

Chronic Toxicity (or Exposure)

Animal

Pregnant rats given 200 ppm dinoseb in their feed showed reductions in fetal survival. Surviving fetuses exhibited lower than normal birth weights. Morphologic abnormalities of the kidney have been noted in the offspring of female rats given dinoseb; however, renal function and morphology subsequently returned to normal. Dinoseb administered intraperitoneally to pregnant rats on gestation days 10-12 at a dose of $10.5 \text{ mg kg}^{-1} \text{ day}^{-1}$ caused a reduction in body weight in offspring. The no-observed-adverseeffect level of dinoseb for developmental toxicity was $3 \text{ mg kg}^{-1} \text{ day}^{-1}$. Maternal toxicity and malformations of the eye have been observed among the offspring of pregnant rats fed 200 ppm of dinoseb. Studies in laboratory animals indicate that dinoseb has the potential to cause damage to the immune system. Dinoseb has been reported to cause decreased sperm count and abnormal sperm shape in male rats and mice following 3 weeks of exposure at low levels of $\sim 10 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 30 days. Dinoseb is a potential teratogen, low levels of which fed to rats and rabbits have been found to cause birth defects in the fetuses of exposed females.

Human

Little is known regarding chronic effects of dinoseb in humans.

In Vitro Toxicity Data

Dinoseb was not genotoxic or mutagenic as assessed by a number of *in vitro* assays.

Clinical Management

Exposure to dinoseb requires symptomatic treatment. Blood glucose, liver function, and renal function tests should be monitored in symptomatic patients. Adequate ventilation and oxygenation should be provided with close monitoring of arterial blood gases. The fluid and electrolyte balances should be maintained. The body temperature should be kept within tolerable limits. Antipyretic drugs are, however, not effective because dinoseb poisoning involves peripheral metabolism, not central nervous system control of temperature. Diazepam is administered to overcome the accompanying seizure and convulsions following dinoseb exposure. In case of an oral exposure to dinoseb, gastrointestinal absorption may be prevented by gastric lavage and/or activated charcoal administration. Exposed eyes and skin should be irrigated with copious amounts of water following an ocular or dermal exposure to dinoseb.

Ecotoxicology

Dinoseb is highly toxic to birds, with oral LD_{50} values less than 10 mg kg^{-1} and 5-8 day dietary LC_{50} values of around 500 ppm. Dinoseb is highly toxic to fish, with 96 h LC_{50} values of 44–118 µgl⁻¹. Dinoseb use can lead to fish kills from runoff following rain. Dinoseb is not bioaccumulated. Dinoseb is also toxic to bees.

Exposure Standards and Guidelines

The reference dose is $1 \,\mu g \, k g^{-1} \, da y^{-1}$.

See also: Developmental Toxicology; Dinitrophenols; Pesticides.

Further Reading

Hollingworth RM (2001) Inhibitors and uncouplers of mitochondrial oxidative phosphorylation. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1169–1261. San Diego, CA: Academic Press.

Relevant Website

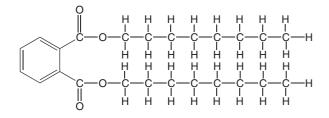
http://www.envirotools.org – Envirotools.org, Michigan State University.

Dioctylphthalate

Robert A Young

Published by Elsevier Inc.

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 117-84-0
- SYNONYMS: Di-N-octylphthalate (preferred name); 1,2-Benzenedicarboxylic acid dioctyl ester; Dioctyl-O-benzenedicarboxylate; DNOP; N-Octyl phthalate; O-Benzenedicarboxylic acid dioctyl ester; Octyl phthalate; Phthal\ic acid dioctyl ester; Benzenedicarboxylic acid di-*n*-octyl ester
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alkyl phthalate
- CHEMICAL FORMULA: C₂₄H₃₈O₄
- CHEMICAL STRUCTURE:



Uses

Dioctylphthalate is used as a plasticizer in cellulose ester resins, polystyrene, and vinyl plastics. It is also a component of some pesticides.

Exposure Routes and Pathways

Humans may be exposed to dioctylphthalate in food (as an indirect food additive) and in drinking water. Although ingestion is the primary route of exposure for the general public, inhalation and dermal exposures may be more significant in occupational settings in which the chemical is used in industrial processes. Exposure via parenteral administration resulting from leaching of dioctylphthalate from plastic tubing and containers used in medical practice has also been documented.

Toxicokinetics

Definitive information regarding the absorption of dioctylphthalate is not available. Absorption may be inferred, however, due to systemic toxic effects following oral administration of the chemical and by analogy to absorption characteristics of similar phthalate esters. The limited information regarding the biotransformation of dioctylphthalate indicates that the chemical undergoes hydrolysis to a monoester within the intestines prior to absorption. However, it is also likely that hydrolysis may occur in the intestinal mucosal cells and in other tissues. Phthalate esters are generally widely distributed in the body. The effects observed in various organs and tissues following exposure to dioctylphthalate affirm its distribution throughout the body.

Chemical-specific elimination data for di-*N*-octylphthalate are not available. However, data from

animal studies using the diisoctylphthalate isomer have shown that it is excreted in the urine and bile as a monoester. Species-dependent quantitative and qualitative differences have been observed for excretion of this isomer as well as di-*N*-butylphthalate and bis(2-ethylhexyl)phthalate. Excretion half-lives of 1.2 and 5.4 h have been reported for these compounds.

Mechanism of Toxicity

Specific data regarding the mechanism by which dioctylphthalate causes toxic responses are not available. There is some evidence that the toxic effects observed for this chemical may be due to its mono*l*-octyl ester metabolite.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats fed diets containing 20 000 ppm di-*N*-octylphthalate, an accumulation of large droplets of fat around central veins was observed that progressed to mild centrilobular necrosis and increased liver weight within 10 days.

Human

Definitive information regarding the acute toxicity of di-N-octylphthalate is not available. An estimated lethal oral dose in humans is between 0.5 and 15 g kg^{-1} , or between 1 oz equivalent to 29.6 mls and 1 qt equivalent to 0.96 liters in a 70 kg adult. Compounds that are structurally similar to di-N-oct-ylphthalate are known to irritate mucous membranes resulting in irritation of the eyes, throat, and upper respiratory tract passages and in gastrointestinal disturbances. There is evidence that some phthalates, such as di-s-octylphthalate, may be reproductive and developmental toxicants. Generally, the acute oral toxicity of alkylphthalates is low and the acute oral toxicity decreases as molecular weight increases.

Chronic Toxicity (or Exposure)

Animal

Renal toxicity has been observed in rats and mice given di-*N*-octylphthalate in the diet (1000 ppm) for 48 weeks, and evidence of liver toxicity was noted for rats given a diet containing 3500 ppm for 7– 12 months. Although many of the phthalate esters exert toxic effects on the male reproductive system, di-*N*-octylphthalate appears to be among the least potent. Evidence for developmental toxicity is equivocal. There is currently no evidence showing that di-*N*-oct-ylphthalate is genotoxic.

Human

A case report noted the development of an asthmatic reaction in a worker continuously exposed to dioctylphthalate during a manufacturing process. Based on the known toxic effects of di-*N*-octylphthalate in animals, chronic exposure of humans may result in liver and kidney damage.

The chronic reference dose for di-*N*-octylphthalate is $0.02 \text{ mg kg}^{-1} \text{ day}^{-1}$. No other regulatory or healthbased guideline values are currently available for di-*N*-octylphthalate. Neither the US Environmental Protection Agency nor International Agency for Research on Cancer have evaluated the carcinogenicity of di-*N*-octylphthalate.

Clinical Management

The potential for esophageal or gastrointestinal tract irritation following ingestion suggests that emesis should not be induced. Other measures to prevent absorption may be beneficial. Gastric lavage may be indicated if performed soon after ingestion or if the patient is comatose or at risk of convulsing. Exposed skin and eyes should be copiously flushed with water.

Environmental Fate

Based on its vapor pressure, dioctylphthalate will likely exist in both the vapor and particulate phases in the atmosphere. The vapor will be degraded by reaction with photochemically produced hydroxyl radicals; the particulate phase will be removed from the atmosphere by wet and dry deposition. It is likely to adsorb to soil and sediment. As a result, volatilization and hydrolysis are not expected to be important fate processes in water.

See also: Phthalate Ester Plasticizers.

Further Reading

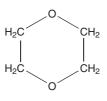
- Kavlock R, Bockelheide K, Chapin R, *et al.* (2002) NTP center for the evaluation of risks to human reproduction: Phthalates expert panel report on the reproductive and developmental toxicity of di-*n*-octyl phthalate. *Reproductive Toxicology* 16(5): 721–734.
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- McKee RH, Butala JH, David RM, and Gans G (2004) NTP center for the evaluation of risks to human reproduction reports on phthalates: Addressing the data gaps. *Reproductive Toxicology* 18(1): 1–22.

Dioxane, 1,4-

Julie A Stickney and Eric M Silberhorn

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 123-91-1
- SYNONYMS: 1,4-Dioxane; Dioxane; Diethylene oxide; *p*-Dioxane; Glycolethyleneether; 1,4-Diethylenedioxide; *p*-Dioxan; Tetrahydro-*p*-dioxin; 1,4-Diethylene dioxide; Dioxyethylene ether
- DESCRIPTION: 1,4-Dioxane is a colorless liquid with a mild, ether-like odor. It is miscible in water and most organic solvents. It is relatively stable under normal temperature and pressure
- CHEMICAL STRUCTURE:



Uses

1,4-Dioxane is a solvent widely used for a range of organic products, including cellulose acetate, nitrocellulose, other cellulose esters or ethers, fats, oils, waxes, mineral oil, natural and synthetic resins, and polyvinyl polymers. It has been used for wetting and dispersing in textile processing, dye baths, stain, and printing compositions. It is also found in cleaning and detergent preparations, adhesives, cosmetics, deodorants, fumigants, emulsions, and polishing compositions. It has been used as an ingredient in lacquers, paints, varnishes, and paint and varnish removers. 1,4-Dioxane is also used in purifying drugs and in cosmetic products such as shampoos and bath preparations. It has been used in the embedding process for the preparation of tissue sections for histology. Additionally, 1,4-dioxane has been used as a stabilizer for chlorinated solvents, particularly, 1,1,1trichloroethane, in solvent applications.

Exposure Routes and Pathways

Exposures to 1,4-dioxane may occur through inhalation, ingestion, and dermal contact. In the occupational setting, workers would be exposed primarily through inhalation of vapors and contact with the skin. For the general population, 1,4-dioxane may be ingested as a contaminant of drinking water, soil, or food. Inhalation of vapors and skin contact are not considered to be major exposure routes for the general population.

Toxicokinetics

1,4-Dioxane is absorbed rapidly and completely following oral and inhalation exposure with much less absorption occurring from the dermal route. In both rats and humans, 1,4-dioxane is primarily metabolized to β-hydroxyethoxyacetic acid (HEAA), which is excreted in the urine. Data indicate that the metabolism of 1,4-dioxane is linear at exposure levels up to $50 \text{ ppm} (180 \text{ mg m}^{-3})$. The half-life for elimination of 1,4-dioxane in humans is $\sim 1 h$ at concentrations of 50 ppm (180 mg m⁻³) or less. A similar half-life was observed in rats given low oral or intravenous doses of 1,4-dioxane (<10 mg kg⁻¹). At oral or intravenous doses of >10 mg kg⁻¹ in the rat, however, plasma clearance and HEAA excretion are reduced and unchanged 1,4-dioxane concentrations are increased in both urine and breath. Studies demonstrate that clearance of 1,4-dioxane from the blood is markedly nonlinear and dose dependent. The biotransformation of 1,4-dioxane to HEAA is a saturable process that is significantly altered by the magnitude of the administered dose. Multiple daily oral doses were excreted more rapidly than the equivalent single dose suggesting induction of metabolism at high doses and 1,4-dioxane has been shown to induce the mixed function oxidase enzyme system in the mouse liver. Physiologically based pharmacokinetic (PBPK) modeling approaches have been used to evaluate the risk of liver cancer in humans exposed to 1,4-dioxane. These models take into account the nonlinear pharmacokinetics observed in experimental studies as well as physiological differences between the rodent models and humans. Various toxicological data indicate that 1,4-dioxane toxicity occurs only after doses large enough to saturate processes for detoxification and elimination.

Mechanism of Toxicity

Pharmacokinetic and toxicological data indicate that liver and kidney toxicity induced by 1,4-dioxane occurs only after doses large enough to saturate processes for detoxification and elimination. 1,4-Dioxane is one of many carcinogens that have not been demonstrated to react covalently with DNA. Its mode of action is not sufficiently well understood to permit assignment to a specific class of epigenetic agents. However, the data suggest a tumor promotion mechanism associated with tissue injury and subsequent regeneration. Eye and respiratory irritation occur from direct contact of 1,4-dioxane with mucous membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute effects of exposure to high concentrations of 1,4-dioxane in animals include eye and respiratory irritation, nervous system effects, and liver and kidney damage. Guinea-pigs can tolerate inhalation of 2000 ppm 1,4-dioxane for several hours without serious symptoms. Higher concentrations produced eye, nose, and lung irritation. Dogs given 1,4-dioxane orally over a period of 9 days died after a total consumption of about $\sim 3 \,\mathrm{g \, kg^{-1}}$ with severe liver and kidney damage. Single doses of 5.66, 5.17, and $3.90 \,\mathrm{g \, kg^{-1}}$ to mice, rats, and guinea-pigs produced symptoms progressing from weakness, depression, incoordination, and coma to death. Autopsy revealed hemorrhage areas in the pyloric region of the stomach, bladders distended with urine, enlarged kidneys, and slight proteinurea without hematuria.

Human

Exposure to high concentrations of 1,4-dioxane may cause eye, skin, and respiratory irritation, nervous system effects, and liver and kidney toxicity. There are five cases of fatal poisoning in men who inhaled excessive amounts of 1,4-dioxane while working in a textile factory. Symptoms were irritation of the upper respiratory passage, coughing, irritation of eyes, drowsiness, vertigo, headache, anorexia, stomach pains, nausea, vomiting, uremia, coma, and death. Autopsies revealed congestion and edema of the lungs and brain and marked injury of the liver and kidneys. Blood analysis of these victims showed no abnormalities other than considerable leukocytosis. Twelve humans were exposed to 200 ppm 1,4-dioxane for 15 min, considered to be the highest acceptable dose; at 300 ppm it caused irritation of the eyes, nose, and throat. Death was reported in one worker after 1 week on a job where the average concentration of 1,4-dioxane vapor was 470 ppm. Possible skin absorption and damage to the kidneys, liver, and brain were indicated.

Chronic Toxicity (or Exposure)

Animal

1,4-Dioxane causes liver and kidney toxicity in laboratory animals. Limited data regarding developmental toxicity show maternal and embryo effects at similar doses. Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea-pigs. With the exception of one vapor inhalation study, drinking water was the exposure medium used in all cancer bioassays. No treatment-related tumors were reported in the inhalation study. The primary target organs for cancer in the oral studies were the liver and the nasal cavity. Taken together, the bioassay data show that production of liver tumors is more consistent across strains and species of experimental animals than the nasal tumor data. Liver tumors were observed in both sexes and multiple strains of both mice and rats, while nasal tumors were present mostly in rats. With the exception of one study liver tumors also occurred with a much higher incidence rate than nasal cavity tumors. US EPA's B2 (probable human carcinogen) classification for 1,4dioxane was based on induction of nasal cavity and liver carcinoma in multiple strains of rats, liver carcinomas in mice, and gall bladder carcinomas in guinea-pigs.

Human

Several epidemiology studies on occupational exposure to 1,4-dioxane are available in the literature. Very limited conclusions can be drawn from the negative findings of these studies. All of the studies lack sufficient cohort size and number of cases to enable identification of low-level excess cancer risk. Of note, the reported cancers have various sites of origin and are not similar to those seen in animal models. A mortality study was conducted on employees exposed to 1,4-dioxane. Observed deaths from overall cancer were not significantly different from expected number of deaths. 1,4-Dioxane can be inhaled in amounts sufficient to cause serious systemic intoxication. Injury may become apparent hours after termination of an exposure that had been erroneously considered to be negligible. Prolonged and repeated contact can cause eczema and repeated inhalation exposures to low concentrations have been fatal.

1,4-Dioxane is currently classified as B2, a probable human carcinogen, by US EPA based on adequate animal studies and inadequate human studies. Three epidemiological studies on workers exposed to 1,4dioxane are available. Two of the deaths were due to cancer: one epithelial carcinoma in a 66-year-old man and one melofibrotic leukemia in a 71 year-old man. No statistically significant increase was noted based on these few cases of cancer. Among 165 production and processing workers exposed to 1,4-dioxane, 12 deaths were reported. Three of these deaths were due to cancer: one stomach cancer, one alveolar carcinoma, and one mediastinal malignancy. Three deaths were not different from the expected numbers.

In Vitro Toxicity Data

1,4-Dioxane has been described as either a very weak genotoxin or not genotoxic. It produced negative results in most test systems including the *Salmonella* assays (Ames test), DNA alkylation and repair, hepatocyte unscheduled DNA synthesis, and the Chinese hamster ovary chromosome aberration assay. Hepatic DNA damage was seen in the alkaline elution test and positive findings were sometimes reported for the liver micronucleus assay, although data for this parameter are inconsistent.

Incubating 2.5% 1,4-dioxane with human lymphocyte cultures caused an increase in phytohemaglutanin stimulation of DNA synthesis. No significant effects were seen at lower concentrations. 1,4-dioxane was not demonstrated to bind covalently to DNA in the presence of microsomal preparations.

Clinical Management

First-aid procedures for eye or skin contact involve irrigation and water washing. Respiratory support may be required for people exposed to high vapor concentrations of 1,4-dioxane. Immediate dilution with milk or water may be of benefit following ingestion of irritant chemicals like 1,4-dioxane. Activated charcoal binds most toxic agents and can decrease their systemic absorption if administered soon after ingestion. Persons exposed to 1,4-dioxane should be thoroughly medically examined before ipecac alkaloid is administered to induce emesis. If signs of oral, pharyngeal, or esophageal irritation, a depressed gag reflex, or central nervous system (CNS) excitation or depression are present, emesis should not be induced. Gastric lavage may be indicated if performed soon after ingestion or in patients who are comatose or at risk of convulsing. The airway should be protected by placing the patient in the Trendelenburg and left lateral decubitus position or by cuffed endotracheal intubation. After control of any seizures present, gastric lavage may be performed.

In cases of ingestion exposure, the patient should be carefully monitored for the development of any systemic signs or symptoms and symptomatic treatment should be provided as necessary. In cases of inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develops, the patient should be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Supplemental oxygen (100%, humidified) should be administered with assisted ventilation as required. When eyes are exposed, they should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility. In case pulmonary edema develops due to exposure, ventilation and oxygenation should be maintained with close arterial blood gas monitoring. If Po_2 remains less than 50 mmHg, positive endexpiratory pressure or CPAP may be necessary.

Environmental Fate

1,4-Dioxane is a cyclic ether compound that is miscible with water in all proportions and is also moderately volatile. It is resistant to hydrolysis and microbial degradation, but may undergo photolysis at water and soil surfaces. An estimate of the half-life for abiotic degradation in water with addition of ozone was 60 h. The half-life for photo-oxidation in air was 3.4 h. 1,4-Dioxane has a low adsorption potential and a high mobility/leaching potential in soil/water systems. No bioaccumulation of this chemical is expected.

Ecotoxicology

Ecological toxicity data for 1,4-dioxane are available for fish, aquatic, and terrestrial invertebrates, microorganisms, algae, and terrestrial plants. Acute and chronic toxicity levels generally range between 1000 and 10 000 mg l⁻¹, with the exception of a long-term study in fish that reported a no-observed effect level of approximately 100 mg l^{-1} .

Other Hazards

1,4-Dioxane is a flammable liquid and a fire hazard. Toxic gases and vapors (which may include carbon monoxide) may be released in a fire involving dioxane.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit for 1,4-dioxane in air is 100 ppm (360 mg m^{-3}) for an 8 h work shift. The National Institute for Occupational Safety and Health recommended exposure limit (REL) is 1 ppm (3.6 mg m^{-3}), which should not be exceeded in a 30 min period. The American Conference of Governmental Industrial Hygienists REL is 20 ppm averaged

over an 8 h work shift. The International Agency for Research on Cancer classifies 1,4-dioxane as possibly carcinogenic to humans (group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals. Similarly, the United States Environmental Protection Agency (USEPA) has classified 1,4-dioxane as a probable human carcinogen (group B2) based on inadequate data from human epidemiological studies and sufficient data from laboratory animal studies including, nasal cavity and liver carcinomas in multiple strains of rats, liver carcinomas in mice and gall bladder carcinomas in guinea-pigs. An oral cancer slope factor (CSF) value of 0.011per(mg per kg per day)⁻¹ was published on USEPA's Integrated Risk Information System (IRIS) database.

See also: Carcinogen Classification Schemes; Kidney; Liver.

Further Reading

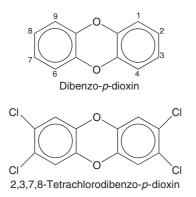
- Stickney JA, Sager SL, Clarkson JR, et al. (2003) An updated evaluation of the carcinogenic potential of 1,4-dioxane. Regulatory Toxicology and Pharmacology 38: 183–195.
- The Netherlands Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and the Environment (RIVM) (1999) *Risk Assessment:* 1,4-Dioxane. The Netherlands: Chemical Substances Bureau, Ministry of Housing, Spatial Planning and the Environment (VROM), Final Version, 5 November, EINECS-No.: 204-661-8.

Dioxins

Robert A Young

Published by Elsevier Inc.

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 1746-01-6 (2,3,7,8-Tetrachlorodibenzo-p-dioxin)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Halogenated aromatic hydrocarbon
- CHEMICAL STRUCTURE: There are 74 chlorinated dibenzo-*p*-dioxin congeners. The basic structure for unsubstituted dibenzo-*p*-dioxin (showing the carbon numbering scheme that is used to name specific congeners) and the structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (one of 22 tetrachlorinated dibenzo-*p*-dioxins) are shown below



Uses

Dioxins are by-products of various chemical syntheses and are usually present as contaminants of end products. There is no known use.

Background Information

There are a wide range of chlorinated dibenzo-*p*-dioxins varying in the extent of their chlorination. Dioxin nomenclature is based on the number and positions of carbon atoms that are chlorinated and include mono-, di-, tetra-, penta-, hexa-, hepta-, and octachlorinated congeners. 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD, more commonly referred to as TCDD or dioxin) is usually of greatest concern because of high toxicity in laboratory animal models, its widespread distribution and persistence in the environment, bioaccumulation potential, and because the greatest amount of data exists for this form.

Exposure Routes and Pathways

2,3,7,8-TCDD and other chlorinated dibenzo-p-dioxins are released during the combustion of many polychlorophenols and also occur as contaminants in various chemicals such as the herbicide 2,4,5trichlorophenoxyacetic acid. Most high-level exposure to 2,3,7,8-TCDD and other dioxins results from accidental releases or explosions in chemical plants or storage facilities for dioxin-containing chemicals. Because of the persistence of dioxin congeners in the environment and their potential for bioaccumulation, exposure may occur via the soil, air (especially when dioxins occur as combustion products), or water. When bound to components of the soil, the health hazard from 2,3,7,8-TCDD is reduced compared to ingestion of the pure compound. However, its bioavailability varies with the specific media in which it occurs.

Toxicokinetics

Dioxins are highly lipid soluble and are efficiently absorbed by most routes of exposure although absorption will vary quantitatively depending on the route. Because dioxins are poor substrates for the enzymes typically involved with biotransformation of xenobiotics, they are very poorly metabolized. Dioxins tend to exhibit high concentrations in the liver and tend to accumulate in fatty tissue. Because of their high lipid solubility and poor metabolism, excretion of dioxins is extremely slow. The elimination half-life in humans is ~ 10 years.

Mechanism of Toxicity

Some, but not all, of the toxic effects of 2,3,7,8-TCDD are mediated by the interaction with an intracellular protein called the Ah receptor. This interaction ultimately alters genetic expression leading to deleterious effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal data have shown that 2,3,7,8-TCDD is a highly toxic chemical and capable of exerting a wide range of toxic effects. Oral LD_{50} values in animals vary considerably (e.g., 0.6, 1, 20, 114, and $1157 \,\mu g \, kg^{-1}$ for guinea pigs, dogs, rats, mice, and hamsters, respectively). Toxic effects observed in animals following acute exposure include damage of the liver, heart, thymus gland, adrenals, and immuno-suppressive effects. The effects, however, vary with species. Toxic effects, including death, have also been observed in animals following short-term dermal exposure to 2,3,7,8-TCDD.

Human

A TD_{Lo} of $107 \,\mu g \, kg^{-1}$ has been reported for humans although a more generally accepted minimum toxic dose for humans is $0.1 \,\mu g \, kg^{-1}$. Nonlethal effects following short-term exposure to 2,3,7,8-TCDD include headache, fatigue, irritation of the gastrointestinal and respiratory tracts, dehydration, and skin irritation. The acneform skin irritation resulting from exposure to 2,3,7,8-TCDD or chemicals that contain TCDD is referred to as chloracne.

Dioxin was the poisoning agent in a high profile political incident in 2004. It was ultimately identified as the cause of the disfiguring acne-like skin condition suffered by Ukrainian opposition leader Viktor Yushchenko a few months before the first presidential election. The suspicion is that the dioxin was placed into soup ingested by Mr. Yushenko. The acne-like skin condition is the most recognizable hallmark of dioxin poisoning in humans. It is expected that at least most of his skin condition is reversible; however, his situation is unique as a known case of high exposure dioxin poisoning, with severe effects, in a human. further, it is not known what other effects to his body related to the poisoning might surface in future months and years. The actual intake of dioxin in this poisoning is unknown.

Chronic Toxicity (or Exposure)

Animal

In addition to the aforementioned acute effects, chronic exposure in animals has also resulted in carcinogenic responses in the liver and lungs. 2,3,7,8-TCDD has been shown to be carcinogenic and teratogenic in several laboratory species.

Human

The toxic effects associated with chronic exposure to 2,3,7,8-TCDD include chloracne, impaired liver function, peripheral neuropathies, and altered blood chemistry parameters. Other long-term effects may include chromosome damage, heart attacks, reproductive disorders, and cancer, although epidemiologic data regarding these effects are equivocal. Some effects of long-term low-level exposure to dioxins appear to be reversible following cessation of the exposure.

The US Environmental Protection Agency classifies hexachlorodibenzo-p-dioxin mixture (HxDD) in cancer group B2 (probable human carcinogen with sufficient evidence in animals but inadequate evidence in humans), and International Agency for Research on Cancer classifies the chemical as 2B (probably carcinogenic to humans; sufficient evidence in animals). The oral slope factor and unit risk for HxDD is $6.2 \times 10^{3} (\text{mg/kg/day})^{-1}$ and $1.8 \times 10^{-1} \,\mu\text{g} \,\text{l}^{-1}$, respectively. The inhalation unit risk is $1.30 (g/m^3)^{-1}$. Inhalation unit risk values for other polyhalogenated dioxin congeners have been developed based on a toxicity equivalent factor (TEF) approach where the TEF for 2,3,7,8-TCDD is equal to 1. No reference doses or reference concentrations have been derived for 2,3,7,8-TCDD or any other dioxin.

Clinical Management

There are no clinical procedures specific for dioxin intoxication, but clinical management for acute intoxication by dioxin-containing chemicals such as 2,4-D and 2,4,5-T may be applied. Basically, these procedures include decontamination of the gut and/ or skin and possibly alkaline diuresis for severe overdose situations.

Ecotoxicology

It has been difficult to assess the ecotoxicology of dioxin in the field because of the presence of other chemicals that cause similar effects and are present at higher concentrations. However, it appears that the early life stages of fish are most susceptible to the effects of dioxin and that birds may exhibit decreased egg production, embryotoxicity, and fetal malformations as a result of dioxin exposure.

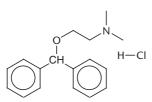
See also: Distribution; Immune System; Pollution, Soil; Polybrominated Diphenyl Ethers (PBDEs); 2,4,5,-T; Toxic Torts.

Diphenhydramine

Michael Wahl

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 58-73-1
- SYNONYMS: Benadryl; Diphenhist; Genahist; Sominex; Nytol; Sleepinal; Caladryl; Dermarest
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: An ethanolamine derivative H-1 receptor antagonist
- CHEMICAL FORMULA: C₁₇H₂₁NO
- CHEMICAL STRUCTURE:



Uses

Diphenhydramine, like other antihistamines, is most often used to provide symptomatic relief of allergic symptoms caused by histamine release. The drug is also used as an antitussive, a nighttime sleep aid for the short-term treatment of insomnia, and as a preventive and treatment for motion sickness. Diphenhydramine may be useful in the treatment of parkinsonian syndrome in geriatric patients including drug-induced extrapyramidal reactions. Diphenhydramine has been used topically for the temporary relief of pruritus and pain associated with various skin conditions including minor burns, insect bites, and minor skin irritation.

Further Reading

- Barnes DG, Kutz FW, and Bottimore DP (1989) Update to the Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans (CDDs and CDFs). Risk Assessment Forum. Washington, DC: US EPA.
- Kirk RE and Othmer F (eds.) (1998) Kirk-Othmer Encyclopedia of Chemical Technology. New York: Wiley.
- US Air Force (1989) *The Installation Restoration Program Toxicology Guide*. Air Force Systems Command, Aerospace Medical Division. Wright-Patterson AFB, 04.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dioxins.

Exposure Routes and Pathways

Ingestion, injection, and dermal application are the routes of both accidental and intentional exposures to diphenhydramine.

Toxicokinetics

Diphenhydramine is absorbed rapidly after an oral dose with peak plasma levels achieved within 2 h. The drug is also absorbed through abraded skin resulting in systemic toxicity. Diphenhydramine undergoes extensive first-pass metabolism with 40–60% of an oral dose reaching systemic circulation as unchanged drug. Diphenhydramine is 98% protein bound and has an apparent volume of distribution of $3-71 \text{kg}^{-1}$. Approximately 64% of the dose of diphenhydramine is excreted as metabolites in the urine. The serum half-life is 4–10 h.

Mechanism of Toxicity

The toxicity of antihistamines is related to their anticholinergic (antimuscarinic) activity. The action of acetylcholine at the muscarinic receptors is blocked, resulting in signs and symptoms of anticholinergic poisoning. Diphenhydramine may produce direct toxicity unrelated to its anticholinergic properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

Central Nervous System (CNS) changes including sedation or hyperexcitability, salivation, and vomiting

Human

Diphenhydramine overdose results in signs and symptoms of anticholinergic poisoning including dry mouth, fixed dilated pupils, flushed skin, fever, and hallucinations. CNS depression is more common in adults, whereas stimulation including tonic-clonic seizures is more common in children. Cardiovascular effects including tachycardia, hypertension or hypotension, arrhythmias, and cardiovascular collapse may occur. Symptoms of an overdose generally occur within 30 min to 2 h after ingestion. Fatalities have occurred in children at doses under 500 mg and seizures have been described after doses as low as 150 mg. A fatal adult dose is estimated to be between 20 and 40 mg kg⁻¹.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies of various concentrations of diphenhydramine in mouse and rat models have shown equivocal evidence of carcinogenesis. Slightly increased rates of unusual cancers were demonstrated (e.g., astrocytomas and alveolar/bronchiolar neoplasms).

Human

Although first-generation anthistamines such as diphenhydramine have been used for decades, they may produce sedation, psychomotor impairment, and may negatively impact sleep patterns.

Diphenoxylate

Alexander B Baer and Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 3810-80-8
- SYNONYM: Lomotil
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Antidiarrheal
- Chemical Formula: $C_{30}H_{32}N_2O_2$

Histamine induces CD86 expression and chemokine production in immature human derived monocytederived dendritic cells that can be blocked by diphenhydramine and other H1 receptor antagonists.

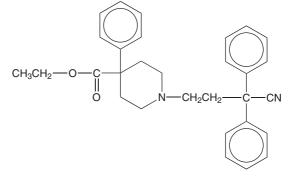
Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Appropriate gastrointestinal decontamination procedures should be administered based on the history of the ingestion and the patient's level of consciousness. Treat seizures with benzodiazepines or barbiturates. Consider treatment of prolonged QRS or wide complex tachydysrythmias with sodium bicarbonate. Consider use of benzodiazepines for CNS excitation, hallucinations and movement disorders. Aggressively treat hyperpyrexia with external cooling such as cool mist and fans. In a limited number of cases physostigmine administration may be necessary to treat severe central and peripheral anticholinergic symptoms refractory to conventional therapy. If physostigmine is given intravenously, the rate of administration should not exceed 2 or 3 min. Continual electrocardiogram monitoring is essential.

See also: Cholinesterase Inhibition; Hypersensitivity, Delayed Type.

Further Reading

- Koppel C, Ibe K, and Tenczer J (1987) Clinical symptomatology of diphenhydramine overdose: An evaluation of 136 cases in 1982 to 1985. *Clinical Toxicology* 25: 53–70.
- Pentel P and Peterson CD (1980) Asystole complicating physostigmine treatment of tricyclic antidepressant overdose. *Annals of Emergency Medicine* 9: 588–590.
- CHEMICAL STRUCTURE:



Uses

The only recognized use for diphenoxylate is in combination with atropine for the treatment of acute and chronic diarrhea.

Exposure Routes and Pathways

Exposure is by the oral route; diphenoxylate is available in liquid and tablet forms.

Toxicokinetics

Diphenoxylate is readily absorbed from the gastrointestinal tract. Peak levels occur 3 h after a single oral dose. Diphenoxylate is metabolized rapidly in the liver to both active and inactive metabolites. Enterohepatic circulation may occur. The volume of distribution is $4.6 \, l \, kg^{-1}$. The half-life of diphenoxylate is 2.5 h. The major metabolite difenoxin (diphenoxylic acid) has a half-life of 4.4 h after therapeutic dosage and is five times more potent than the parent chemical. After overdose, difenoxin's half-life has been reported to extend greater than 12 h. Diphenoxylate is excreted in the feces (49%) and urine (13%) primarily as metabolites.

Mechanism of Toxicity

Diphenoxylate is a narcotic-like substance that slows gastrointestinal motility and depresses the central nervous system producing coma and respiratory depression. Anticholinergic effects (secondary to the presence of atropine as an abuse deterrent) can be seen early after exposure with opioid effects occurring later. There is no correlation between the dose ingested and the severity of effects in children. Severe poisonings with coma and respiratory depression have been reported in children with small ingestions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Diphenoxylate-atropine has been used in the past to treat cats with diarrhea, but toxicity has limited its usefulness. Lomotil should generally be avoided in cats.

Human

Single therapeutic doses produce little or no opiatelike effects in adults. Effects from larger doses (40– 60 mg) are typical of opioid drugs and include miosis, ataxia, lethargy, respiratory depression, seizures, and coma. Onset of symptoms may be delayed 6–8 h. Anticholinergic effects of atropine (tachycardia, urinary retention, irritability, mydriasis, and cutaneous flushing) may be evident before opioid symptoms. Children are more susceptible to the effects and may manifest slower onset. The therapeutic index is low in children; symptoms have resulted with only one tablet.

Chronic Toxicity (or Exposure)

Human

A morphine-like physical dependence can occur with chronic administration.

In Vitro Toxicity Data

In a Caco-2 model of P-glycoprotein influenced cell transport and uptake, diphenoxylate was not dependent on P-glycoprotein.

Clinical Management

In addition to general supportive measures directed to the airway, breathing and circulation, the clinician may consider measures to decrease absorption in the alert patient they suspect has been exposed to a lifethreatening dose of diphenoxylate. Charcoal may decrease absorption. Charcoal administration should be cautiously used in a patient with altered mental status to avoid charcoal aspiration pneumonitis. It is important to recognize the potential for delayed toxicity and monitor diphenoxylate poisoned patients for a minimum of 24 h. Transient recovery may be observed before this time. Repeat boluses or continuous infusion of naloxone may be necessary to reverse opioid sedating effects.

See also: Atropine.

Further Reading

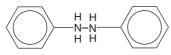
- Cutler EA, Barrett GA, and Craven PW (1980) Delayed cardiopulmonary arrest after lomotil ingestion. *Pe-diatrics* 65: 157–158.
- McCarron MA, Challoner KR, and Thompson GA (1991) Diphenoxylate–atropine (lomotil) overdose in children: An update (report of eight cases and review of the literature). *Pediatrics* 87: 694–700.

Diphenylhydrazine

Robert A Young

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Diphenylhydrazine occurs as the isomers 1,1-Diphenylhydrazine (CAS 530-50-7) and 1,2-Diphenylhydrazine (CAS 122-66-7)
- SYNONYMS: 1,1-Diphenylhydrazine; N,N'-Bianiline; 1,2-Diphenylhydrazine; Hydrazobenzene; N,N'-Diphenylhydrazine; sym-Diphenylhydrazine
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Polycyclic amine
- Chemical Formula: $C_{12}H_{12}N_2/(C_6H_5)_2NNH_2$
- CHEMICAL STRUCTURE:



Uses

1,2-Diphenylhydrazine has been used for the production of benzidine, which, in turn, is used in the production of benzidine-based dyes. These dyes, however, are no longer produced in the United States. 1,2-Diphenylhydrazine is also used in the production of the anti-inflammatory pharmaceutical agent phenylbutazone and in the production of sulfinpyrazone, a uricosuric agent.

Exposure Routes and Pathways

The primary route of exposure is likely to be via ingestion or dermal contact with dust of contaminated soil. Because of the low volatility and solubility of diphenylhydrazine, inhalation exposure or exposure via water is not likely to be significant.

Toxicokinetics

For humans, there are no data regarding the absorption of diphenylhydrazines by any exposure route. Gastrointestinal absorption of 1,2-diphenylhydrazine in rats can be inferred by the presence of the parent compound and its metabolites in the urine and by systemic toxic effects following oral administration. No data are available regarding inhalation or dermal absorption in animals. Data are unavailable regarding the metabolism of 1,1-diphenylhydrazine. Limited data regarding the metabolism of 1,2-diphenylhydrazine by rats suggest benzidine and aniline to be major metabolites with minor metabolites including unspecified hydroxy derivatives. Conversion of 1,2-diphenylhydrazine to aniline may occur through intestinal microflora and by acid conversion in the stomach.

Data are not available regarding the distribution of either form of diphenylhydrazine. Limited data in rats indicate that urinary excretion of metabolites and unchanged parent compound occurs following oral administration of 1,2-diphenylhydrazine. No data are available regarding the excretion of 1,1diphenylhydrazine.

Mechanism of Toxicity

The mechanism of diphenylhydrazine toxicity is not currently known. It is possible that some of the toxic effects observed for diphenylhydrazine may be the result of its major metabolites, aniline and benzidine, which are known animal carcinogens.

Acute and Short-Term Toxicity (or Exposure)

No data are available regarding the acute or chronic toxicity of 1,1- or 1,2-diphenylhydrazine in humans.

Chronic Toxicity (or Exposure)

Animal

Only limited data are available regarding the toxicity of diphenylhydrazine in animals. Oral LD₅₀ values of 959 and 301 mg kg^{-1} have been reported for rats, and gastrointestinal hemorrhage and death have been reported for rodents following 4 week dietary exposure to a dose of 390 mg 1,2-diphenylhydrazine per kilogram body weight per day. Chronic exposure (78 weeks) of rats and mice to 1,2-diphenylhydrazine in the diet (equivalent to doses of 4 and 52 mg kg⁻¹ day^{-1} for rats and mice, respectively) resulted in hepatocellular carcinomas. Exposure of rats to 1,2diphenylhydrazine at doses of $5-15 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 78 weeks resulted in effects ranging from death to histopathological changes in the liver and gastrointestinal tract. Other effects of chronic oral exposure of animals to 1,2-diphenylhydrazine include decreased weight gain, interstitial inflammation of the lung, and fatty degeneration and necrosis of the liver. The acute toxicity of diphenylhydrazines in animals following inhalation exposure has not been determined, and no information is available regarding the carcinogenic or noncarcinogenic effects in animals

following chronic inhalation exposure to diphenylhydrazines.

Environmental Fate

If spilled on land, diphenylhydrazine would absorb moderately to soil and be oxidized to azobenzene by air or cations in the soil. If released in water, it will adsorb moderately to sediment and particulate matter where it should undergo rapid reversible oxidation by dissolved oxygen and environmentally common cations (e.g., copper (II)) to azobenzene. If released in the atmosphere, it will degrade by a combination of air oxidation and photolysis.

Other Hazards

1,2-Diphenylhydrazine has been found to be present in drinking water at levels of $1 \mu g l^{-1} = 1$ ppb. Hydrazobenzene was detected in groundwater samples at Love Canal, Niagara Falls, NY, and in 1.2% of 1205 effluents sampled in a national survey at a median concentration of less than $10 \mu g l^{-1}$.

Exposure Standards and Guidelines

Health-based guidance values for 1,2-diphenylhydrazine include an inhalation unit risk of 2.2×10^{-4} (µg/m³)⁻¹ and a drinking water unit risk of 2.2×10^{-5} (µg/l)⁻¹. The US Environmental Protection Agency (EPA) classifies 1,2-diphenylhydrazine as a probable human carcinogen (B2). The cancer slope factor for 1,2-diphenylhydrazine is 8.0×10^{-1} (mg/ kg/day)⁻¹. No regulatory values or guidance values are available for 1,1-diphenylhydrazine.

The US EPA Reportable Quantity for 1,2-diphenylhydrazine is 1 lb (statutory) with a proposed Reportable Quantity of 10 lbs.

See also: Aniline; Benzidine.

Relevant Website

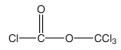
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Diphenylhydrazine, 1,2-

Diphosgene

Fu-Min Menn

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- CHEMICAL NAME: Carbonochloridic acid trichloromethyl ester
- REPRESENTATIVE CHEMICAL: Phosgene (COCl₂, CAS 75-44-5)
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 503-38-8
- SYNONYMS: Trichloromethyl chloroformate; Trichloromethyl carbonochloridate; Chloroformic acid trichloromethyl ester; UN1076; Perstoff; Superpalite, DP
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Choking agent
- Chemical Formula: $C_2Cl_4O_2$
- CHEMICAL STRUCTURE:



Uses

Diphosgene has been widely used as a substitute compound for phosgene in different chemical synthesis reactions, including formation of chloroformates, carbonates, urea, and isocyanates, and for chlorination, carboxylation, and dehydration, in various industry settings, such as pharmaceuticals, dyes, perfume, adhesive, and pesticide. It was also used as a chemical weapon agent in World War I.

Background Information

Diphosgene was produced by Germany (~ 11600 tons) as a chemical weapon in World War I, and caused mass casualties when first used in 1916. It is classified as a choking agent or lung-damaging agent (pulmonary toxicant). It is denser than air and accumulates in low-lying areas. Diphosgene is a combination of phosgene and chloroform, which destroys gas filters in gas masks. Diphosgene is chemically similar to phosgene and has a slower decomposition rate than phosgene.

Exposure Routes and Pathways

Diphosgene is listed as highly toxic in the United States and as very toxic in the European Union. The primary routes of entry for diphosgene are through skin, eyes, inhalation, and ingestion. It causes damage primarily to the respiratory tract through inhalation, which irritates the nose, throat, and especially lung tissue. Direct skin exposure to liquid diphosgene can cause surface burns.

Toxicokinetics

Diphosgene is not detoxified in the human body.

Mechanism of Toxicity

The mechanism of action has not been well established. However, its toxicity is very similar to phosgene because diphosgene decomposes to phosgene and nontoxic levels of chloroform on heating and reaction with a nucleophile. Even a trace of moisture leads to the formation of phosgene. Both phosgene and diphosgene react with water to generate hydrochloric acid, which can cause damage to the tissue in the upper respiratory tract. Diphosgene and phosgene are insoluble in water and react directly on alveolar and capillary membranes allowing plasma to flood the alveoli resulting in pulmonary edema.

Acute and Short-Term Toxicity (or Exposure)

Inhalation is the major route of exposure. Diphosgene is extremely damaging to mucous membranes, eyes, skin, and the respiratory tract, and may cause minor irritation to severe tissue damage and death. Toxicity effects vary with the concentration of vapor and the length of exposure. Signs and symptoms of toxicity may be immediate or delayed. The delayed (up to 6 h) acute respiratory distress syndrome is characteristic of chocking agent inhalation.

Animal

Mild pulmonary edema was observed in experimental rats at 6 h after exposure to diphosgene (20 min at 44.9 mg m^{-3}), and became extensive at 24 h. However, lung damage caused by low-dose diphosgene inhalation in most experimental rats was reversed and could not be distinguished from lungs of control animals after 10 days of exposure.

A 10–20 min exposure to diphosgene at a concentration of $0.9 \text{ mg} \text{ l}^{-1}$ caused fatality to tested rabbits.

Human

Diphosgene usually causes irritation to eyes and the upper respiratory tract at low concentration. Inhalation can cause fatality if the concentration is >25 ppm. Surface burns are the common symptoms

on humans when exposed to high concentrations of diphosgene. Direct eye exposure to liquid diphosgene can cause corneal abrasions, ulcers, or perforations.

For the respiratory tract, inhalation can cause spasms, inflammation and edema of the larynx and bronchi, dyspnea, cyanosis, pneumonia, and pulmonary edema. Serious symptoms, such as pulmonary edema and asphyxiation, may not be observed for hours after overexposure. Occasionally, cardiac failure occurs as a complication of severe pulmonary edema. With regards to the cardiovascular system, diphosgene can cause rapid heartbeat and hypotension. Gastrointestinal exposure may cause nausea and vomiting in patients and may be fatal.

It has been reported that the LCt₅₀ (inhalation dose) of phosgene in humans is $\sim 3200 \text{ mg min m}^{-3}$.

Chronic Toxicity (or Exposure)

Human

Pulmonary fibrosis and emphysema can develop after persistent exposure.

Clinical Management

There is no known laboratory test available that can confirm diphosgene exposure. However, evaluation of oxygen saturation and arterial blood gas is recommended for initial treatment for all patients. When inhaled, the patient should be removed to fresh air area immediately. Oxygen supplement can improve tissue oxygenation and reduce the damage due to hypoxemia in patients. Artificial respiration devices with or without positive pressure should be used if necessary.

If diphosgene is swallowed, vomiting should not be induced. For skin contact, contaminated clothing should be removed and the exposed area flushed with water and soap for at least 15 min. Patient should be under medical observation for at least 48 h.

Antibiotics should be used only with the development of bacterial bronchitis or pneumonia. β -adrenergic agonist has been used to relieve bronchospasm by relaxing bronchial smooth muscle and reducing hyperactivity in diphosgene inhalation. An adult dose of albuterol 0.5% (2.5 mg in 2.5 ml saline solution) can be used and repeated as needed.

Environmental Fate

Diphosgene decomposes into phosgene gas, hydrogen chloride gas, carbon monoxide, and carbon dioxide.

Exposure Standards and Guidelines

Phosgene occupational exposure criteria include the following:

- The US Occupational Safety and Health Administration permissible exposure limit is 0.1 ppm time-weighted average (TWA).
- The American Conference of Governmental Industrial Hygienists threshold limit value is 0.1 ppm TWA.

Miscellaneous

Diphosgene is safer than phosgene during production, transportation (in glass container only), and storage because it is stable in liquid form at room temperature. The odor of diphosgene smells like green corn or newly mown hay. It is a colorless liquid at 20°C, and has a specific gravity of 1.66 at 15°C; the boiling point is 128°C. It is heat sensitive and should be stored at 2–8°C. It is insoluble in water and soluble in ether and ethanol.

In 1992, a monitoring device that detected phosgene and diphosgene in the air was patented in Germany.

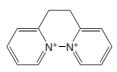
See also: Phosgene.

Diquat

Carey N Pope

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- CHEMICAL NAME: 1,1'-Ethylene-2,2'-bipyridinium ion
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 231-36-7
- SYNONYMS: Deiquat; Reglone; Aquakill; Dextrone; Reglox; Reward; Tag; Torpedo; Vegetrole; Weedtrine-D
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Bipyridinium herbicide and desiccant
- CHEMICAL FORMULAS: C₁₂H₁₂N₂; C₁₂H₁₂N₁Br₂ (dibromide salt)
- CHEMICAL STRUCTURE:



Uses

Diquat is used to control aquatic weeds, for preharvest desiccation of various crops, and for postemergence weed control in cotton production. It is sometimes used in conjunction with paraquat.

Exposure Routes and Pathways

Dermal and respiratory are common accidental routes of exposure.

Toxicokinetics

Absorption from the gastrointestinal (GI) tract is poor (6% absorption in rats) with the remainder primarily eliminated in the feces. Oral diquat is transformed to a minimal degree within the intestines, with fecal elimination of metabolites. Complete elimination occurred within 4 days after oral dosing. Diquat was rapidly eliminated via the urine with dermal, inhalation, or intravenous dosing. With subcutaneous dosing, ~90% was eliminated within 24 h via urine. Following intravenous administration in mice, diquat did not accumulate in lung or muscle.

Mechanism of Toxicity

Diquat free radical is formed by glutathione reductase. Aerobic autooxidation of diquat free radicals leads to superoxide production and oxidative damage (lipid peroxidation).

Acute and Short-Term Toxicity (or Exposure)

Animal

Diquat is moderately toxic via oral dosing, with oral LD_{50} values of 120–233 mg kg⁻¹ in rats, mice, rabbits, guinea pigs, and dogs. Cows are more sensitive, with an oral LD_{50} of 30–56 mg kg⁻¹. The acute dermal LD_{50} in rabbits is 400–500 mg kg⁻¹. A single dose of diquat was not irritating to the skin of rabbits, but repeated dosing led to erythema and eschar formation. Moderate to severe eye irritation was

noted in rabbits. Diquat can lead to severe irritation of the mouth, throat, esophagus, and stomach, and nausea, vomiting, diarrhea, severe dehydration, and disruption of fluid balance, GI discomfort, chest pain, kidney failure, and hepatotoxicity. Very large doses can lead to tremors and convulsions. Diquat elicits delayed toxicity, with an onset at ~ 24 h following dosing with death occurring from 2 to 14 days after dosing.

Human

Acute overdose in humans has been associated with ulceration of the GI tract, acute renal failure, hepatotoxicity, and breathing difficulties. Nausea, emesis, and diarrhea are initial signs of overdose. Inhalation of diquat can lead to nosebleed. Exposure to dusts can cause skin irritation, cough, and chest pain.

Chronic Toxicity (or Exposure)

Animal

Chronic diquat exposures $(2.5 \text{ or } 5 \text{ mg kg}^{-1} \text{ day}^{-1})$ led to cataracts in dogs and rats. Higher dosages may lead to retinal detachment and hemorrhage. In another study, oral diquat $(4 \text{ mg kg}^{-1} \text{ day}^{-1} \text{ for } 2 \text{ years})$ in rats produced no behavioral changes or alterations in kidneys, liver, or mycocardium but elicited some histopathology in lung. Repeated dermal contact can lead to skin inflammation, and, at sufficient dosages, systemic toxicity. Diquat has little reproductive toxicity, teratogenic, or carcinogenic potential.

Human

There is relatively little information on the chronic effects of diquat in humans. In contrast to animal studies, cataracts have not been noted in workers occupationally exposed to diquat for long periods.

In Vitro Toxicity Data

Diquat was negative in mutagenesis assays using *Escherichia coli* mutants but positive in the Ames assay.

Clinical Management

There are no antidotes for diquat. The airway should be maintained and ventilation assisted. Oxygen therapy should not be used. For oral exposure, gastric lavage should be performed with activated charcoal. Bentonite (7%, 200 ml) every 2 hours for 24 h may be used instead of charcoal. Supportive measures including fluid and electrolyte replacement should then be employed. Hemodialysis is of proven value when renal failure is present.

Environmental Fate

Diquat is highly adsorbed by soil. While being water soluble, its adsorption to soil minimizes leaching. Diquat typically remains in the top inch of soil for long periods after application. Diquat stays bound to soil particles, remaining biologically immobile in surface waters. When applied to open water, it disappears rapidly by binding to suspended particles. Microbial degradation and photodegradation are important pathways of elimination. Diquat is rapidly absorbed into plant leaves but does not translocate due to its rapid toxicity to plant tissues.

Ecotoxicology

Acute oral LD_{50} values in birds range from 200 to 564 mg kg⁻¹. Diquat is moderately to practically nontoxic to fish and aquatic invertebrates. The 8 h LC_{50} for diquat was $12-29 \text{ mg} \text{l}^{-1}$ in two fish species. The 96 h LC_{50} was $16-245 \text{ mg} \text{l}^{-1}$ in six species of fish. Yellow perch appear sensitive to concentrations of diquat found during control of aquatic vegetation. There is little or no bioconcentration of diquat in aquatic species. Diquat is practically nontoxic to honey bees.

Exposure Standards and Guidelines

The oral reference dose for diquat is $2.2\mu g kg^{-1} day^{-1}$, the acceptable daily intake for diquat is $2 \mu g kg^{-1} day^{-1}$, and the 8 h threshold limit value is $0.1 m g m^{-3}$.

See also: Paraquat.

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- Tanen DA, Curry SC, and Laney RF (1999) Renal failure and corrosive airway and gastrointestinal injury after ingestion of diluted diquat solution. *Annals of Emergency Medicine* 34: 542–545.

Relevant Websites

- http://extoxnet.orst.edu Extension Toxicology Network, Oregon State University.
- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Diquat.

Disc Batteries

Toby Litovitz

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• SYNONYM: Button batteries

Uses

Disc batteries have become ubiquitous. They are used as a power source for hearing aids, games and toys, watches, cameras, calculators, recording devices, remote controls, digital thermometers, lighted jewelry, musical or 'talking' books and greeting cards, phones, and many other devices. While their diameters range from 6 to 23 mm, cells over 15 mm in diameter are more often implicated in severe clinical outcomes.

Background Information

Disc batteries are composed of two wafer-like plates separated by an electrolyte-soaked fabric. The electrolyte is generally an alkaline solution, typically up to 45% sodium or potassium hydroxide. These contents are placed in a steel can with a plastic grommet separating the cathode and anode. Batteries may also contain metals including zinc and cadmium. Significant amounts of mercury have not been present in button batteries sold in the United States since the enactment of the Mercury-Containing and Rechargeable Battery Management Act of 1996.

Exposure Routes and Pathways

Typical exposure to disc batteries is via unintentional ingestion; these ingestions are not limited to small children. Ingestion often occurs unintentionally in those who place them in their mouths while changing the batteries of their instruments or toys, or when batteries are mistaken for pills. Disc battery ingestions are unique among childhood poisonings in that the mean age of victims is higher than that of typical pediatric poison exposures. Batteries may also be placed in aural or nasal cavities.

Toxicokinetics

Seventy-eight percent of ingested disc batteries are eliminated in the feces within 72 h; 86% pass within

4 days. Transit times in excess of a year have been reported without adverse effects.

Mechanism of Toxicity

Toxicity from disc battery exposure occurs through four potential mechanisms: (1) alkaline injury to adjacent tissue following leakage of alkaline constituents; (2) generation of an external current that flows through electrolyte-rich tissue fluids and forms hydroxides locally, also leading to alkaline damage of tissue; (3) aspiration, producing respiratory tract obstruction; and to a lesser extent (4) pressure necrosis as occurs following the ingestion of coins. Heavy metal poisoning is not expected, and symptomatic cases of heavy metal poisoning following battery ingestion have not been reported despite tens of thousands of battery ingestions that have occurred. Battery lodgment, whether in the esophagus, a diverticulum, the nose, or the ear canal, is required for injury to occur. Lodgment, especially in the esophagus, is usually associated with larger diameter cells (20-23 mm diameter). Most of these larger cells are lithium batteries, with 3 V rather than the standard 1.5 V button cell. The greater voltage, in addition to the greater diameter, contributes to the increased likelihood of significant injury.

Acute and Short-Term Toxicity (or Exposure)

Human

Most button battery ingestions are benign. However, if a disc battery becomes lodged in the esophagus, auditory canal, or nasal cavity, severe corrosive injury may occur. Esophageal burns may occur within 4-6 h of the ingestion. Esophageal perforation, esophageal stenosis requiring repeated dilatation or surgical repair, tracheoesophageal fistula, tension pneumo- or hemothorax, perforation through the aortic arch, massive exsanguinations, and cardiac arrest have been reported following button battery lodgment in the esophagus. Two fatal battery ingestion cases have occurred in toddlers. Batteries in the ear or nose may also be associated with severe injury including perforation or destruction of the tympanic membrane, destruction of the ossicles, hearing impairment, nasal septal perforation, saddle deformity of the nose, destruction of the nasal turbinates, facial nerve paralysis, chondritis, or atrophic rhinitis.

Chronic Toxicity (or Exposure)

Human

ged impaction of the battery in the esophagus, ear, or nose markedly increases the severity of the injury.

Clinical Management

Because of the high rate of prompt, uncomplicated passage of disc batteries, clinical management of battery ingestions is focused on ensuring that esophageal lodgment has not occurred. Patients with disc battery ingestion should have prompt radiographic localization, including a chest X-ray to ensure that the disc battery has not lodged in the esophagus. The absence of symptoms is not adequate confirmation that the battery is not in the esophagus as more than one-third of patients with batteries in the esophagus are asymptomatic at the time of the initial diagnosis. Furthermore, although battery diameter is predictive of lodgment, severe esophageal injury has occurred with button cells as small as 11.6 mm diameter. If located in the esophagus, the battery must be promptly removed endoscopically, ideally within 2–4 h of the ingestion.

If the battery is located in the stomach or more distal gastrointestinal tract, removal is not indicated except in the unusual event that the patient develops signs or symptoms suggestive of significant injury such as hematochezia or abdominal pain with tenderness. Once batteries have passed beyond the esophagus, more careful outpatient follow-up for lithium cells and larger diameter cells (especially in children <6 years of age) is indicated, even in the absence of symptoms. The battery diameter and chemical system can be determined from the imprint code. Assistance with identification of cells and clinical guidance is available through the National Button Battery Ingestion Hotline at 202-625-3333. Batteries in the ear or nose must be removed immediately. Nasal and otic drops should be avoided as these increase injury by enhancing corrosion, leakage, and the generation of an external current.

Conservative management includes a repeat abdominal radiograph in 1–2 weeks if the battery has not been observed to pass. There is no role for gastric emptying efforts (induced emesis or gastric lavage) or use of cathartics. Blood or urine mercury concentrations are not needed.

See also: Alkalies.

Further Reading

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Distribution

Jules Brodeur and Robert Tardif

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Distribution is the process by which absorbed chemicals are delivered to the various organs of the body and may produce an effect, be stored, or be eliminated.

One of the major factors regulating the distribution of chemicals throughout the body is the amount of blood perfusing the various organs. Per unit of organ weight, brain, and viscera, especially kidneys, are very well perfused and are therefore presented with large amounts of chemicals. Irrigation of resting skeletal muscle, skin, and bone is less important, while that of fat tissue is poor. If one also considers total tissue mass, however, the amount of blood reaching an organ can be of importance in terms of distribution. For instance, in certain individuals, fatty tissue can represent 30% of the body mass and, despite a low perfusion rate, can become an important repository for lipid-soluble chemicals.

Increasing or lowering blood perfusion will result in more or less rapid distribution of chemicals to their sites of action, storage, or removal. Muscular effort increases the amount of blood ejected by the heart per unit time. The influence of muscular activity on the overall perfusion of body organs is rather complex. For instance, the fraction of blood perfusing skeletal muscles is disproportionately increased at the expense of most of the other organs, including those responsible for elimination; skin perfusion is also markedly increased during muscular effort. On the other hand, the fraction of blood perfusing the brain remains the same. Distribution of chemicals will vary accordingly; elimination processes will be less effective allowing higher concentrations of chemicals to reach target organs, including the brain.

When the ambient temperature is elevated, the rate of skin perfusion increases; the skin then appears red and feels warm. Although skin is not an important route for transfer of chemicals, increasing the local circulation is likely to facilitate percutaneous exchange of chemicals between blood and the environment.

Another major factor determining the distribution of chemicals is their affinity for a given tissue. Affinity depends on the physico-chemical properties of a chemical, the biochemical composition of the various cells in organs, and the ability of the cellular membrane acting more or less as a barrier. Chemicals may penetrate into cells by passive diffusion through the lipid-rich membrane, by special carrier-mediated transport systems, or by filtration through small water channels in the cell membrane. Lipid-soluble neutral molecules easily diffuse across cell membranes and tend to accumulate in lipid-rich tissues. Active transport systems (energy-requiring systems) in the liver help remove certain molecules for elimination into the bile. Distribution is therefore a dynamic process enabling chemicals to reach their sites of action, storage, or removal.

Sites of storage have considerable importance in modulating the action of a chemical or its removal. These sites are usually different from those of major action, but they could be located in organs responsible for removal (e.g., liver and kidneys). While they are stored, most chemicals are temporarily inactivated; they remain available to be released and redistributed as the concentration of free, circulating chemicals in blood decreases. Storage prolongs the residence time of chemicals in the body and helps smooth out rapid fluctuations of the concentration of circulating chemicals. On the other hand, sites of storage can represent a threat in the sense that deposited chemicals may be rapidly released for further redistribution to potential sites of action.

Adipose tissues (fat) can store relatively large amounts of highly lipid-soluble chemicals like chlorinated pesticides, polychlorinated biphenyls, dioxins, furans, a number of organic solvents (benzene, trichloroethylene, and styrene), and certain drugs like anesthetics. In the past, biopsy of subcutaneous abdominal fat tissue has been used to monitor exposure to chlorinated pesticides in rural populations around the world; today, the tendency is to monitor such chemicals using blood lipids since the concentration of chemicals in the latter is in equilibrium with that in fatty tissues. Volatile chemicals, like benzene, temporarily stored in fatty tissues, are slowly released into blood and may be more easily monitored in the air exhaled by the lung. Chlorinated pesticides are also largely stored in adipose tissues of birds during summer. When birds migrate, however,

adipose tissues are extensively used as a source of energy; pesticides are then mobilized and redistributed to body tissues including the central nervous system (CNS), where concentrations may reach toxic levels.

Bone is also an important site of storage for certain metals that possess physicochemical properties similar to calcium. More than 90% of absorbed lead is incorporated into bone. Lead will stay there for years, slowly exchanging with lead in the blood and other tissues. When the demand for calcium in bone is high, lead may be rapidly released from its deposit and may reach toxic concentrations in target organs; this is especially true for lead workers who have accumulated large burdens of lead in bone throughout the years. Radioactive strontium is another metal with high affinity for bone. Unfortunately, bone is not only a site of deposit for strontium but also a site of action since radiation emitted by strontium induces bone cancers. Fluorides, which are also deposited in bone, may eventually cause skeletal fluorosis, a disease characterized by an increase in the density and the calcification of bone.

The affinity of liver and kidney for a number of chemicals is also considerable. A protein in the liver, ligandin, has a remarkable degree of affinity for organic acids; it plays a role in the transfer of these chemicals from blood into liver. Both liver and kidney may become storage depots for metals, like cadmium and zinc, due to the presence of small binding proteins called metallothioneins. When the binding capacity of these proteins is exceeded, local toxicity may appear, as is the case for cadmium in the kidney.

Finally, what may be considered as one of the most important storage depots in the body is plasma protein. Albumin, the most abundant protein in plasma, and other plasma proteins may bind reversibly a very large number of chemicals, many of which are therapeutic agents. The protein-bound fraction of a chemical exists in a state of equilibrium with the unbound (also called 'free') fraction; only the free form of a chemical is available for biological effect and disposition. By sequestering chemicals for several hours, in certain cases, protein binding in plasma regulates the pharmacological and toxicological effects of chemicals; distribution to sites of action is delayed and access to elimination processes is slowed down. It is a fact of considerable therapeutic importance that certain drugs may be displaced from their sites of protein binding by other chemicals with higher affinity for the same protein. Displacement of drugs that require precise dosing schedules to produce their therapeutic effect without also inducing toxicity, like anticoagulants and oral hypoglycemic agents, can lead to severe toxic manifestations.

The brain, as a site where chemicals are distributed, is a very sensitive organ. A more or less permeable membrane barrier located at the junction between the bloodstream and the brain acts as a shield to certain noxious chemicals; it is called the 'blood-brain barrier'.

The barrier effect is mainly due to the fact that the cells lining the walls of the capillaries present in the brain tissue are tightly joined, contrary to what prevails with capillaries in other tissues; this leaves very little space between the cells for filtration of small-size, water-soluble molecules. Moreover, the cells of brain capillaries possess very few endocytotic vesicles, which in capillaries of other tissues engulf large molecules and serve as a transfer mechanism; as a result, many neurotoxins, such as diphteria and tetanus toxins, are excluded. Furthermore, the capillaries of the brain are surrounded by prolongations of certain brain cells, thus forcing lipid-soluble chemicals to cross an additional lipid membrane. Finally, the intercellular fluid bathing the brain cells contains lower concentrations of proteins; this results in a reduction of the movement of certain water-insoluble chemicals that are more easily transported when bound to proteins.

The existence of the blood-brain barrier does not preclude the passage of chemicals into the brain. As is the case with all other cellular membranes in the body, lipid-soluble nonionized chemicals enter the brain by passive diffusion. Anesthetics, ethanol, and CNS depressants, for instance, rapidly diffuse into the brain in a matter of a few seconds or minutes. They also exit the brain rapidly when the concentration gradient between blood and brain is reversed. Elemental mercury, methylmercury, and tetraethyl lead are examples of lipid-soluble forms of metals that easily enter the brain, while the ionized, much less lipid-soluble inorganic salts of mercury and lead penetrate only poorly.

In newborn infants, the blood-brain barrier is not fully developed; certain chemicals, like lead, and some endogenous substances, like bilirubin, may therefore enter the brain more easily. Like the brain, but for different reasons, the embryo is also very sensitive to exogenous chemicals circulating in the maternal blood. The placenta is the route by which the developing embryo and fetus exchanges with maternal blood. Its main physiological function is to provide nutrients to the fetus and remove its waste products. In humans, only three layers of cells separate maternal and fetal blood and form what has been termed the placental barrier.

The placental barrier is far from being an absolute shield to the passage of foreign chemicals into the fetal circulation. The free form of lipid-soluble, nonionized molecules crosses the placenta by passive diffusion and reaches equilibrium between maternal and fetal circulations. Large molecules and microorganisms may traverse the placenta by endocytosis. Certain differences between maternal and fetal tissue concentrations of chemicals can be explained by, among other factors, lower plasma concentrations of binding proteins, lower amounts of body fat, and the absence of a fully developed blood-brain barrier in the fetus. Once delivered, most chemicals will diffuse back into the maternal circulation, leaving the fetus unharmed. A few chemicals, however, may have a devastating effect, killing the fertilized egg, inducing birth defects, or retarding the growth of the developing fetus.

Thus, for a number of chemicals and a few molecules of microbiological origin, the placenta does not serve as an efficient barrier. For many years, it has been known that the rubella virus (German measles) may cause human congenital anomalies. Similarly, some chemicals known as teratogens may also produce abnormalities in the development of the human fetus. Among them are vitamins A and D taken at high dosages, certain anticancer drugs, some steroid hormones, and thalidomide, certainly the best known teratogen.

Although less spectacular than birth defects like missing limbs or cleft palate, retardation in the functional development of the fetus may be just as damaging. In this regard, the fetotoxicity of excessive alcohol consumption and tobacco smoking is well known. Governments now issue severe warnings to pregnant women concerning the danger of these actions.

See also: Absorption; Blood; Developmental Toxicology; Excretion; Gastrointestinal System; Kidney; Liver; Metallothionein; Neurotoxicity; Pharmacokinetics/Toxicokinetics; Skeletal System.

Further Reading

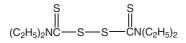
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Disulfiram

F Lee Cantrell

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 97-77-8
- SYNONYMS: Tetraethylthiuram disulfide; Teturamin; Abstensil; Alcophobin; Abstenyl; Antabuse; Antadix
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Thiuram derivative; Ethanol abuse deterrent
- CHEMICAL FORMULA: C₁₀H₂₀N₂S₄
- CHEMICAL STRUCTURE:



Uses

Disulfiram is used as a deterrent to ethanol abuse.

Exposure Routes and Pathways

Disulfiram is available only in an oral form.

Toxicokinetics

Disulfiram is rapidly absorbed, although up to 20% is excreted unchanged in the feces. Pharmacological effects usually occur within 12 h after ingestion and can persist for up to 14 days. Disulfiram has a large volume of distribution secondary to its high lipid solubility. Its protein binding is ~50%. Disulfiram is metabolized in the liver to produce diethyldithiocarbamate, diethylamine, and carbon disulfide. Metabolites are excreted in the urine although a small amount is excreted from the lungs as carbon disulfide.

Mechanism of Toxicity

Disulfiram has multiple mechanisms of toxicity. Its most well-defined action is inhibition of aldehyde dehydrogenase, which thereby diminishes the breakdown of acetaldehyde. Accumulation of carbon disulfide, a disulfiram metabolite, as well as inhibition of dopamine- β -hydroxylase has also been associated with its toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Disulfiram is not used therapeutically in domestic animals. Its toxicity when ingested in overdose is undefined.

Human

Acute overdose of disulfiram, in the absence of concomitant ethanol ingestion, may produce hypotension. When taken with ethanol, a constellation of severe reactions including flushing, vasodilation, pulsating headache, vomiting, and chest pain may occur. Less commonly, severe reactions including hypotension with shock, coma, seizures, and myocardial infarction may occur. An ethanol level as low as $5-10 \text{ mg dl}^{-1}$ may produce this reaction with fully developed symptoms appearing when ethanol concentrations exceed 50 mg dl^{-1} . These toxic manifestations correlate with increased serum concentrations of acetaldehyde and may persist for 1–2 weeks after cessation of disulfiram use.

Chronic Toxicity (or Exposure)

Animal

Large doses in laboratory animals produce advanced degenerative changes in liver and kidneys.

Human

In the absence of ethanol ingestion, chronic disulfiram use may produce adverse effects including fatigue, impotence, headache, dermatitis, and a metallic or garlic aftertaste. Neurologic complaints including vertigo, irritability, insomnia, slurred speech, and personality changes may occur. Less commonly, peripheral neuropathy, optic neuritis, delirium, and bizarre behavior may occur. Hematologic and gastrointestinal toxicity include blood dyscrasias and cholestatic hepatitis, respectively. Clinically important drug interactions include impaired metabolism of barbiturates, warfarin, and phenytoin; this may result in toxicity from these agents.

In Vitro Toxicity Data

Disulfiram has been investigated as an agent to prevent the development of multiple drug resistance in cancer cells. Recent studies have demonstrated inhibition of ATP hydrolysis and modulated P-glycoprotein transport.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. In patients presenting within 1 h of ingestion, activated charcoal should be administered. Supportive care should be provided as needed. Extracorporeal elimination is not indicated for acute disulfiram poisoning but has been

Disulfoton

Jamaluddin Shaikh

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 298-04-4
- SYNONYMS: Dimaz; Disyston; Disystox; Dithiodemeton; Dithiosystox; Solvirex
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organothiophosphate insecticide and acaricide
- CHEMICAL FORMULA: C₁₀H₁₈O₂S₃P
- CHEMICAL STRUCTURE:

Uses

Disulfoton is a selective, systemic organophosphorus insecticide and acaricide that is particularly effective against aphids, leafhoppers, beet flies, spider mites and coffee leaf miners affecting cotton, tobacco, beets, corn, peanuts, wheat, ornamentals, cereal grains, and potatoes.

Exposure Routes and Pathways

Disulfoton is formulated as a granular product. Dermal and oral routes are the most common exposure pathways. Vapors can also be absorbed by inhalation.

Toxicokinetics

Following absorption, disulfoton distributes throughout the body. It generally does not accumulate in tissues, but is initially bioactivated to the more toxic effective in treating the acute disulfiram-ethanol interaction.

See also: Alcoholic Beverages and Alcoholism; Carbon Disulfide; Dithiocarbamates.

Further Reading

Heath MJ, Pachar JV, and Martinez ALP (1992) An exceptional case of lethal disulfiram–alcohol reaction. *Forensic Science International* 56: 45–50.

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oxygen analogue, sulfoxide and sulfone, which further degrade to less toxic products. Animal studies showed that within 10 days after dosing, disulfoton and metabolites were eliminated through the urine (81.6%), feces (7%), and exhaled air (9.2%).

Mechanism of Toxicity

Disulfoton mainly causes harmful effects to the nervous system. Sulfoxide and sulfone metabolites inhibit acetylcholinesterase activity in the nervous system, and this action causes neurological effects. Cholinesterase activity in blood is also inhibited by disulfoton and can serve as indicator of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Disulfoton exhibits gender-dependent differences in toxicity. The oral LD_{50} ranges from 6.2 to 12.5 mg kg⁻¹ in male rats and from 1.9 to 2.5 mg kg⁻¹ in female rats. The dermal LD_{50} is 15.9 mg kg⁻¹ for male rats and 3.6 mg kg⁻¹ for female rats. The inhalation LC_{50} for 1 h is 180 ppb for male rats, and 90 µg l⁻¹ for female rats.

Human

Disulfoton is considered a highly toxic insecticide and acaricide by all routes of exposure. Early signs and symptoms in humans, whether absorbed through skin, ingested, or inhaled, may include headache, fatigue, blurred vision, dizziness, salivation, tearing, sweating, defecation, urination, and fluid accumulation in the airways. Convulsion and coma can occur at high doses. Ingestion of disulfoton can lead to a rapid onset of signs. Signs and symptoms following dermal exposure may be delayed up to 12 h. At least 1 week is needed for complete recovery from acute poisoning but complete restoration of blood cholinesterase activity to normal levels may take longer.

Chronic Toxicity (or Exposure)

Animal

Rats have survived for 90 days at a dose of $0.5 \text{ mg kg}^{-1} \text{ day}^{-1}$. Repeated exposures to disulfoton lead to tolerance, at least partially through downregulation of cholinergic receptors in the central and/or the peripheral nervous systems.

Human

Disulfoton is readily absorbed through the skin. Daily repeated absorption may cause loss of appetite, weakness, flu-like symptoms, and malaise. Some studies report that workers continuously exposed to disulfoton can develop anxiety, irritability, delayed reaction times and cognitive defects. Chronically exposed workers may also suffer from cataract.

Clinical Management

Dermal decontamination should be accomplished by repeated washing with soap. The possibility of disulfoton sequestered under the fingernails should not be overlooked. In case of eye contamination with disufoton, eyes should by flushed with copious amounts of clean water for 15 min. If eye irritation is persistent after decontamination, ophthalmologic consultation is required. Ipecac can be used to induce emesis in case of recent ingestion. Emesis is not encouraged if the patient is comatose or convulsing. Activated charcoal is an effective absorbent and hence used with or without saline cathartic and sorbitol.

Environmental Fate

Disulfoton is relatively stable in water at neutral and acidic pH. It is resistant to hydrolysis with a half-life of 323 days at pH 7. Alkalinity enhances hydrolysis. Disulfoton has been shown to persist for 1 week in sandy loam soil.

Ecotoxicology

Disulfoton is highly toxic to most species of warm water fish. Cold-water species are less sensitive to disulfoton. It has been detected in ground water in several places in the United States. Massive fish kills have been noted in the past with disulfoton use. The metabolites of disulfoton are very toxic to honey bees. It is very toxic to birds. The LD₅₀ for Northern bobwhite, Red-winged blackbird, and Mallard are 28, 3.2, and 6.54 mg kg⁻¹, respectively.

Exposure Standards and Guidelines

The reference dose for disulfoton is $0.043 \,\mu g \, kg^{-1}$ day⁻¹.

See also: Cholinesterase Inhibition; Organophosphates; Pesticides.

Relevant Websites

- http://extoxnet.orst.edu Extension Toxicology Network, Oregon State University.
- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Disulfoton.

Dithiocarbamates

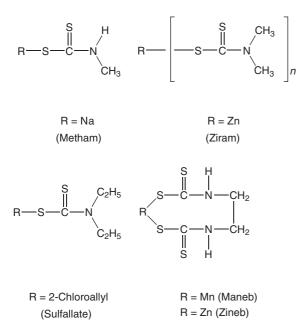
David Janz

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- PREFERRED NAMES: Thiram (CAS 137-26-8); Ziram (CAS 137-30-4); Maneb (CAS 12427-38-2); Zineb (CAS 12122-67-7)
- REPRESENTATIVE CHEMICALS: Methyldithiocarbamates (metham); Dimethyldithiocarbamates (DDC, ferbam, thiram, and ziram); Diethyldithiocarbamates (sulfallate); Ethylenebisdithiocarbamates (anobam, maneb, nabam, and zineb)
- SYNONYMS: Thiram–Arasan; Fernasan; Nomersan; Puralin; Tersan; Thiosan Ziram–Corozate; Fuclasin; Karbam White; Mathasan; Milbam; Nibam; Zimate Maneb–Dithane M-22; Manzate

Zineb–Dithane Z-78; Lodacol; Parzate

• CHEMICAL STRUCTURE:



Uses

Most dithiocarbamates are used as fungicides. Dithiocarbamates are also used as herbicides and, at least one compound, metham, is used as nematocide. Ziram is also used as an accelerant in the rubber industry, as a biocide in water treatment, and as an ingredient in adhesives.

Exposure Routes and Pathways

Occupational exposure to dithiocarbamates may occur through inhalation and dermal routes, whereas the general population may be exposed via ingestion of food and water, and through dermal contact with commercial fungicide products. Human poisonings have been reported following oral, inhalation, and dermal exposures to these compounds.

Toxicokinetics

Dithiocarbamates are absorbed from the gastrointestinal tract, lungs, and skin. The absorption of ethylenebisdithiocarbamates from the gastrointestinal tract may be altered by the presence of cations occurring naturally in food.

Little specific information is available regarding the biotransformation of these compounds. Indirect evidence suggests, however, that these chemicals undergo rapid biotransformation and excretion in humans, usually within hours or days following absorption. The metal moiety of ethylenebisdithiocarbamates is eliminated in the metabolic process. The initial metabolites of these ethylenebisdithiocarbamates are not identical to those of dimethyldithiocarbamates. A common metabolic product of all dithiocarbamate fungicides is carbon disulfide. Carbon disulfide may undergo further metabolism to form thiourea (an antithyroid substance), which may partially explain the tendency of different dithiocarbamates to affect thyroid function. Mammalian biotransformation and environmental degradation of ethylenebisdithiocarbamates to ethylene thiourea (ETU) is of concern due to the known mutagenic, carcinogenic, teratogenic, and antithyroidal properties of this chemical. ETU is reported to be metabolized in vivo and in vitro to ethylene urea with release of atomic sulfur. This sulfur atom can bind to macromolecules in the liver and may alter the activity of some enzymes in the endoplasmic reticulum. It is suggested that binding of this reactive sulfur atom in the thyroid gland may cause a decrease in iodination of tyrosine leading to thyroid dysfunction.

Generally, dithiocarbamates are rapidly excreted through the kidneys. One study reported that the elimination half-life for ethylenethiourea was ~ 100 h. However, the effect of thiram on acetaldehyde dehydrogenase tends to persist for 10–14 days.

Mechanism of Toxicity

As mentioned above, in vivo biotransformation or environmental degradation of ethylenebisdithiocarbamates to form ETU is of toxicological significance due to the known mutagenic, carcinogenic, teratogenic, and antithyroidal properties of this chemical. Neurotoxicity following chronic exposure to maneb has been reported to involve dopaminergic neurotransmission. However, a link to Parkinsonism remains unclear and may be related to manganese content of maneb, to certain metabolites such as carbon disulfide, and/or to the capacity of dithiocarbamates to bind divalent metals and form more lipophilic complexes able to enter the central nervous system. Thiram can precipitate an antabuse (disulfiram) reaction in persons who have consumed a substantial amount of alcohol by inhibiting the enzyme, acetaldehyde oxidase. The ethylenebisdithiocarbamates have the same potential. Several dithiocarbamates have been reported to inhibit cytochrome P450-dependent monooxygenases in animal models.

Acute and Short-Term Toxicity (or Exposure)

Animal

All dithiocarbamates have moderate to low acute toxicity. As opposed to carbamate pesticides, exposure to dithiocarbamates does not precipitate symptoms of cholinergic crisis. The oral LD_{50} values of these agents vary from 285 to 7500 mg kg⁻¹ (average, >2500 mg kg⁻¹). Large doses of thiram caused ataxia, and hyperactivity followed by clonic convulsion, loss of muscle tone, and dyspnea in rats and mice.

Human

Historically, systemic poisoning by dithiocarbamates has been rare. However, an antabuse-like reaction (flushing, sweating, headache, weakness, hypotension, and tachycardia) may occur when ethanol is consumed following exposure to thiram and metallobisdithiocarbamates. Interestingly, this is not typically observed following carbamate, monothiocarbamate, or ethylenedithiocarbamate exposure.

Ataxia, weakness, hypothermia, and ascending paralysis are possible neurologic symptoms after exposure to thiram and ethylenedithiocarbamates. Gastrointestinal signs following dithiocarbamate exposure include nausea, vomiting, and diarrhea. Renal failure has been reported following maneb exposure. Exposure to sprays, solutions, suspensions, and powders of these agents may cause allergic contact dermatitis and mucous membrane irritation. In one case, coma, seizures, and right hemiparesis were reported following exposures to maneb and zineb.

Chronic Toxicity (or Exposure)

Animal

Decreased fertility and impaired thyroid function were reported in cattle after repeated exposure to 200 mg kg^{-1} of zineb for 80 days. Dogs given ziram for 5–9 months ($25 \text{ mg kg}^{-1} \text{ day}^{-1}$) exhibited convulsions and some lethality.

Human

Thiuram, the ethyl analog of thiram, was reported to cause peripheral neuropathy in humans (characterized by pain, numbness, and weakness in the extremities). Occupational exposure to maneb-containing fungicides caused extrapyramidal symptoms in two agricultural workers. In addition to the previously mentioned symptoms, mental confusion, drowsiness, lethargy, and flaccid paralysis were reported to occur with thiram poisoning.

Clinical Management

Symptomatic treatment is recommended as there is no specific antidote available for poisoning by these compounds. In the case of accidental oral poisoning, gastric lavage should be performed soon after ingestion. Absorption of these compounds may be prevented by administering activated charcoal slurry. Conventional anticonvulsant drugs may be used to treat seizures.

Environmental Fate

In general, dithiocarbamates have low to moderate mobility in soils. If released into water, dithiocarbamates are expected to adsorb to particulates and sediments. Volatilization from water or soils is not expected to occur for most dithiocarbamates. If released into air, the low vapor pressures for most dithiocarbamates indicate they will be bound predominantly to particulate matter in the ambient atmosphere. Environmental degradation in air, water, and soil is relatively rapid due to photolysis and/or hydrolysis. Bioconcentration factors ranging from 2 to 90 suggest low accumulation of dithiocarbamates in aquatic organisms.

Ecotoxicology

Dithiocarbamates are potent toxicants in *Daphnia*, with an LC_{50} of $<1 \text{ mg l}^{-1}$. Dithiocarbamates are also potent toxicants in fish, with LC_{50} values generally $<1 \text{ mg l}^{-1}$. Immature trout are more sensitive than older individuals. Some dithiocarbamates have embryotoxic and teratogenic effects.

See also: Carbamate Pesticides; Pesticides.

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Relevant Website

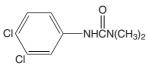
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Diuron

Jing Liu

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 330-54-1
- SYNONYMS: N'-(3,4-Dichlorophenyl)-N,N-dimethylurea; 3-(3,4-Dichlorophenyl)-1,1-dimethylurea; DCMU; DMU; Cekiuron; Crisuron; Dailon; Diater; Diurex; Duirol; Karmex
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Substituted urea herbicide
- CHEMICAL FORMULA: C₉H₁₀Cl₂N₂O
- CHEMICAL STRUCTURE:



Uses

Diuron is used as a general pre-emergence herbicide for weed control on noncrop lands and among some agricultural crops such as asparagus, pineapple, cotton, and sugarcane. It is also used as a soil sterilant.

Exposure Routes and Pathways

Dermal and inhalation routes are the primary exposure pathways in occupational settings. Ingestion is also a possible route of accidental exposure.

Toxicokinetics

Diuron is absorbed from the gastrointestinal and respiratory tracts. There was no apparent tissue storage of diuron being noted in either rats or dogs after up to 2 months of feeding. It undergoes hydroxylation and dealkylation with the urea moiety generally unchanged. In rats and dogs, the predominant metabolite was N-(3,4-dichlorophenyl)-urea, accompanied by small amounts of N-(3,4-dichlorophenyl)-N'-methylurea, 3,4-dichloroaniline, 3,4-dichlorophenol, and unmetabolized diuron. In mouse liver microsomes, eight metabolites were identified with the N-demethylated derivative being the major one, followed in importance by three N-hydroxymethyl compounds. Metabolites found in mammals are similar to those found in soil and plants wherein dealkylation and hydroxylation are also the major metabolic pathways. The metabolites are mainly excreted in urine and feces.

Mechanism of Toxicity

Diuron is a selective inhibitor of the Hill reaction in plant photosynthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Diuron exhibits low acute toxicity. The oral LD_{50} in male rats is $> 3 g kg^{-1}$. Dietary protein levels have been reported to influence the acute toxicity of diuron in albino juvenile rats. High acute dosage of diuron (at LD_{50} s) in these young rats may cause drowsiness and ataxia; animals that survived were irritable and hyperexcitable. Diarrhea and clinical signs of renal dysfunction were reported. Subacute exposure of diuron may cause growth retardation and increase erythropoiesis. No skin irritation and sensitization was found in guinea pigs. Diuron was able to induce multiple hepatic microsomal enzymes in rats after as early as 3 days of oral exposure.

Human

Diuron produces little acute toxicity in humans except irritation of the skin, eyes, and nose.

Chronic Toxicity (or Exposure)

Animal

A 14 months feeding study in female rats $(0.5-1 \text{ g kg}^{-1})$ caused hemolytic anemia and methemoglobinemia. At extremely high subacute dosages, male rats exhibited spleen and bone marrow changes. With repeated high dosages, diuron led to blood chemistry changes, increased mortality, and growth retardation. At a very high dosage (250 mg kg⁻¹ day⁻¹ on gestation days 6–15), diuron caused wavy ribs, extra ribs, and delayed bone formation in rats.

Human

Little is known regarding effects of long-term exposure to diuron in humans.

In Vitro Toxicity Data

Diuron was not mutagenic in a number of bacterial and mammalian cell assays.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Diuron is stable to hydrolysis (at pHs 5, 7, and 9) and photolysis and therefore persistent in the environment. Mobility of diuron in the soil is related to organic matter. It has the potential to leach into ground and to contaminate ground and surface waters. Diuron, however, has low water solubility (42 ppm).

Ecotoxicology

Diuron has an oral LC_{50} of 1730 ppm in bobwhite quail and 5000 ppm in mallard ducks. The compound is relatively nontoxic to honey bees. Diuron is moderately toxic to aquatic animals such as rainbow trout, bluegill sunfish, sheepshead minnow, Eastern oyster, and brown shrimp. The LC_{50} s of diuron in fish range from 4.3 to 42 ppm.

Exposure Standards and Guidelines

The oral reference dose derived from a 2 year dog feeding study using abnormal pigments in blood as the critical endpoint was $0.002 \text{ mg kg}^{-1} \text{ day}^{-1}$.

The threshold limit value – time-weighted average is 10 mg m^{-3} .

Oncogenic systemic no-observed-adverse-effect level (NOAEL) in rats is $1.25 \text{ mg kg}^{-1} \text{ day}^{-1}$.

Reproductive NOAEL in rats is 6.25 mg kg^{-1} day⁻¹.

See also: Pesticides.

Further Reading

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Relevant Websites

http://pmep.cce.cornell.edu – EXTOXNET. http://www.epa.gov – US Environmental Protection Agency.

DNA *See* Aneuploidy; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; DNA Phosphoramidites; Genetic Toxicology; Genomics, Toxicogenomics; Molecular Toxicology–Recombinant DNA Technology; Toxicity Testing, Mutagenicity.

DNA Adduct See Carcinogen–DNA Adduct Formation and DNA Repair.

DNA Phosphoramidites

Sang-Tae Kim

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• REPRESENTATIVE CHEMICALS: Deoxyadenosine benzoyl cyanoethyl phosphoramidite; Deoxycytidine benzoyl cyanoethyl phosphoramidite; Deoxyguanosine isobutyryl cyanoethyl phosphoramidite; Thymidine cyanoethyl phosphoramidite

 CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Deoxyadenosine benzoyl cyanoethyl phosphoramidite (CAS 98796-53-3); Deoxycytidine benzoyl cyanoethyl phosphoramidite (CAS 102212-98-6); Deoxyguanosine isobutyryl cyanoethyl phosphoramidite (CAS 93183-15-4); Thymidine cyanoethyl phosphoramidite (CAS 98796-51-1)

- Synonyms:
 - A phosphoramidite; Deoxyadenosine phosphoramidite; Adenosine, N-benzoyl-5'-O-[bis(4-methoxyphenyl)phenylmethyl]-2'-deoxy-,3'-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]
 - C phosphoramidite; Deoxycytidine phosphoramidite; Cytidine, N-benzoyl-5'-O-[bis(4-methoxyphenyl)phenylmethyl]-2'-deoxy-,3'-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]; N4-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidine-3'-O-[O-(2-cyanoethyl)-N,N'-diisopropylphosphoramidite]
 - G Phosphoramidite; Deoxyguanosine phosphoramidite; Guanosine, 5'-O-[bis(4-methoxyphenyl)phenylmethyl]-2'-deoxy-N-(2-methyl-1oxopropyl)-,3'-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]
 - T Phosphoramidite; Deoxythymidine phosphoramidite; Thymidine, 5'-O-[bis(4-methoxyphenyl)phenylmethyl]-, 3'-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]
- CHEMICAL FORMULAS:
 - A phosphoramidite: C₄₇H₅₂N₇O₇P
 - C phosphoramidite: C₄₆H₅₂N₅O₈P
 - G Phosphoramidite: C44H54N7O8P
 - T Phosphoramidite: C₄₀H₄₉N₄O₈P

Uses

DNA phosphoramidites are used in the chemical synthesis of DNA oligonucleotides.

Exposure Routes and Pathways

Exposure to DNA phosphoramidites is most likely to occur in occupational settings. Inhalation and dermal exposure are the primary routes of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

DNA phosphoramidites were determined to be nontoxic to rats when dosed at 2000 mg kg^{-1} via the oral route. DNA phosphoramidites had no skin irritation potential based upon studies in the rabbit. DNA phosphoramidites had slight eye irritation potential based upon studies in the rabbit. DNA phosphoramidites were tested for their capacity to induce hypersensitivity responses in mice as measured by the proliferation of lymphocytes in the draining lymph nodes. DNA phosphoramidites did not induce hypersensitivity responses and therefore are not considered to be potential sensitizers. In a 28 day oral toxicity study in rats, the major target organ of toxicity was the liver; however, the trends or findings might not necessarily be considered adverse in the 28 day dosing regimen. No other significant tonic effect were noted.

Human

Little information is available regarding the toxic effects of DNA phosphoramidites in humans. Prolonged eye contact to the solid form of DNA phosphoramidites may cause eye irritation.

In Vitro Toxicity Data

DNA phosphoramidites were not mutagenic to *Salmonella typhimurium* and *Escherichia coli* strains with and without metabolic activation.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Molecular Toxicology–Recombinant DNA Technology.

Further Reading

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DNA Repair See Carcinogen–DNA Adduct Formation and DNA Repair.

Dominant Lethal Tests

Samantha E Gad

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Dominant lethal mutations are generally considered to be the result of mutations in germinal tissue which do not cause dysfunction of the gametes but which result in prenatal death of embryos heterozygous for the mutations. Thus, dominant lethal tests, usually conducted in rodents, assess the inheritability of genomic mutations (i.e., mutations that can be passed to the next generation). Obviously, since embryos heterozygous for dominant lethal mutations do not survive, the mutations that result in dominant lethality cannot be passed to succeeding generations. However, the assumption is made that if dominant lethal mutations are present, other dominant and recessive nonlethal mutations could also be present and inherited by future generations.

Pioneering studies that provided the scientific foundation for the dominant lethal test were conducted in the 1930s, but the only known mutagen before the end of World War II was radiation. It was not until the late 1950s and the 1960s that, as a result of genetic research, scientists expressed concerns that chemicals might be hazardous to the germline of humans and suggested that routine toxicity testing of chemicals should include assays for mutagenicity. The dominant lethal test was one of the first genetic toxicology tests to be developed to assess the potential hazards of chemicals. In the early 1970s, the dominant lethal test (together with the host-mediated assay and the in vivo cytogenetic assay) was one of the original three screening tests recommended for evaluating the mutagenic effects of chemicals and, in the ensuing time, numerous approaches for examining dominant lethal mutations in rodents have been developed and evaluated.

Dominant lethal tests are usually conducted in mice or rats. The mouse dominant lethal test is more economical, but the rat dominant lethal test is more informative, as corpora lutea may be accurately enumerated in pregnant female rats, but not in mice, to assess preimplantation loss. Although dominant lethal tests may be performed with treated females, the tests are commonly performed with treated males in order to identify stages sensitive to mutation induction during germ cell development because the knowledge of stage sensitivity is important for risk evaluation. In a typical dominant lethal test, males are dosed with three levels of the test chemical with the objective of the highest dose being one that will exhibit some signs of toxicity but that will be low enough for a sufficient number of males to survive through the duration of the test. Following dosing, each treated male is mated to one or more virgin females over each of a series of mating cycles. The females are euthanized mid-gestational term, and the uterine contents are examined to enumerate the number of live implantations (fetuses), early and late dead implantations (dominant lethals), and total implantations. If the test is conducted in rats, the corpora lutea are also enumerated to determine the number of ovulated eggs that fail to develop into fetuses (preimplantation loss). Data, including fertility indices, are then analyzed statistically based on groups of treated males, and each parameter and each mating group is evaluated independently.

Dominant lethal protocols can vary in the route and duration of exposure of the treated males, the number of males per dose level, the number of females per male per mating interval, and the number of mating intervals. However, it is necessary that dominant lethal tests be conducted with sufficient numbers of animals and mating intervals to maximize the test's sensitivity for detecting genomic mutational events and to provide information on germ cell-stage sensitivity of any mutagenic effects that may be observed. Thus, it is necessary to define protocols for each test material that will address these concerns as well as current regulatory requirements. Economies of testing may be achieved by using a laboratory's recently obtained (e.g., within $\sim 12 \text{ months}$) historical positive control values for the same species and strain, with the same number of mating cycles, rather than using a concurrent group of positive control animals. However, even then, one dominant lethal test may involve more than 2000 animals.

The dominant lethal test is no longer used as an initial, or first-tier, test largely because of the time and expense that are involved as well as the numbers of animals that are required to assess the results for statistical significance. However, the dominant lethal test has gained acceptance as a second-tier test for national and international regulatory submissions since the current consensus of experts is that the dominant lethal test can best be used to confirm positive results from lower tier chromosomal aberration-detecting systems (confirming in the sense of indicating the ability of the chemical to penetrate gonadal tissue and to produce cytogenetic damage). Further, the expert consensus is that the dominant lethal assay should not

be used as a risk assessment method. Supporting these views is the evidence indicating that the dominant lethal test is less sensitive than first-tier tests for assessing gene and chromosomal mutations in vitro. In addition although the correlation between positive dominant lethal results and carcinogenicity is apparently low, which would be expected, it has been found that dominant lethal results are highly predictive of the outcome of the even more expensive and time-consuming third-tier specific locus and heritable translocation tests for genetically transmissible mutations. Thus, the apparent lack of sensitivity of the dominant lethal test in comparison to in vitro tests and the lack of concordance of dominant lethal test results with carcinogenicity may reflect differences between in vitro and in vivo exposures of target cells, differences in the cellular and genetic mechanisms of carcinogenesis in somatic tissues and the induction of heritable mutations in germinal tissues, and/or the failure of some mutagenic chemical metabolites to reach germinal tissues because the dominant lethal test has high concordance with other mammalian germ cell mutagen tests.

See also: Analytical Toxicology; Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Chromosome Aberrations; Developmental Toxicology; Host-Mediated Assay; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Genetic Toxicology; Sister Chromatid Exchanges; Toxicity Testing, Mutagenicity.

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Donora: Air Pollution Episode

Michael A Kamrin

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Introduction

Donora, Pennsylvania is located in a narrow valley along the Mohongahela River ~ 35 miles south of Pittsburgh. In the first half of the twentieth century, it was the site of two United Steel Corporation facilities: the Donora Zinc Works and the American Steel and Wire Company. As was the case in many other towns in which industrial facilities were sited during that era, the poor air quality that was associated with these installations was considered a fact of life. Although most people thought of it as a nuisance, some health impacts of the pollution were recognized as early as 1918 and in the 1920s farmers sued the company for loss of crops and livestock. As a result of these health concerns, air quality measurements were made regularly from 1926 to 1935 but were then discontinued. The end of monitoring was not due to great improvements in air quality. Indeed, this remained poor especially when weather conditions led to the accumulation of pollutants in the area.

Donora Air Pollution Incident

The attitude towards local air pollution changed in 1948, when weather conditions led to a prolonged period of very poor air quality which resulted in significant morbidity and mortality among the residents. The incident, which lasted from October 26 to October 31, started as the result of cold anticyclone that approached the area from the west. The cold ground, coupled with the anticyclone, led to an elevated inversion layer that trapped the pollutants in the air above Donora and the surrounding area. This inversion layer lasted for 5 days due to a high-pressure ridge that remained in the area, moving less than a few hundred miles during this time.

As the days went by, visibility gradually decreased as the chemical smog increased in intensity. By the third day of the incident, the percentage of people becoming ill started to rise precipitously despite the best efforts of the local emergency response volunteers and medical personnel. An attempt was made to evacuate citizens with heart or respiratory ailments but the conditions made travel impossible. Volunteers brought oxygen to those suffering respiratory distress but the smog delayed the delivery of oxygen and supplies were limited. The first death occurred on the third day and on the fourth day, the number of deaths increased so that a temporary morgue had to be set up. The town's eight physicians were too few in number to attend to all who were affected. All during this period, the plants continued to spew pollution, containing particulates, sulfur dioxide/sulfuric acid, zinc, lead and cadmium, into the air. It was not until the morning of the 30th that the Donora Zinc Works shut down and later that afternoon, rains finally arrived and washed the smog from the air.

The incidence of adverse health impacts decreased rapidly as the smog disappeared. It appears from the time course of the toxicity that it was a threshold phenomenon; that is, effects were related to the maximum pollution dose and abated quickly when the dose decreased. Other air pollution episodes; for instance, the 1952 London fog, followed a different response pattern in that the effects were influenced mainly by the total pollution dose (concentration averaged over time).

The toll from the Donora incident was very high; 20 people died, ~ 50 were hospitalized, and ~ 6000 (out of the population of 14 000) experienced some adverse effects from the chemical smog. These adverse effects included nasal discharge, constriction of the throat, sore throat and symptoms related to compromised lung function. As might be expected, the older people were most affected; over two-thirds of those hospitalized were over 55. These data reflect only the acute effects of the episode and it is quite possible that some of those who survived suffered chronic or delayed effects that decreased their life expectancy. Indeed, a follow-up study ten years after the incident suggested that there was higher mortality in the individuals who showed adverse effects during the episode.

This incident gained national attention when it was mentioned in a national news broadcast by the well-known Walter Winchell. The seriousness of the episode led to a collaborative investigation by local, state, and national agencies, including the US Public Health Service. This represented the first organized attempt to document the health effects of air pollution in the US. The Public Health Service recommended warnings tied to meteorological conditions and better air sampling to prevent future problems of this sort.

However, this episode did not remain in the public or scientific consciousness long. By 1949, public attention turned to a smog incident in Southern California where irritant effects were of most concern. The lack of scientific interest in the effects of smog was reflected in the absence of discussion of this incident in technical conferences, such as the meetings of the American Chemical Society.

However, the great London fog of 1952 that resulted in ~4000 deaths refocused attention on the public health consequences of air pollution and the lessons from Donora. The tremendous death toll in London made it clear that air pollution was more than just a nuisance and led to the enactment of legislation on both the state and federal level to control this type of environmental insult. The Commonwealth of Pennsylvania passed a state Clean Air Act in 1955, the first such law to control air pollution. This was followed in 1970 by the passage of the Federal Clean Air Act; hearings on this bill were marked by references to the events that happened in Donora in 1948.

Summary

The Donora air pollution incident was an early warning of the potential health impacts of industrial air pollutants and served as an important contributor to the development of regulations to control air pollution and also to increased public concern about environmental contamination of all types. This concern intensified in the 1960s and culminated in the formation of the Environmental Protection Agency in 1970. This was followed soon after by the passage of a number of environmental protection laws and regulations, including the Clean Air Act, during the decade of the 1970s.

See also: Clean Air Act (CAA), US; Pollution, Air; Great Smog of London.

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Dose-Response Relationship

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The dose-response relationship measures the correlation that occurs as one modifies the amount (dose) of a chemical substance to which a living material is exposed and the severity of the effect (response). This is commonly used with pharmaceuticals to determine the most effective amount of medication to be administered to have the desired beneficial effect. If the amount of medicine administered is too small (below the therapeutic level), the intended beneficial effect does not occur; if the dose is increased and the amount administered is too large (above therapeutic range) toxicity may become evident. Toxicologists hold that the dose-response relationship applies not only to therapeutic agents but also to all chemical substances, that is, 'the dose makes the poison'. The underlying principal is that the biological effects (beneficial or deleterious) of chemicals are due to the amounts of active material at the site, or sites, of action and that the concentration or the amount of the substance at the site (internal dose) is related to the amount of chemical administered (external dose).

The dose-response relationship can be viewed most simply as what happens in a single individual, where the severity of the effect increases as the dose increases; this is referred to as a 'graded' relationship. This relationship between dose and response can also be described in terms of the numbers of individuals (usually measured and reported as a percentage) of a defined population affected at a given dose level, where the frequency increases as the exposure increases; this is referred to as an 'all-or-none' or 'quantal' relationship. The demonstration of doseresponse relationship suggests causality between the degree of exposure and the adverse effect. An adverse effect can be defined as a change in the morphology, physiology, growth, development, or life span of an organism that results in an impairment of its functional capacity or ability to compensate for additional stress, or in an increased susceptibility to the harmful effects of other environmental influences.

In 1983, the National Research Council of the (US) National Academy of Sciences published a report titled *Risk Assessment in the Federal Government: Managing the Process*; this work has had a marked influence on the risk assessment process used by regulatory agencies worldwide. The risk assessment process, in this report, consists of four components: hazard identification, dose–response assessment, exposure assessment, and risk characterization. 'Hazard identification' in the context of the report is concerned with evaluating the potential adverse health effects of a chemical, mixture of chemicals, or process; thus, it is very similar to the traditional term 'toxicity' used by toxicologists.

Dose–Response Curves

The typical dose-response curve is usually sigmoidal in shape, when the response, expressed as a percentage of the frequency, is plotted against the dose, expressed as a logarithmic scale (Figure 1a). The sigmoidal curve represents the cumulative curve of a normal (Gaussian) distribution of the response, where the response for each individual dose level is expressed as a percentage of the total and is plotted against the log dose (Figure 1b). All of the responses occurring in the dose levels below and above any dose level of particular interest should be included to do the curve, and the background response level in the control group must be kept in mind. The resulting bell-shaped, normal distribution curve that is typically developed from toxicity data reflects the variation in susceptibility among individual subjects in a given population to the effects of the chemical. The subjects responding at the lower doses (left side of the curve) represent hypersusceptible subjects, whereas those responding at the higher doses (right side of the curve) are considered resistant subjects; those situated at the middle doses represent the average responders.

Mathematically, if one plots the cumulative response along a probability unit ('probit') scale, the cumulative sigmoidal dose-response curve is transformed into a straight line. Such a transformation is illustrated in Figure 1c. Converting the data to straight lines is essential if one wishes to compare the dose-response characteristics of several chemicals because mathematical procedures exist for determining various parameters of such a linear relationship. One can assess the degree of change in response to the degree of change in dose; this is called the 'slope' of the dose-response curve. With some agents, the change in response may be extremely abrupt (a relatively small change in dose results in a large change in response); this type of chemical is said to possess a 'steep' dose-response curve. With other agents, the change may be quite small (a relatively large change in dose is required to elicit a small change in response); such a substance is said to exhibit a 'flat' dose-response relationship. Linear curves can also be compared mathematically to determine if they are parallel to each other.

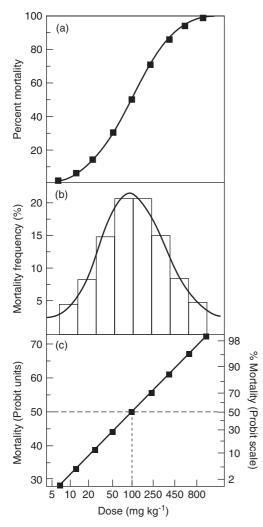


Figure 1 Graphical representation of a typical dose-response relationship for assessing mortality in laboratory animals receiving varying dosages of a toxicant. In all panels, the dosage $(mg kg^{-1})$ is plotted as a logarithmic scale. (a) The response (%mortality) is plotted as a cumulative percentage of the total number of dead animals (the number of animals killed at a specific dosage level and all dosages below it are added together and the percentage of the total number is calculated). (b) Each bar (mortality frequency %) represents the percentage of the total number of animals that died at each dosage minus the percentage that died at the immediately lower dosage. The curve that joins the bars is the bell-shaped relationship known as the normal frequency distribution curve. (c) The cumulative percentage of the total number of dead animals seen in the top panel is expressed in probit units on the left ordinate (mortality (probit units)) and as a probit scale on the right ordinate (% mortality (probit scale)). (Reproduced from Klaassen CD and Eaton DL (1991) Principles of toxicology. In: Amdur MO, Doull J, and Klaassen CD (eds.) Casarett and Doull's Toxicology: The Basic Science of Poisons, 4th edn, 13. New York: Pergamon.)

While the typical dose–response curve can usually be described by a sigmoidal or linear curve, it may be convex, concave, or even bimodal. These other configurations are usually the exception rather than the rule and depend on the mechanism of action of the material in question and even the presence of multiple toxicity sites. In some instances, the curve may be 'Jshaped' or inverted 'U-shaped', depending on the endpoint being measured. This type of dose-response relationship is observed in a phenomenon known as hormesis, with one explanation being that exposure to small amounts of a material can actually confer resistance to the agent before frank toxicity begins to appear following exposures to larger amounts. However, analysis of the available mechanistic studies indicates that there is no single hormetic mechanism. In fact, there are numerous ways for biological systems to show hormetic-like biphasic dose-response relationship. Hormetic dose-response has emerged in recent years as a dose-response phenomenon of great interest in toxicology and risk assessment.

Threshold

A toxicant must be present at its cellular site of action in sufficient amounts to exert its deleterious effects. When the concentration is too small it is said that the 'threshold' has not been reached; therefore, the material does not exert any adverse action. The distribution of active substances in the body is not uniform, and certain cells can exhibit preferentially high affinities for particular agents. Pharmacokinetic thresholds determine the effective dose of a chemical at its biological target site based on the absorption, distribution, biotransformation, and excretion of the particular chemical.

Specific Dose-Dependent Values

Specific dose-dependent values on the linear doseresponse curves can be estimated and statistically compared as well as the degree of variability (confidence limits) representative of the data being analyzed. Perhaps the most common specific toxicity value so determined in laboratory animals is the 'median lethal dosage' or the LD_{50} . This value is the estima ted dosage that would be expected to kill 50% of a given population of animals under the conditions of a particular laboratory test. With medicinal agents, another useful value related to the LD_{50} is the 'therapeutic index'. Here one is interested in the 'median effective dosage' (ED₅₀) for a beneficial pharmacological therapeutic effect and how it compares to the toxic potency of the agent. One way of assessing this situation is to calculate an LD_{50}/ED_{50} ratio; the larger the ratio, the greater the relative safety of the chemical. Another way of assessing the relative safety of medicinal agents is to compare the ED_{99} (the dose that is effective in 99% of a given population) to the

 LD_1 (the dose that is lethal to 1% of the same population). The ratio LD_1/ED_{99} is called the 'margin of safety' – the larger the ratio, the greater the relative safety of the medicinal agent.

Chronicity Index

The result of repetitive exposures to a given chemical may be different from when exposure to the material only occurs once or twice. The cumulative effects of repetitive exposures over time may render the agent more hazardous. This property can be estimated in animals by comparing the lethal potency (LD_{50}) of an agent, given only once, to its lethal potency when administered repetitively. The 'chronicity index' is a term that has been applied to a ratio of the 'one-dose LD₅₀' (animals receive the material only once) and the '90-dose LD₅₀' (animals receive the material repetitively each day for 90 days). If the one-dose/90dose ration is close to 1, this is an indication that repetitive administration does not result in cumulative effects or cumulative retention, whereas if the ratio increases and is larger than one, it is quite likely that the agent exerts cumulative effects or is retained over the repeated exposures.

Dose-Response Limits in Regulatory Toxicology

Although mathematical ratios are not usually derived from subchronic toxicity studies conducted with laboratory animals for regulatory purposes, the doseresponse relationship is a very important part of such studies. Different dose levels are utilized in such experiments, and it is desirable to have at least one dose level where no adverse biological effects occur following exposure. One finds various terms used to describe the severity of biological effects observed or extrapolated from such studies. The 'no-observedeffect level' (NOEL) is described as the highest dosage that creates no significant difference in the observed and measured effects between the exposed animals and the unexposed control group. The 'lowest-observed-effect level' (LOEL) is the lowest dose used in a study that results in the appearance of some statistically or sometimes nonstatistically significant biological effect (beneficial or deleterious). The 'lowest-observed-adverse-effect level' (LOAEL) is the lowest dose used that results in the appearance of an adverse effect.

The 'no-observed-adverse-effect level' (NOAEL) is the highest dosage where an adverse effect is not observed. Depending on the doses used in a study, even the lowest effective dose could cause a moderate or severe response although this would not be the ideal situation toxicologically. The same is true with the LOAEL, that is, this dose group could have effects that are more than mild. Finally, the 'frankeffect level' (FEL) is a treatment level that results in the appearance of overt toxic effects. Figure 2 is a graphical representation of where these various dosage levels might appear in a typical doseresponse curve. Given study designs and their fixed number of dose levels, not all of them are observed in each subchronic or chronic study. Nevertheless, one usually sees an FEL, an LOAEL, and either an LOEL or an NOAEL. One can also extrapolate an NOAEL or an NOEL from the data derived in a subchronic or chronic study because these dosage levels can be important for establishing safety guidelines for humans. Extrapolation of an NOAEL, NOEL, or other level of effect or no effect is usually a matter of data analysis. In risk assessment, this is usually achieved by applying uncertainty factor or using a mathematical model. There are many decision-making processes in order to estimate an NOAEL of NOEL by data extrapolation, and readers are referred to some of the 'related topics' of this encyclopedia (see See also section). For example, the benchmark dose (BMD) methodology can be used to identify pointof-departure (POD) estimates for use in derivation of reference doses (RfDs) or for evaluation of margins of exposure.

The dose–response relationship is the basis by which regulatory bodies define under what limits humans can be exposed to potentially toxic chemicals and yet not suffer adverse effects. A number of different government bodies establish regulations to define safe exposure conditions. The 'acceptable daily intake' (ADI) is

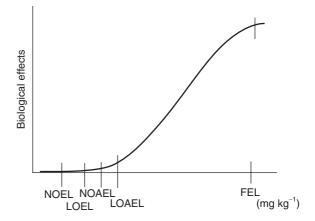


Figure 2 Schematic representation of various dosage limits used in regulatory toxicology and where they usually appear in a typical dose–response curve. (Reproduced from Ecobichon DJ (1992) *The Basis of Toxicity Testing*, Boca Raton, FL: CRC Press.)

defined as the daily intake of a chemical that, during an entire lifetime, appears to be without appreciable risk; the ADI is used by the US Food and Drug Administration (FDA) for calculating permissible levels of nonfood ingredient residues in food (for instance, food additives or pesticides). The US Occupational Safety and Health Administration (OSHA) estimates 'permissible exposure limits' for chemical contaminants in occupational environments, to which workers are exposed for given periods during normal working conditions. Further, the US Environmental Protection Agency (EPA) makes use of the reference concentration (RfC) and RfD to estimate levels of noncarcinogenic environmental chemicals to which humans can be exposed during a lifetime without deleterious effects. All of these various safety indicators use NOAEL or NOEL values as part of the calculation.

Dose-response has received an increasing level of attention in recent years, including the founding of the Dose-Response Specialty Group (DRSG) of the Society for Risk Analysis (SRA) in 1994. This group is open to all members of the SRA interested in biological and mathematical relationships between exposure and effect, with a focus on issues related to the shapes of dose-response curves and their underlying biological meaning, population distributions that describe ranges of response, and probabilistic approaches to extrapolating responses in unknown populations based on observations in experimental populations or epidemiology studies. The group is interested in all exposed populations including humans and environmental species and is closely aligned with the toxicology and environmental sciences fields.

In addition to SRA's DRSG, a group of scientists representing several US federal agencies, the International Society of Regulatory Toxicology and Pharmacology, the private sector, and academia met in 1990 to develop a strategy to encourage the assessment of the biological effects of low-level exposures (BELLE). The meeting was convened because of the recognition at the time that most human exposures to chemical and physical agents are at relatively low levels, yet most toxicological studies assessing potential human health effects involve exposures to levels of orders of magnitude greater than actual human exposures. Consequently, the BELLE founders noted that risks at low levels are estimated by various means, frequently utilizing assumptions about which there may be considerable uncertainty. BELLE is committed to the enhanced understanding of low-dose responses of all types, whether of an expected nature (e.g., linear, sublinear) or of a so-called paradoxical nature. Paradoxical dose–response relationships might include U-shaped dose–response curves and biphasic dose–response curves. The focus of BELLE now encompasses dose– response relationships to toxic agents, pharmaceuticals, and natural products over wide dosage ranges in *in vitro* and *in vivo* systems, including human populations.

See also: Benchmark Dose; Exposure Assessment; Exposure Criteria; Hazard Identification; Hormesis, LD_{50} / LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Levels of Effect in Toxicological Assessment; Maximum Allowable Concentration (MAC); Maximum Tolerated Dose (MTD); Pharmacokinetics/Toxicokinetics; Reference Concentration (RfC); Reference Dose (RfD); Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Toxicity, Acute.

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Relevant Websites

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Drinking Water Criteria

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Drinking Water Standards and Criteria

Drinking water is necessary for people, livestock, wildlife, crop irrigation, and for recreation. Although \sim 70% of the Earth's surface is covered with water, most is salt water (salinity of \sim 3.5%) and not fit to drink. Only 3% of the water on the Earth is freshwater (water that contains only minimal quantities of dissolved salts) and much of this water is in snow and ice (e.g., glaciers) as well as lakes, streams and groundwater. In some places in the world, freshwater is plentiful (e.g., the Great Lakes); however, in many places water is scarce. Wars have been fought over freshwater resources.

Freshwater generally includes constituents and materials other than water (H_2O) . These may be naturally occurring or introduced by human activities. These may include chemicals, microbial agents, radioactive species, and other agents (e.g., asbestos, suspended materials such as sediments). Where drinking water is supplied through public water supply systems, contaminants may also be introduced during treatment (residual disinfection chemicals) and during delivery from water treatment facilities to consumers (e.g., lead leaching from solder, pipes and faucets). Contaminants in drinking water can pose a health threat, may cause cosmetic effects (e.g., consuming water with high levels of fluoride may result in mottled teeth) or may make water less palatable (e.g., disagreeable odor or taste).

Drinking water standards and criteria define acceptable levels of contaminants. That is, they specify levels of contaminants that are at concentrations that pose acceptable risks of adverse health and cosmetic effects during typical consumer exposures. Governments and regulatory agencies around the world develop drinking water standards and criteria. What is acceptable is generally defined under the applicable laws, rules and guidance. Generally, criteria are established to protect human health. However some criteria, particularly those that are legally enforceable standards, also account for feasibility and weigh costs against benefits. A set of criteria may be useful for one contaminant. For example, health-based criteria may be developed for short-term exposure and for longterm exposure, criteria based on aesthetic effects, and for consideration of feasibility.

Generally, health-based criteria incorporate consideration of all potential effects known to be associated with the contaminant and the criteria established to protect for the most sensitive effect (effect that occurs at the lowest concentration). If a constituent is regulated as a carcinogen, the criteria may be set to protect for an acceptable excess lifetime cancer risk based on an assumed rate of water consumption. Approaches used to develop criteria and standards may not be the same from one governing body to another although most agencies do strive to use the best science and risk assessment approaches consistent with science policy and regulations. The following are examples of agencies that develop drinking water criteria and standards:

- The World Health Organization develops drinking water quality guidelines that can be used in developing and developed countries worldwide.
- Health Canada publishes Guidelines for Canadian Drinking Water Quality.
- The United States Environmental Protection Agency (US EPA) develops drinking water criteria consistent with requirements defined under the Federal Safe Drinking Water Act (SDWA).
- States in the United States may develop or adopt drinking water standards and guidance that is equal to or more restrictive than criteria prepared under the Federal SDWA. For example, California develops Public Health Goals (PHGs).

United States Perspective

Drinking water quality in the United States is primarily regulated under the Federal SDWA, passed in 1974 and amended in 1986 and 1996. The SDWA, among other things, provides USEPA the authority to set drinking water standards. Drinking water standards are part of a system, referred to as a 'multiple barrier' approach, which protects drinking water quality. The SDWA provides for evaluation and regulation of drinking water sources, water collection systems, treatment, and distribution systems. Some states have primary enforcement authority for the SDWA and regulate drinking water quality under state laws, regulations, and administrative rules. If a state administers the SDWA, criteria and standards may not be less restrictive than the federal program.

Drinking water supplied by most public water systems in the United States is regulated under the SDWA. Standards apply to public water systems that provide drinking water to at least 15 service connections or regularly serve at least 25 individuals at least 60 days out of the year. Public water systems may include systems that service schools, businesses, camps, and shopping malls as well as municipal water treatment systems.

Not all drinking water is regulated under SDWA. Private wells are not regulated, but standards and criteria established under the SDWA may be used as guidelines to judge water quality. In addition, drinking water criteria have been developed under a number of remedial programs (e.g., states and US EPA Regional Offices). For example, health-based drinking water criteria are provided for numerous constituents in US EPA Region 9's Preliminary Remediation Goals and US EPA Region 3's Risk-Based Concentrations. Standards and criteria developed under the SDWA fall into the following general categories:

- National Primary Drinking Water Regulations (Primary Standards) are legally enforceable water quality standards that are applied to water regulated under SDWA (public water systems). Primary standards limit the levels of specific constituents in drinking water. Standards are developed for constituents anticipated to be present in drinking water and that can adversely affect the public health. Standards include maximum contaminant levels (MCLs) and treatment technologies. MCLs are generally health/risk based. However, they also take into consideration whether levels can be achieved (feasibility) and include a cost benefit analysis. Some standards are based on the best available treatment technology. Some are expressed as action levels (e.g., lead).
- National Secondary Drinking Water Regulations (secondary standard) are nonenforceable criteria/ guidelines. They are set at levels that protect against aesthetic effects (e.g., taste, odor, or color) and/or cosmetic effects (e.g., teeth and skin discoloration). Secondary standards are not enforceable under the federal SDWA, however, states can adopt them as enforceable standards.

Generally, when criteria are developed, the first step is to collect the available data and information and evaluate the potential for exposure through drinking water to cause harm. Then, criteria and standards are developed as appropriate.

Maximum contaminant level goals (MCLGs) are nonenforceable public health goals. MCLGs are set at levels where there is no known or expected risk of adverse effects. They do not consider detection limits or available treatment technology to reduce levels of constituents and may be set at levels that cannot be achieved in certain public water systems. Once the MCLG has been developed an enforceable standard, usually an MCL, is established.

An MCLG may be based on a reference dose (RfD) to protect for effects that are believed to occur only if exposure levels exceed a particular threshold. The RfD is expressed as a daily dose $(mg kg^{-1} day^{-1})$ that is believed to be protective over a lifetime exposure. Generally this includes most noncancer effects. The standard approach is to multiply the RfD by the assumed adult body weight (70 kg) and then divided by the assumed daily water consumption (2 l). This is called the drinking water equivalent level (DWEL). The DWEL reduced by a percentage (usually 20%) to account for exposure from sources other than drinking water is used to arrive at the MCLG.

For chemicals regulated as carcinogens, the MCLG is generally set as zero. This is based on the assumption that in the absence of data indicating otherwise, any exposure to a carcinogen, no matter how small, is associated with some risk of cancer. If there is sufficient information on the mode of action for a carcinogen that allows for the use of a different approach for developing health protective criteria, the MCLG may not be set at zero. The MCLG is also set at zero for microbial contaminants.

Health Advisories (HAs) are drinking water criteria that provide guidance on levels of constituents that may cause adverse effects in drinking water. HAs are developed to be protective of 1-day exposure, 10-day exposure, and lifetime, which is the DWEL.

USEPA has set \sim 90 standards These include the following:

- Inorganic contaminants such as antimony, asbestos, barium, beryllium, cadmium, chromium, copper, cyanide, mercury, nitrate, nitrite, selenium, thallium, arsenic, fluoride, and lead.
- Volatile organic contaminants such as benzene, carbon tetrachloride, chlorobenzene, *o*-dichlorobenzene, *p*-dichlorobenzene, 1,1-dichloroethylene, *cis*-1,2-dichloroethylene, *trans*-1,2-dichloroe ethylene, dichloromethane, 1,2-dichloroethane 1,2-dichloropropane, ethylbenzene, styrene, tetrachloroethylene, 1,2,4-trichlorobenzene, 1,1,1,trichloroethane, 1,1,2-trichloroethane, trichloroethylene, toluene, vinyl chloride, and xylenes.
- Synthetic organic contaminants including pesticides and herbicides such as the following: 2,4-D, 2,4,5-TP (Silvex), acrylamide, Alachlor, atrazine, benzoapyrene, carbofuran, Chlordane, dalapon, di-2-ethylhexyl adipate, di-2-ethylhexyl phthalate, dibromochloropropane, Dinoseb, dioxin (2,3,7,8-TCDD), Diquat, Endothall, Endrin, epichlorohydrin, ethylene dibromide, glyphosate, Heptachlor, Heptachlor epoxide, hexachlorobenzene,

hexachlorocyclopentadiene, Lindane, Methoxychlor, Oxamyl, (Vydate) polychlorinated biphenyls (PCBs), pentachlorophenol, Picloram, Simazine, and Toxaphene.

- Microbiological agents are commonly found in drinking water sources. Levels of certain bacteria (such as coliform bacteria, Fecal Coliform and *Escherichia coli*) and parasites (such as *Cryptosporidium and Giardia lamblia*) are regulated. In addition, turbidity is regulated as it may provide a good medium for microbial growth, may be an indicator of microbial presence, and may interfere with disinfection agents.
- It is common for water suppliers to use disinfectants such as chlorine, chloramines and chlorine dioxide to kill microorganisms such as giardia and *E coli*. Levels of disinfectants used may be higher after rainstorms in summer months. By-products include: trihalomethanes, haloacetic acids, bromate, and chlorite. Levels of disinfection products and by-products are regulated.
- The levels of radionuclides are regulated. These include certain alpha emitters, beta/photon emitters, combined radium 226/228 and radon gas.

In addition to laws, criteria and systems discussed above, compliance with several standards play an important role in maintaining drinking water quality. These standards provide for development of criteria when none are available from the regulating body. For example, compliance with National Sanitation Foundation International/American National Standards Institute (NSF/ANSI) Standard 61, which addresses the potential for constituents to leach from components of drinking water systems into water moving toward the tap, is required under many state laws and regulations.

NSF International is a not-for-profit, nongovernmental organization that is known world wide for standards development and product certification. NSF is accredited by ANSI, US Occupational Safety and Health Administration (OSHA) and the Standard Council of Canada (SCC). Water program standards important to drinking water quality in the United States include the following:

NSF/ANSI Standard 60: Drinking Water Treatment Chemicals – Health Effects: Standard 60 addresses the potential for adverse health effects to occur because of the use of drinking water treatment chemicals and related impurities. The standard includes a procedure for developing

criteria when none are available under the SDWA. Chemicals of interest include those used to control scale and corrosion, used to adjust pH, to soften water, precipitation and sequestering agents, coagulation and flocculation chemicals, disinfection chemicals oxidation chemicals and drilling products.

• NSF/ANSI Standard 61: Drinking Water System Components – Health Effects: Standard 61 addressing constituents that may be indirectly added to drinking water from the well or intake to the tap/ faucet. These include pipes and related products, mechanical devices, protective materials, joining and sealing materials, process media, and plumbing devices, including faucets. In addition, certain materials that can support microbial growth (e.g., include solvent-based coatings, gaskets, etc.) must be evaluated to demonstrate compliance with this Standard. The Standard includes a procedure for developing criteria when none are available under the SDWA. Standard 61 is incorporated by reference into many state drinking water laws.

Freshwater resources are precious and necessary for life. Work on cost-effective systems that can desalinate salt water for use as drinking water is ongoing and likely to be important to the future development of certain areas of the world.

See also: Exposure Criteria; Gastrointestinal System; Risk Assessment, Ecological; Risk Assessment, Human Health; Safe Drinking Water Act, US.

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Drugs of Abuse

Molly Broderick and Teresa Dodd-Butera

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Certain pharmaceutical drugs and other substances are classified as 'drugs of abuse' because of the tendency for people to use (or overuse) these substances for other than their intended purpose and in some cases become addicted. Because of the adverse health, sociological, and other consequences of using these substances, availability and quantity of many – but not all – of these substances are controlled by regulatory agencies. This article surveys major classes and provides specific examples of drugs of abuse, the main adverse effects, treatments available in overdose situations, and withdrawal symptoms, if applicable.

Stimulants

Controlled stimulants that are frequently abused include amphetamines, methylphenidate, methamphetamine, and cocaine. Amphetamine, methamphetamine, methylphenidate, and cocaine can be smoked, inhaled, ingested, and injected. Methamphetamine's effects can last up to 6 h. Methylphenidate (Ritalin) is a sustained release product and can last up to 12 h. Cocaine's effects last only about 1 h. These drugs have significant potential for abuse and addiction.

Adverse effects include increased risk of seizures, myocardial infarction, rhabdomyolysis, renal failure, and stroke. Other life-threatening adverse effects include hyperthermia, hypertension, vasoconstriction, tachycardia, cardiac ischemia, and paranoia. Prolonged cocaine abuse has been shown to cause cardiomyopathy.

Treatment is aimed at controlling sympathetic stimulation, specifically controlling hypertension, tachycardia, seizures, hyperthermia, and agitation. Medical and psychological withdrawal syndrome starts within 24–48 h after drug discontinuation. Withdrawal symptoms include sleepiness or insomnia, apathy, depression, irritability, and nausea but are not life threatening. A number of medications are available to decrease the craving during withdrawal.

Depressants

This class includes drugs with sedating or amnesic effects. GHB (gamma hydroxybutyric acid), the GHB precursor, 1,4-butanediol (1,4-BD), and Rohypnol or flunitrazepam are sometimes used for sexual assault or for the central nervous system (CNS) effects. Both GHB and Rohypnol have been referred to as date

rape drugs. GHB usage has decreased because the Food and Drug Administration (FDA) has banned the drug's usage and has stopped Internet sales of the drug's precursor sold as a dietary supplement. Rohypnol is not legal in the United States but is smuggled into this country and distributed illegally. These medications can be added to a drink or can be taken in pill form. Lethargy, amnesia, ataxia, and confusion can last up to 8 h. In large doses coma and respiratory depression can occur. Effects are compounded when taken with alcohol.

Rohypnol is a benzodiazepine and has a risk of sedation, ataxia, slurred speech, amnesia, respiratory depression, bradycardia, and hypotension. These effects are compounded if other medications are co-ingested. GHB's risk includes coma, respiratory depression, bradycardia, and seizures. Rohypnol ('roofies') and GHB (liquid ecstasy) are not manufactured or sold legally in the United States. Rohypnol is legally sold in Europe, Latin America, and Mexico and is smuggled into this country and transported illegally throughout the country. GHB is easy to produce in clandestine laboratories. Until 1997 it was readily available on the Internet or in health food stores. The FDA made it illegal to sell or produce GHB in 1997.

Respiratory support with oxygen may be required for respiratory depression associated with Rohypnol ingestion. A benzodiazepine antagonist can reverse respiratory depression and coma caused by overdose but is not routinely recommended because it can precipitate withdrawal symptoms and seizures. There is no antidote to GHB overdose. Ventilator respiratory support, seizure control, and supportive care may be required. Symptoms often resolve within 3–4 h. Abuse of both rohypnol and GHB can cause withdrawal symptoms. Long-term use of Rohypnol can cause seizures, tremors, and anxiety. Long-term abuse of GHB withdrawal can last from days to weeks. GHB withdrawal includes anxiety, tremors, disorientation, hallucinations, and insomnia.

Opioids/Narcotic Drugs

Opioids cause a release of endorphins producing a feeling of pleasure. Examples of abuse include heroin, a highly addictive opioid that metabolizes to morphine and readily passes into the brain producing an immediate euphoria. Pharmaceutical or medicinal abused opioids include oxycontin, hydrocodone, codeine, methadone, and propoxyphene.

Opioid toxidrome effects include miosis, respiratory depression, and CNS depression. More serious effects include hypoxia, hypotension, and coma. Morphine and oxycontin come in extended release forms and can cause prolonged effects. Opioids are available both legally and illicitly. Illegal street drugs can contain adulterants or a highly concentrated preparation. Naloxone is an opioid antagonist and will reverse coma and respiratory depression. Oxygen is also required for respiratory depression.

Opioid Analogs

Dextromethorphan (DM, DTM, skittles) is an opioid analog that is available in hundreds of over-thecounter cough suppressants and cold medications. Abusers describe a feeling of euphoria, disassociation, and visual distortion, CNS depression, and ataxia. It is a popular over-the-counter drug of abuse. This is normally a safe medication when taken as prescribed, but in overdose or intentional misuse adverse effects include distortions in sight and sound and a feeling of being separated from the body. A series of plateaus are described ranging from mild stimulating effects to more serious effects of confusion, lack of coordination, ataxia, and increased heart rate. Respiratory depression, inability to move extremities, and seizures has been reported.

Naloxone, an opioid antagonist, may reverse sedation and the respiratory depressant effects. Oxygen may be required for respiratory depression alone. No specific antidote is available. The treatment mainstay is supportive care.

Inhalants

Young teenagers abuse inhalants because they are easily available in the home and readily available in over 1000 products. They are relatively inexpensive or free and anyone can purchase them regardless of age. Some are also available in most offices and schools as well as in the home. Commonly abused inhalants include gasoline, butane, propane, benzene, toluene, degreasers, cleaning fluids, nail polish removers, whipped cream propellants, glues, and paint thinner. When inhaled they cause a feeling of lightheadedness, tingling, and disorientation. Unfortunately, these solvents can be life threatening and associated with 'sudden sniffing death' resulting in hypoxia, ventricular arrhythmias, and/or cardiac arrest. Inhaling solvents from plastic bags can result in suffocation. Chronic abuse causes brain atrophy, neurological impairment, hepatotoxicity, and nephrotoxicity.

Inhalants cause fast acting intoxicating symptoms because they are inhaled directly into the lungs. The initial symptom is stimulation but with repeated inhalations the symptoms include disinhibition, euphoria, giddiness, dizziness, tingling, stupor, apathy, muscle weakness, and slurred speech. Inhalants can produce a rapid irregular heart rate that can cause heart failure and death within minutes. Death can also occur from suffocation. Abusers inhale fumes from rags or from plastic or paper bags or balloons, or directly from the can. Long-term abuse of inhalants can cause permanent damage to the brain, heart, kidneys, and liver.

There is no specific antidote to inhalant abuse. Treatment may require oxygen and electrocardiogram and blood tests. Detox and drug abuse treatment programs are sometimes not effective and the relapse rate is high. Inhalant abusers build up a tolerance and require increased amounts to achieve the same effects in addition to having cravings for solvents. Detoxification and withdrawal can cause tremors, agitation, irritability, and difficulty in sleeping.

Hallucinogens

Methylenedioxymethamphetamine (MDMA), ecstasy, is a hallucinogenic amphetamine that increases levels of neurotransmitters in the brain. It is most often ingested in pill form but can be inhaled. It is often ingested at 'rave' parties. It is referred to as Adam and XTC. Lysergic acid diethylamide (LSD) (acid) is a mood-altering hallucinogenic agent. It is sold in tablet form, on blotter paper, and sometimes as a liquid. Ecstasy and LSD are illegal but manufactured and sold illicitly.

MDMA causes a feeling of clarity or sharpness, tingling, pleasure, and a feeling of disassociation. It can result in dehydration, seizures, hyperthermia, tachycardia, and renal impairment. LSD is also a hallucinogen but does not have an amphetamine component. The drug causes visual hallucinations. Emotions can be labile and paranoia can occur. Increased body temperature, heart rate, and blood pressure can occur. Some people experience flash-backs.

There are no specific antidotes for ecstasy or LSD. Treatment is supportive and includes fluid replacement, seizure, and temperature control. Keeping patients in a dark quiet room with decreased stimulation may help lessen anxiety. The risk of longterm dependence, addiction, or withdrawal of MDMA is unclear. Growing evidence is that MDMA can affect memory. LSD is not known to be addicting and is not known to cause withdrawal.

Dissociative Drugs

Ketamine (special K, super K, vitamin K) is a shortacting general anesthetic producing what is described as an out of body experience or a feeling of delusion or dissociation. It is odorless and tasteless and can be added to drinks prior to date rape. It is available in liquid, powder, and pill forms. Phencyclidine (PCP, angel dust, rocket fuel, supergrass) can be smoked, snorted, or ingested. The effects are immediate and can last hours. It is not used medically due to severe risks. PCP is abused for the euphoric, hallucinogenic, out-of-body sensations and dissociative anesthetic effects but often causes violence, agitation, paranoia, and severe hypertension. Treatment often requires sedating and restraining the patient before evaluation of toxicity can be assessed. Ketamine is mainly used in veterinary medicine. It causes a dream-like state and/or hallucinations. In excess it causes delirium, amnesia, lack of coordination, impaired consciousness, and respiratory depression. PCP can cause prolonged hypertension in addition to hyperthermia, seizures, paranoia, combativeness, muscle rigidity, and psychosis.

Both Ketamine and PCP can be life threatening. There is no antidote. Treatment is dependent on symptoms and may require hospitalization. PCP is addictive and withdrawal can cause drug-seeking cravings, fatigue, irritability, and depression.

Marijuana

Marijuana contains a psychoactive resin called D9 tetrahydrocannabinol (THC) and cannabinol. Most resin is found in the flowering female plant. When smoked or ingested it produces a feeling of relaxation, euphoria, and happiness. Other possible effects include poor concentration and decreased reaction time. Used medically under the names dronabinol and marinol, it has been prescribed as an antiemetic and as an appetite stimulant and has also been used

in the treatment of glaucoma. Effects last a few hours, are not considered highly toxic, and most often do not require medical treatment.

See also: Amphetamine; Benzodiazepines; Cocaine; Codeine; Dextromethorphan; Heroin; Hydrocodone; LSD (Lysergic Acid Diethylamide); Marijuana; Methadone; Methylenedioxymethamphetamine; Morphine; Phencyclidine; Propoxyphene.

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Dyes

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Introduction

Dyes are used primarily to impart color in textile, leather, paints, cosmetic, and food industries. Many natural dyes (animal, mineral, or vegetal extracts) have been largely replaced by synthetic dyes that were developed at the end of the nineteenth century. Dyes should be safe, with no toxicity, carcinogenicity, or allergenicity. However, the most frequently reported causes of unexpected side effects of garments are textile dyes, and some dyes formerly used for food like Butter Yellow are known to be carcinogenic. It is actually arduous to routinely detect the exact composition of dyes, because the chemicals used are generally not declared in textiles, contrary to the case with cosmetics or foods. Moreover, the manufacturing modifications in developed countries and the banishment of strongly allergenic, mutagenic, or carcinogenic dyes in Europe and in the United States may be counterbalanced by the high numbers of imported clothing generally treated with historically older and cheaper dyes from the Far East or underdeveloped countries.

Classification and Characteristics of Dyes

There are thousands of dyes, marketed under different names (more than 100 for some of them), that it is sometimes difficult to rapidly and accurately recognize a specific dye.

Color Index System

Dyes are indicated in the color index (CI), with two systems. A numeric one, with five numbers, corresponds to the CI number, for example, CI 11110. The second system is a CI name, indicating the chemical category, the color, and an identification number, for example, CI Disperse Red 1 for the previous molecule. However, the CI does not contain all information about dyes and some textile dyes have no CI number.

According to their chemical structures and the CI system, dyes can be classified into 17 groups: nitro dyes, triphenylmethane derivatives, xanthenes, acridine derivatives, quinoline derivatives, azines, anthraquinones, indigoid dyes, phthalocyanines dyes, oxydation bases, insoluble azo dye precursors, and azo dyes (classes XII–XVII). In practice, dyes are classified into different application classes: disperse, acid, basic, direct, vat, fiber-reactive, sulfur, premetallic, solvent dyes, and naphthols.

Purity of Dyes

A final color often results from a subtle mixture of several dyes. Because of this, a priori unexpected dyes can be employed as yellow, red, orange, or red dyes for black or blue garments. For example, Serisol Black L 1944, used to dye black 'velvet' clothes, contains five disperse dyes, namely Blue 124, Blue 106, Red 1, Yellow 3, and Blue 1. Moreover, a commercial 'pure' dye often comprises one or two major components, and frequently other chemicals and/or impurities. Disperse Yellow 3 is generally pure, Disperse Red 153 or Disperse Blue 35 contain two major fractions, and Disperse Red 1 comprises one major compound and at least two other minor substances. These impurities can also be responsible for sensitization. Moreover, there can be some mistake and confusion between dyes with similar names.

Transformation of Dyes

If they are ingested, dyes and particularly those that have an azo group can be metabolized by the intestinal microflora or by the liver enzymes. So, their effects can occur in organs responsible for metabolism or elimination, like the liver and urinary tract. Skin metabolism may also be responsible for the transformation of dyes, for example, those from colored textiles that can leach from the fabric and migrate to the skin. For example Disperse Orange 3 is degraded to *p*-phenylenediamine (PPD) and nitroaniline in the skin (Figure 1). Direct Blue 14 (CI 23850), after azo reduction, converts to the aromatic amine *o*-toluidine and other amines when incubated with cultures of *Staphylococcus aureus*.

The manufacturing processes for textile fabrication are complex and additional procedures such as bleaching can also lead to allergenic products.

Dye Application Classes

Disperse Dyes

Disperse (or plastosoluble) dyes are partially soluble in water and are used to color synthetic fibers like polyester, acrylic and acetate, and sometimes nylon, particularly in stockings. They are not employed for natural fibers. These molecules are the main sensitizers among the dyes. Women seem to be more prone than men to become sensitized but these data are not consistent.

Anthraquinone Dyes These dyes consist of substituted anthraquinones (see Figure 2 for the structure of anthraquinone). They are plastosoluble and used to stain synthetic fibers such as polyester, acetate, or nylon. Among these substances, Disperse Red 11, Disperse Blue 3, and Disperse Blue 35 have been reported as causes of contact dermatitis from dresses, trousers or nylon stockings. Disperse Blue 35 is also a phototoxic compound. Disperse Blue 3 has a structure close to that of Disperse Blue 7, and was positive in several patient tested with a dye series (chemical structures of these dyes are shown in Figures 3–6). With Disperse Orange 76 (an azo dye), Disperse Red 11 was thought to be one of the most common causes of dye allergy in men.

Azo Dyes Azo dyes are characterized by an R1–N=N-R2 chemical structure. They represent the

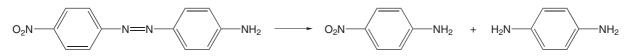


Figure 1 Degradation of Disperse Orange 3 into nitroaniline and *p*-phenylenediamine.

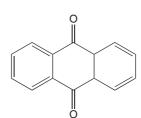


Figure 2 Anthraquinone.

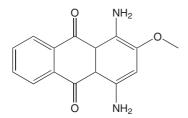
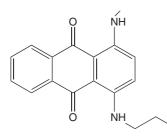


Figure 3 Disperse Red 11.



ЮH

Figure 4 Disperse Blue 3.

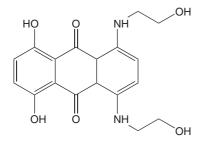


Figure 5 Disperse Blue 7.

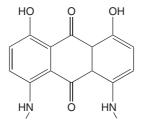


Figure 6 Disperse Blue 35 (major compound).

majority of commercial colorants, enabling a broad spectrum of shades and fastness properties. They are suitable for coloring various substrates, including both synthetic and natural fibers. These molecules are trapped within the fibers in which they are formed during the dyeing process. Azo dyes, disperse type, are used in synthetic fibers. They are the molecules most often implicated in textile dye dermatitis, mainly in nylon stocking, socks, trousers, dresses, and underwear. Disperse Yellow 3, Disperse Orange 3, and Disperse Red 1 were the principal sensitizers in a retrospective 1940–1984 study. Today, Disperse Blue 124 and/or 106, Disperse Orange 3, Red 1, or Yellow 3 are frequently encountered. A recent classification divided them into four chemical subgroups.

The monoazoic compound Disperse Blue 124 (Figure 7) is the most frequently positive dye on patch testing with the textile series, particularly in women. It is probably the main cause of textile contact dermatitis today. It is closely related to another azo dye, Disperse Blue 106 (Figure 8), marketed since 1985, and both are frequently used together. This latter dye seems to have the stronger sensitizing potential and can provoke infiltrated lesions. Concomitant positive reactions to both Disperse Blue 106 and 124 are expected because of their structural similarity, and are very consistent.

Disperse Orange 3 (Figure 9) was cited in reports of stocking dermatitis, and remains a frequent allergen. An average of two-thirds of the patients

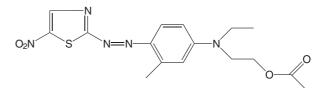


Figure 7 Disperse Blue 124.

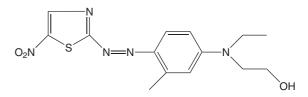


Figure 8 Disperse Blue 106.

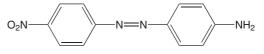


Figure 9 Disperse Orange 3.

sensitized to Disperse Orange 3 are sensitized to PPD, and primary sensitization to Disperse Orange 3 seems to be acquired from PPD contained in hair dyes. *p*-Aminoazobenzene (PAAB, Solvent Yellow 1) and *p*-dimethylaminoazobenzene (PDMAAB or Butter Yellow) are positive in about two-thirds of the patients sensitized to Disperse Orange 3.

Disperse Red 1 (Figure 10) was implicated in dermatitis from stocking, and is frequently observed on patch testing, especially in subjects under 12 years of age.

Disperse Red 17 (Figure 11) gave positive patch test reactions in patients sensitized to other azo dyes, and was cited as a stocking dye.

Disperse Brown 1 (Figure 12) is less frequently positive, as is Disperse Brown 2.

Disperse Orange 76 (Figure 13) is often positive and was thought to be one of the main causes of dye allergy in men, together with Disperse Blue 3 (an anthraquinone dye).

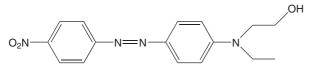


Figure 10 Disperse Red 1.

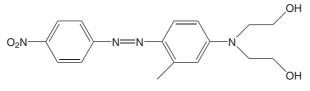


Figure 11 Disperse Red 17.

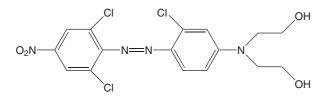


Figure 12 Disperse Brown 1.

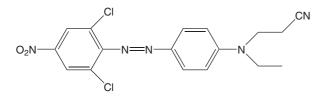


Figure 13 Disperse Orange 76.

Reactions to Disperse Yellow 3 (Figure 14) are frequent. The first cases reported concerned nylon stocking dermatitis, and this azo dye is still currently used to dye such garments.

Disperse Red 153 (Figure 15) is based on two structurally close compounds.

Disperse Black 1 and 2 are rarely positive.

Methine, Nitro, and Quinoline Dyes

Disperse Yellow 39, a no longer available methine dye, was implicated in trouser dermatitis. The nitro dye Disperse Yellow 9 was cited in some reports.

Acid Dyes

These are used to color silk, wool, and other animal fibers, or nylon (polyamide) when high wet-fastness is needed. Such dyes include monoazoic, diazoic, triphenylmethane, and anthraquinone compounds. Acid Yellow 23, Acid Black 48, Acid Black 63, and Acid Violet 17 (triphenylmethane derived; Figure 16) were reported in the literature, mainly before 1985. Acid Yellow 61 (Supramine Yellow GW), Acid Red 359 (Neutrichrome Red SGN), and Acid Red 118 (Supramine Red GW), each tested in 5% petrolatum and removed after 3 days, were positive for skin

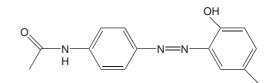


Figure 14 Disperse Yellow 3.

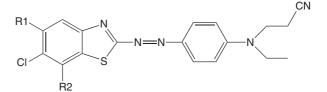


Figure 15 Disperse Red 153. R1 = CI or H and R2 = H or CI, respectively.

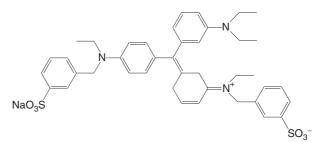


Figure 16 Acid Violet 17.

reactions in 5, 2, and 1 out of 1814 consecutive patients, respectively. The relevance of the patch test results to observed dermatitis was considered possible in four patients.

Basic Dyes

These are mainly used to dye wool and silk, modacrylic, nylon, and polyester. They can be applied to cotton with a mordant (a substance used to set dyes). Basic dyes include monoazoic, diazoic, and azine compounds. Basic Red 46, a monoazoic dye, was implicated in a sweater-induced dermatitis. Basic Brown 1 (Figure 17), Basic Black 1, Brilliant Green, Turquoise Reactive and Neutrichrome Red have also been reported as allergens.

Direct Dyes

These dyes are directly applied on fibers, most often cotton, wool, flax, or leather in a neutral or alkaline bath. They have low wet-fastness, and frequently need after-treatments. Direct Black 38 (Figure 18), a triazoic compound dye used for cotton, wool, and silk, has been implicated in patients wearing black clothes, with concomitant immediate-type reactions in some cases.

Direct Orange 34 (Arancio Diazol Luce 7 JL), an azo dye, was positive during systematic testing in 8 out of 1814 patients.

Vat Dyes

Such water-insoluble dyes are applied in a reduced soluble form and then re-oxidized to the original

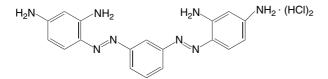


Figure 17 Basic Brown 1 (Bismarck Brown Y).

insoluble form once absorbed into the fiber. They have high wet-fastness and are used to dye cotton, flax, wool, and rayon fibers. They mostly commonly include Vat Blue 6, responsible for cosmetic dermatitis, and Vat Green 1. Vat Blue 1 (Figure 19) is used to dye Levi Strauss 501 'shrink to fit' blue jeans. Vat Green 1, an anthraquinone derivative (Figure 20), has been reported as a cause of clothing contact dermatitis, from navy-blue uniforms in nurses.

Reactive Dyes (Fiber)

Reactive dyes were introduced at the end of the 1950s. These synthetic dyes consist of a two-part, direct coloring agent. The first moiety is a chromophore with an azo, anthraquinone, or phthalocyanine derivative structure. This moiety is connected to a second reactive group, which is able to form covalent bonds with the amine or sulfhydryl groups of proteins in the textile fibers (Figure 21). The main

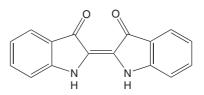


Figure 19 Vat Blue 1 (synthetic indigo).

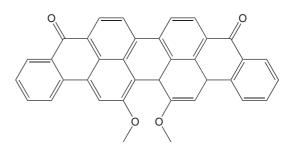


Figure 20 Vat Green 1.

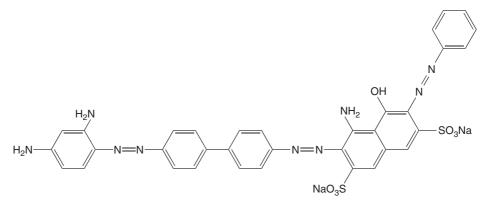


Figure 18 Direct Black 38.

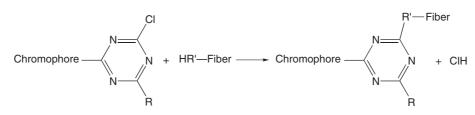


Figure 21 Reaction between textile fibers and the reactive dye.

reactive structures are vinylsulfone, monochlorotriazine, dichlorotriazine, difluoropyrimidine, and dichloroquinoxaline. Such dyes are used for coloring cellulosic fibers (cotton and silk), wool, or polyamides, and are widely used for the production of clothes. They account for more than 10% of the world's production of dyes. Reactive dyes are used in industry in a powdered or granulated form, or as liquids or pastes. They are irritant and sometimes allergenic, and most sources of sensitization are from occupational exposures. Respiratory symptoms in the textile industry have been reported in up to 10% of employees: rhinitis, dyspnoea, and asthma. These dyes can be irritants, inducing nonspecific symptoms. Allergic cases due to a specific IgE production are ascertained by positive prick tests and blood serum IgE levels. Contact dermatitis may occur and may be of irritant or allergic type. In allergic dermatitis, patch tests realized with the dye are positive, proving a delayed type allergic reaction.

In a study of allergic contact dermatitis in consumers, 1813 consecutive patients were tested with an additional textile series of 12 reactive dyes, and 18 patients (0.99%) were found to be sensitized to reactive dyes. However, only five patients had a history of intolerance to garments, and two of the four patch tests performed with pieces of garment were positive. In practice, reactive dyes in clothing should not be sensitizers. If they can be extracted from fibers, they are in a hydrolyzed, nonsensitizing form.

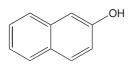
Sulfur, Solvent, and Nondisperse Azoic Dyes

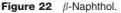
Sulfur dyes are used for cotton in work clothes. Solvent dyes are mono- or diazoic compounds used to dye oils, greases, varnishes, solvents, and cosmetics. Solvent Yellow 1 (PAAB), a monoazoic compound, was positive in patients sensitized to stockings.

Dye-Fixing and Dye-Coupling Agents

 β -Naphthol (2-naphthol, azoic coupling component 1) (Figure 22) is no longer used.

Naphthol AS (3-hydroxy-2-naphthoic acid anilide, azoic coupling component 2) (Figure 23), a coupling agent used for cotton dyeing, has replaced β -naphthol





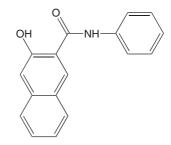


Figure 23 Naphthol AS.

because of a stronger affinity for cellulose. Naphthol AS first caused pigmented contact dermatitis in workers at a textile factory in Mexico in the 1970s, where it was widely used. It has been reported as an agent of pigmented contact dermatitis in several patients, generally due to non-European and non-North American textiles. Its presence in textile can be easily ascertained by thin-layer chromatography.

Side Effects of Dyes

Allergic Contact Dermatitis

Allergic contact dermatitis is a result of a delayed-type immune reaction, due to hapten-specific lymphocytic T clonal expansion. Sensitization to textile dyes in clothing necessitates a transfer of the dye from the garment to the skin, inducing sensitization and/or elicitation of the immune response. However, 'bleeding' of textile dyes, which induces skin discoloration, is a nonallergic phenomenon. Sensitization occurs from the dye itself, from intermediate products during the dying process or after-treatments, or from metabolites arising in the skin. Attributing an allergy to a textile dye is a difficult process and, even if a textile dye is found to be positive on patch testing, the precise identification of the sensitizer in the garment is extremely difficult. Reports of clothing dermatitis are frequently individual, excepting rare epidemics occurring from furs dyed by PPD and derivatives in the 1920s, from dyed nylon stockings in the 1940s, or from black 'velvet' clothing and blouses in the 1980s. Epidemiological studies regarding this topic are most often not controlled, and habitually report a frequency of positive patch tests to textile additives, mainly dyes or finishes. Thus, the prevalence of sensitization to substances potentially implicated in textile dermatitis is $\sim 1-5\%$ of patch-tested patients, but the clinical relevance of such tests is sometimes questionable. For example, a study in 1012 patients indicated that 31 patients (3%) reacted to at least one clothing dye, but that only 10 reactions were relevant. It is difficult to determine its exact incidence for these reasons, but some data suggest that clothing dermatitis is not rare.

Contact dermatitis from clothing has the clinical feature of a typical eczema, though dry rather than vesicular. The lesions can progress and be severe, generalized or even erythrodermic, as long as contact with the allergen is not avoided. Pigmented contact dermatitis arises mainly in patients with a high phototype (IV or V), and has been described from Naphthol AS as well. In some instances, the lesions can be monomorphic and infiltrated, and even simulating cutaneous lymphoma. They may imitate an atopic dermatitis in popliteal areas, demonstrate a persistent erythematous or urticarial-type dermatitis, or even present solely as diffuse itching. Purpuric clothing dermatitis, described during World War II, was due to textile finishes in British soldiers' uniforms. This rare instance occurred with rubber compounds such as isopropyl-phenyl *p*-phenylenediamine (IPPD), and with the azo dye Disperse Blue 85, or another azo Disperse Yellow 27 available as Serisol Fast Yellow GDW. Cockade lesions are rarely described.

The dermatitis generally occurs on the sites of intimate contact with the garment, and the lesions are sometimes symmetrical. Friction or perspiration sites are preferentially involved, and a clinical pattern of textile dermatitis is generally described: neck, major skin folds, and inner thighs. The areas protected by underclothing or the lining of the skirt of the clothing are often free of symptoms. The face can be involved from the handling of the dyes. Some peculiar localizations in accordance with the form of the garment, are reported in **Table 1**. The delay necessary for the diagnosis may be long, and some patients may have difficulties in understanding the role of an invisible although colored substance in their allergy.

Examination of the garment, since the labeling indicates the fiber composition, can guide the practitioner to specific dyes or textile finishes. The practitioner can examine the different parts of the

Table 1 Localization of dermatitis according to garment type

Type of garment	Localization of the lesions
Socks Stockings Blouses Dresses	Feet, legs Lower legs, feet, toes, popliteal fossea Back, chest, axillary borders Back, neck, elbows, axillary borders, forearms, wrists
Jackets Trousers	Dorsum of hands, wrists, forearms Thighs, lower legs, dorsum of hands

fabric and take some of them, of different colors or textures, for patch testing or for further chemical analysis. Main reported allergens are indicated in **Table 2**. It has to be noted that all allergens have not been identified and no published data are available on them.

Mutagenesis and Carcinogenesis

Monoarylamines Monoarylamines, less or more substituted, can have a weak carcinogenic potential. The prototype of these single ring aromatic amines is aniline (Figure 24), which can induce splenic carcinoma by feeding at high doses for a long term. Other monoarylamines (substituted anilines) like *o*-toluidine, *o*-anisidine, and *p*-cresidine are genotoxic upon metabolic activation and induce carcinomas of spleen or urinary balder.

Polycyclic Amines Several polycyclic arylamines have carcinogenic potential. It is, however, important to note that (even slight) molecular modifications can influence solubility, bioavailability, and metabolism, and the mutagenic and carcinogenic potential of molecules.

Naphthylamines The dicyclic arylamine 2-naphthylamine (**Figure 25**) has demonstrated carcinogenicity in several species, including humans. On the other hand, 1-naphthylamine has not revealed carcinogenic potential in experimentation, but the process for its production can generate 2-naphthylamine and other possibly carcinogenic aromatic amines.

Diphenylamines The biphenyl series comprises molecules with two phenyl rings joined by a carbonto-carbon bond and an exocyclic amino group. The prototype is 4-aminobiphenyl (xenylamine) and one of the most known is 4,4'-diamino-diphenyl (benzidine) (**Figure 26**). Case reports and follow-up studies of workers provide evidence that occupational exposure to benzidine is strongly associated with an increased risk of bladder cancer. In animals, when administered in the diet or by intraperitoneal injections, benzidine **Table 2**Main textile dyes reported as allergens

Name of the dye	Color index no.	Application class	Chemical class
Acid Red 118 (Supramine Red GW)		Acid	Azo dye
Acid Red 359 (Neutrichrome Red SGN)		Premetallic	Azo dye (chrome)
Acid Black 48	65005	Acid	Anthraquinone
Acid Yellow 36	13065	Acid	Azo dye
Acid Yellow 61	18968	Acid	Azo dye
Acid Violet 17	42650	Acid	Triphenylmethane
Basic Black 1	50431	Basic	Azine
Basic Brown 1 (Bismarck Brown R)	21000	Basic	Diazoic
Basic Red 46		Basic	Mono azoic
Direct Black 38	30235	Direct	Triazoic
Direct Orange 34	40215	Direct	Azo (stilbene)
Disperse Black 1	11365	Disperse	Azo
Disperse Black 2	11255	Disperse	Azo
Disperse Blue 1	64500	Disperse	Anthraguinone
Disperse Blue 3	61505	Disperse	Anthraquinone
Disperse Blue 7	62500	Disperse	Anthraquinone
Disperse Blue 35	02000	Disperse	Anthraquinone
Disperse Blue 85	11370	Disperse	Azo
Disperse Blue 106	11070	Disperse	Azo related to DB 124
Disperse Blue 124		Disperse	Azo related to DB 106
Disperse Blue 153		Disperse	Azo
Disperse Brown 1	11153	Disperse	Azo
Disperse Orange 1	11080	Disperse	Azo
Disperse Orange 3	11005	Disperse	Azo
Disperse Orange 13	11000	Disperse	Azo
Disperse Orange 76		Disperse	Azo
Disperse Red 1	11110	Disperse	Azo
Disperse Red 11	62015	Disperse	Anthraquinone
Disperse Red 17	11210	Disperse	Azo
Disperse Red 153	11210	Disperse	Azo
Disperse Yellow 1	10345	Disperse	Nitro
Disperse Yellow 3	11855	Disperse	Azo
Disperse Yellow 9	10375	Disperse	Nitro
Disperse Yellow 27	10375	Disperse	Azo
Disperse Yellow 39		Disperse	Methine
Disperse Yellow 49		Disperse	Methine
Disperse Yellow 54		Disperse	Quinoline
Disperse Yellow 64	47023	Disperse	Quinoline
Naphthol AS	37505		Quinointe
•		Coupling agent	Polated to some and diver
<i>p</i> -Aminophenol	76550		Related to some azo dyes
<i>p</i> -Aminoazobenzene (Solvent Yellow 1)	11000		Related to some azo dyes
<i>p</i> -Phenylenediamine	76060	Vot dvo	Related to some azo dyes
Vat Green 1	59825	Vat dye	Anthraquinone

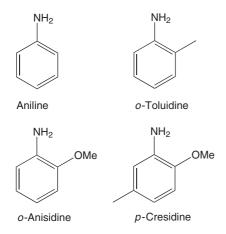


Figure 24 Molecular structures of aniline derivatives.

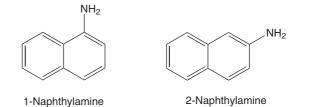
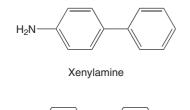
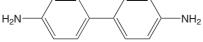


Figure 25 Molecular structures of naphthylamines.

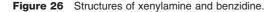
induces urinary bladder carcinomas, mammary carcinoma, and hepatocellular carcinomas.

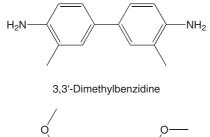
Benzidine derivatives like 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine (o-dianisidine) (Figure 27), are used as dyes or intermediates for dyestuffs or pigments (e.g., Trypan Blue, Acid Red 14, and Direct

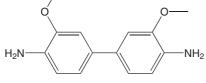




Benzidine







3,3'-Dimethoxybenzidine

Figure 27 Structures of 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine.

Blue 1, 8, 15, 76, 98, 218, and Pigment Orange 16), coatings, plastics, or in chemical studies. They are currently suspect carcinogens. Carcinogenicity studies in animals have showed induction of several neoplasms and carcinomas (intestine, lung, mammary, liver, and skin). No adequate studies have been reported in humans. Dyes metabolized into such amines are also reasonably anticipated to be human carcinogens.

Azo Compounds Azo dyes are widely used in the food, pharmaceutical, cosmetic, textile, and leather industry. They are synthetic compounds characterized by one (monoazo) or several intramolecular N=N bonds. Azo dyes, if they are systemically absorbed, can be metabolized by the way of azoreductases of intestinal microflora by liver cells and skin surface bacteria. This metabolism leads to aromatic amines that can be hazardous. In the 1930s, some azo derivatives like 4-dimethyl aminoazobenzene (Butter Yellow, CI Solvent Yellow 2, CI 11020) and o-aminoazotoluene were experimentally found to be directly carcinogenic to liver and bladder after feeding. Other complex azo dyes like Direct Black 38 or Direct Blue 6 (Figure 28) release the aromatic amine benzidine. Some examples of azo dyes metabolized in benzidine and benzidine-congeners are listed in Table 3.

Anthraquinone Derivatives 2-Aminoanthraquinone (CAS 117-79-3) (Figure 29) is used as an intermediate

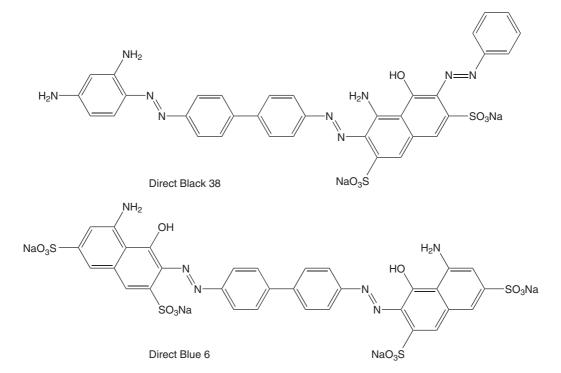


Figure 28 Structure of Direct Black 38 and Direct Blue 6. See the benzidine precursor at the center of the molecules.

 Table 3
 Benzidine and benzidine-congener-based dyes

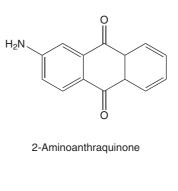
	Color index name	CI number	CAS number
Benzidine- based	Direct Red 28 (Congo)	22120	573-58-0
	Direct Blue 6	22610	2602-46-2
	Direct Brown 95	30145	16071-86-6
	Direct Black 38	30235	1937-37-7
<i>o</i> -Toluidine- based	Direct Red 2	23500	992-59-6
	Direct Blue 14 (Trypan)	23850	72-57-1
<i>o</i> -Dianisidine- based	Direct Blue 8	24140	2429-71-2
	Direct Blue 15	24400	2429-74-5

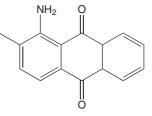
in the industrial synthesis of anthraquinone dyes: Vat Blue 4, 6, 12, and 24, and Pigment Blue 22. It is a carcinogen in animals, inducing hepatocellular carcinomas and lymphomas. 1-Amino-2-methylanthraquinone (CAS 82-28-0) is used as a dye and a dye intermediate, for example, for Solvent Blue 13 and Acid Blue 47. It is a liver and kidney carcinogen in animals. Disperse Blue 1, used for semipermanent hair colorations and for coloring fabrics and plastics, induced urinary bladder carcinomas and sarcomas in rats. They are reasonably anticipated to be human carcinogens.

Other Compounds Magenta and Basic Red 9 (CAS 569-61-9), a common constituent of Magenta, have been used to dye textile fibers, to prepare printing inks, and in biological stains. In workers engaged in the manufacture of Magenta, there was a marked excess of cancer of the urinary bladder. It is possible that the workers were also exposed to *o*-toluidine. CI Basic Red 9 (Figure 30) was, however, an inducer of hepatocellular carcinoma in mice and rats after oral administration, and induced local sarcomas after subcutaneous administration.

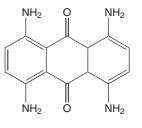
Legislation

Following the introduction in 1994 of the German Consumer Goods Ordinance that restricted the use of certain azo dyes in consumer goods, several other European Union (EU) member states introduced similar but different regulations. In the interests of transparency and the maintenance of the single market, the European Parliament accepted the nineteenth amendment of the Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations, namely azo dyes. Azo dyes, which can release any of a group of defined aromatic amines, are prohibited from being used in those consumer goods that are





1-Amino-2-methylanthraquinone



Disperse Blue 1

Figure 29 Structures of 2-aminoanthraquinone, 1-amino-2-methylanthraquinone, and Disperse Blue 1.

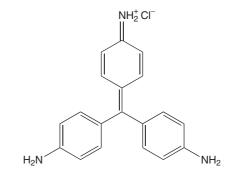


Figure 30 Structure of Basic Red 9 monohydrochloride.

considered to have direct and prolonged skin or mouth contact. Such dyes may not be detectable in textiles and leather that can be in contact with skin or mouth, that is, at a level lower than 30 ppm. The EU Directive was published in September 2002 and, since September 2003, all EU countries are required to prohibit the manufacture and sale of those defined

Table 4 List of atomatic annues followed in the European officin					
CAS number	Index number	EINECS ^a number	Substances		
92-67-1	612-072-00-6	202-177-1	Biphenyl-4-ylamine (4-aminobiphenyl xenylamine)		
92-87-5	612-042-00-2	202-199-1	Benzidine		
95-69-2		202-441-6	4-Chloro-o-toluidine		
91-59-8	612-022-00-3	202-080-4	2-Naphthylamine		
97-56-3	611-006-00-3	202-591-2	o-Aminoazotoluene (4-amino-2', 3-dimethylazobenzene (4-o-tolylazo-o-toluidine)		
99-55-8		202-765-8	5-Nitro-o-toluidine		
106-47-8	612-137-00-9	203-401-0	4-Chloroaniline		
615-05-4		210-406-1	4-Methoxy- <i>m</i> -phenylenediamine		

Table 4 List of aromatic amines forbidden in the European Union

101-77-9 612-051-00-1 202-974-4 4,4'-Methylenedianiline (4,4'-diaminodiphenylmethane) 91-94-1 612-068-00-4 202-109-0 3,3'-Dichlorobenzidine (3,3'-dichlorobiphenyl-4,4'-ylenediamine) 612-036-00-X 3,3'-Dimethoxybenzidine (o-dianisidine) 119-90-4 204-355-4 3,3'-Dimethylbenzidine (4,4'-bi-o-toluidine) 119-93-7 612-041-00-7 204-358-0 838-88-0 612-085-00-7 212-658-8 4,4'-Methylenedi-o-toluidine 120-71-8 204-419-1 6-Methoxy-m-toluidine (p-cresidine) 101-14-4 612-078-00-9 4,4'-Methylene-bis-(2-chloroaniline) (2,2'-dichloro-4,4'-methylene-dianiline) 202-918-9 101-80-4 202-977-0 4,4'-Oxydianiline 139-65-1 205-370-9 4,4'-Thiodianiline 95-53-4 612-091-00-X 202-429-0 o-Toluidine (2-aminotoluene) 612-099-00-3 4-Methyl-m-phenylenediamine 95-80-7 202-453-1 137-17-7 205-282-0 2,4,5-Trimethylaniline 90-04-0 612-035-00-4 o-Anisidine (2-methoxyaniline) 201-963-1 60-09-3 611-008-00-4 200-453-6 4-Amino azobenzene

^a European Inventory of Existing Commercial Substances.

consumer goods, which on chemical analysis are found to contain the listed aromatic amines (Table 4). Since most colored textile and leather articles are treated with azo dyes and pigments, it is important to underline that only few azo dyes are affected ($\sim 4\%$ of known azo dye structures, a total of ~ 300 dyes, and these are mainly direct dyes). Articles colored with other azo dyes can be manufactured and sold without restriction. Additionally, over the last years, all European dye manufacturers have stopped manufacturing such azo dyes and test institutes report that the vast majority of samples tested today comply with the EU Directive.

See also: Aniline; Cosmetics and Personal Care Products; Safety Testing, Clinical Studies; Skin; Toluidine.

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Relevant Website

http://www.etad.com – Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD) Website.

Ε

E. coli (Escherichia coli)

Lee R Shugart

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Description

Escherichia coli is a member of the bacterial family, Enterobacteriaceae, the enteric bacteria. Members of the Enterobacteriaceae are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g., *Salmonella, Shigella, Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g., *Escherichia, Enterobacter, Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans. *E. coli* is a facultative anaerobic, motile, gram-negative rod.

Mechanism of Toxicity

In general, most strains of *E. coli* are avirulent; however, there are strains that cause an impressive variety of different types of diseases, including diarrhea, dysentery, hemolytic uremic syndrome, bladder and kidney infections, septicemia, pneumonia, and meningitis. A strain of *E. coli* associated with a particular disease is due to the fact that the organism has acquired a set of virulence genes.

Before the advent of molecular biology and the identification of virulence factors, surface antigens were a convenient method for 'fingerprinting' *E. coli*. Three surface components formed the basis for the serological classification scheme: O antigens for lipid polysaccharides, H antigens for flagella, and K antigens for capsulated strains. At least 700 serogroups have been identified and are still in use for tracing outbreaks of intestinal disease. There is some correlation between serogroup and virulence.

Nature of Disease

E. coli is responsible for three types of infections in humans: intestinal diseases (gastroenteritis); urinary tract infections (UTI); and neonatal meningitis.

As a pathogen, *E. coli* is best known for its ability to cause intestinal diseases. A classification scheme based on virulence factors (virotyping) is more directly associated with the intestinal disease process than serotyping. The characteristics that form the basis for the virotyping system include patterns of bacterial attachment to host cells, effects of attachment on host cells, production of toxins, and invasiveness. Currently, there are five virotypes: enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EagEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC).

Uropathogenic *E. coli* cause 90% of the urinary tract infections. The bacteria colonize from the feces or perineal region and ascend the urinary tract to the bladder. With the aid of specific adhesins (pyelone-phritis-associated pili) they are able to colonize the bladder. Another factor involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to complement-dependent bactericidal effect of serum. This phenomenon is associated with the presence of a capsule, which decrease the ability of antibodies and/or complement to bind to the bacterial surface, which in turn prevents the phagocytes from recognizing and engulfing the bacterial cells.

Epidemiological studies have shown that pregnancy is associated with increased rates of colonization by K-1 strains of *E. coli* and that these strains become involved in the subsequent cases of meningitis in the newborn. The organism invades the blood stream of infants from the nasopharynx or gastrointestinal tract. Neonatal meningitis requires antibiotic therapy and catastrophic sequelae are rare. The K-1 antigen is considered the major determinant of virulence among strains of *E. coli* that cause neonatal meningitis. The K-1 antigen is a capsular homopolymer of sialic acid that inhibits phagocytosis, complement, and other host's immunological mechanisms.

Control

E. coli colonizes the gastrointestinal track of most warm-blooded animals within hours or a few days after birth. A symbiotic relationship exists under normal conditions, as our enteric flora provide for our source of Vitamin K and B-complex vitamins. Once established, a strain may persist for years, but shifts in resident populations tend to occur over long periods of time, and more rapidly after enteric infection or antimicrobial chemotherapy that perturbs the normal flora. Acquisition of genetic information from other sources (bacterial viruses, plasmids, etc.) may result in the organism becoming a virulent strain. Because of the potential for fecal contamination of water and food, common-sense actions should be taken to minimize risk. *E. coli* food poisoning usually requires hospitalization and most diseases associated with *E. coli* infections respond to antibiotic therapy. See also: Salmonella; Shigella.

Further Reading

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Relevant Website

http://www.ifst.org – Verocytotoxin-Producing *E. coli*; Food Poisoning and its Prevention (from the Institute of Food Science and Technology, UK).

Echinacea

Lee R Shugart

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 SYNONYMS: Black Sampson; Coneflower; Niggerhead; Rudbeckia

Description

Family: Asteraceae

Genus and species: *Echinacea angustifolia*, *E. pallida*, *E. purpurea*

Echinacea, better know as the purple coneflower, is a plant native to the United State and can be found growing as a wildflower in the prairies of the Great Plains states and as far south as Texas. The flowers are a rich purple and the florets are seated round a high cone. It has a faint aromatic smell, with a sweetish taste that leaves a tingling sensation, an indication of isobutylamides.

Chemistry and Pharmacology

Echinacea contain a complex mixture of chemicals, the composition of which varies depending upon the

part of the plant that is examined and may include caffeic acid derivatives, flavonoids in the free and glycoside forms, alkamides, polysaccharides, inulin and fructans, glycoproteins, sugars, and essential oils.

Usage

Echinacea is a medicinal herb that was widely used by the North American Plains Indians and later by colonial settlers before the nineteenth century. Its popularity decreased markedly with the widespread use of antibiotics during 1940–1950, but the renewed interest currently demonstrated by society for herbal medicines has reversed this trend.

Nearly all parts of the plant are used, including the roots, leaves, flowers, and seeds, to prepare liquid extracts (tinctures), tablets, and teas.

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Ecotoxicology

Chris Theodorakis

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Ecotoxicology generally refers to the effects of environmental pollutants at the population,

community, or ecosystem levels, although individual-level effects (toxicity, sublethal effects, toxicokinetics) of pollution may also be included if they are part of a field study (rather than in the laboratory). Field studies can be classified as either manipulative or observational. In manipulative studies, previously unexposed organisms are used, and the experimenter determines the level of contamination to which they are exposed. In contrast, in observational studies the level of contamination to which the organisms are exposed is not under the control of the experimenter. These studies may be part of an independent research project, or may be mandated by a regulatory authority for monitoring organismal health and environmental quality. Manipulative field studies may employ microcosms and mesocosms, enclosures, and field applications of chemicals, while observational studies involve field surveys and collections.

Microcosms are composed of large chambers, terreria, aquaria, or artificial pools; aquatic mesocosms include artificially constructed ponds or streams, while terrestrial mesocosms are large containers filled with soil, plants, and (sometimes) leaf litter. Microcosms and mesocosms typically contain more than one species of test organism, are located outdoors (but may also be located indoors), and often contain sediment and/or vegetation. The rationale is to produce a test system with similarities to the natural environment, but is more controllable. End points examined may include acute toxicity, sublethal effects, or community/population level effects.

The uses and end points measured for enclosure studies are similar to mesocosms, but here a portion of the natural environment is enclosed and manipulated, rather than constructing an artificial system. Manipulations include adding previously unexposed organisms to an enclosure in a contaminated environment, or applying test chemicals to an enclosed portion of a noncontaminated environment. Terrestrial enclosures are usually corrals fenced in by wire or plastic mesh or impermeable barriers such as metal or plastic sheets. They may range from $< 1 \text{ m}^2$ to more than a hectare. Aquatic enclosures may include a section of the shoreline fenced off by plastic curtains (littoral enclosures) or boxes made of flexible plastic sheets suspended in open water (limnocorrals). Small enclosures are used to monitor acute toxicity or sublethal effects, while larger enclosures may study population, community, or ecosystem level end points.

Field applications of chemicals may be used to study effects on populations, communities, or ecosystems, or to study movement of chemicals through the environment. In terrestrial systems, the chemical is applied to various plots of land and samples are taken at various times. In aquatic systems, such applications may include adding chemicals to natural ponds or streams. In some studies, lakes were separated by plastic curtains, and the chemical was applied to one side of the lake only. This allowed determination of effects by comparison of impacted and nonimpacted sides. Because of potential of environmental contamination or lasting impacts, field applications are rarely used, and then only for chemicals that are not persistent and easily degradable.

Finally, biotic surveys and collections involve going to contaminated field sites and collecting organisms or samples of environmental media (air, water, soil, sediment) for analysis. They may be used for determining the level of contamination in organisms or media, and/or for determining the toxic effects on organisms living there. Field surveys may also be used for determining the effects of pollution on populations, communities, and ecosystems.

A population is a group of interacting or potentially interacting individuals of the same species. Population effects may be determined by collection of empirical data or simulated with the use of population models. In the former case, the effects of environmental contamination on population density (number of individuals per unit area), size (total number of individuals), age structure, sex ratios, biomass (total mass of all individuals), population growth (change in size or density over time), sustainability, and probability of extinction are determined. Movement between populations, such as immigration, emigration, and colonization, may also be affected by pollution. Population-level effects of pollution may be determined using laboratory exposures (for small organisms) or manipulative or observational field studies. Alternatively, the effects of pollutants on populations can be predicted or simulated using mathematical models, or by using computers to study the effects of pollution on populations. These models use empirical data such as mortality (toxicity), abundance, age distribution, and age-specific mortality and fecundity in order to predict the effects of pollutant exposure on abundance of individuals and rate of population change (e.g., growth or decline).

These types of alterations could also result in modifications of the genetic structure of aquatic and terrestrial populations. Such modifications are manifested as reductions in genetic diversity within the population or changes in gene or allele frequencies. The mechanisms whereby these changes occur include genetic bottlenecks as a result of reductions in population size or recruitment and selection for pollutant-resistant genotypes. A reduction in genetic diversity may affect population growth, sustainability, and ability to adapt to environmental variables. Furthermore, because changes in the genetic makeup of the population involve alterations in survival and recruitment, such changes may be indicators of adverse chronic effects on population structure and dynamics. They may also be indicators of community-level effects, because it has been found that patterns of genetic diversity and communitylevel pollution effects are correlated in contaminated streams.

Populations exposed to pollutants may undergo genetic adaptation (short-term evolution) and become resistant to the effects of pollution. Adaptation to anthropogenic toxicants was first documented for pesticide resistance in insect and rodent populations, and plants exposed to heavy metals. Subsequently, populations of other organisms such as fish, frogs, invertebrates, and rodents have been found which have genetic adaptations to cope with polluted environments. Such adaptation may affect the patterns of population responses, and needs to be taken into consideration during biomonitoring and ecological risk assessment programs. In addition, there may be costs associated with developing such resistance, and individuals that have the pollutant-resistant genotypes may be more susceptible to natural stressors, or be at a disadvantage in nonpolluted environments.

Any effects on populations may ultimately be manifested as effects on communities because, by definition, communities are collections of interacting populations of several species (e.g., an aquatic community may consist of populations of fish, worms, plants, insects). Individual populations within a community may interact by competing for resources (food, habitat, etc.) or by predator/prey relationships. Environmental contaminants can affect the structure of communities as well as the interactions of species within them. For example, it is well known that exposure to chemicals may cause a reduction in community diversity (e.g., relative number of species), and changes in community composition. In addition, the trophic structure of fish and invertebrate communities may also be affected by exposure to anthropogenic chemicals. Changes in community structure and diversity may be determined by field sampling or manipulative studies. Alternatively, computer simulations using food web or linked population models may be used to assess community-level effects.

The trophic structure of communities is related to the relative abundance of species that feed on various food items (piscivores, omnivores, detritivores, insectivores, etc.), or have various foraging methods (shredders, scrapers, etc.). These changes in species/ trophic composition may come about by direct or indirect mechanisms. The direct effects involve loss of some species due to an increase in pollution-induced mortality or reduced reproductive output. In this case the communities will be dominated by species that are less affected by pollutant exposure. This is the basis of a phenomenon termed 'pollution-induced community tolerance' (PICT), in which algal communities become more pollution tolerant over time due to the replacement of pollution-sensitive species with more tolerant ones.

On the other hand, community structure may change through indirect mechanisms. Indirect effects are those that are not due to toxic effects per se. For example, an insecticide may not be toxic to birds, but the birds may disappear because the insecticide kills off the insects on which it feeds. Conversely, the body size and population density of a species of minnow may increase in contaminated sites due to toxic effects on competing species (more food available for the minnow) or on predators such as bass. It has also been suggested that such changes in community structure come about because some species are more genetically plastic than others, and so are better able to adapt to novel stressors such as pollution. Thus, the more sensitive species would not be able to adapt to this stressor and become extinct locally. These types of perturbations in community structure and dynamics may ultimately compromise the stability, sustainability, and productivity of affected ecosystems.

An ecosystem is a collection of interacting populations and communities, plus the abiotic environment (e.g., climate, environmental chemistry, soil type, geology, hydrology). Ecosytem studies focus on end points such as biomass (the total mass of all organisms in an ecosystem), trophic structure, energy flow and carbon cycling (e.g., from plants to herbivores to carnivores), productivity (the amount of biomass in each trophic level produced over time), and cycling of oxygen, nitrogen, or nutrients (e.g., phosphorus) through the ecosystem. Manipulative studies use microcosms or mesocosms to study net ecosystem photosynthesis, respiration, or productivity. Field studies attempt to quantify these effects in natural ecosystems, which may be difficult. The most studied effects of pollutants on ecosystems include (1) effects of acid rain on nutrient cycling and productivity, (2) effects of elevated greenhouse gases or decreased atmospheric ozone on ecosystem structure and function, and (3) hypoxia and anoxia (little or no oxygen in the water) as a result of algal blooms or microbial decomposition due to inputs of fertilizers, sewage or animal wastes, and biodegradable chemicals into aquatic ecosystems. Complex ecosystem computer models also exist, which link biotic populations with climate and other abiotic components of the ecosystem.

Results from individual-level effects in field studies and surveys of population, community, and ecosystem-level effects of pollution are often complicated by confounding effects. Confounding factors are, for example, temperature, season, soil chemistry that may affect or obscure responses to toxic agents. Moreover, any two sites differ naturally in terms of chemistry, habitat, etc. Thus, when comparing contaminated and noncontaminated sites, it may be difficult to separate natural variation from anthropogenic effects.

At present, one of the 'hot topics' that illustrates the problem of separating anthropogenic effects from natural variation is the status of amphibian populations. Worldwide, amphibian populations are declining, and in many locations, deformed amphibians are being found. Such deformities include missing or extra limbs or digits and facial malformations. Possible causes of these phenomena include air and wapollution, agricultural chemicals, habitat ter destruction, introduction of nonnative predators, diseases caused by viruses and parasites, and increased exposure or susceptibility to solar ultraviolet light. Many industrial and agricultural chemicals have also been found that are lethal, inhibit metamorphosis, interfere with embryonic development, or alter mating behavior of amphibians at very low levels. Although similar patterns have been found in many 'hot spots' around the world, there is probably no single cause, and multiple factors may be at work in each location. Unraveling this mystery and linking cause and effect remains a major challenge.

The ultimate application of ecotoxicology is in the use of ecological risk assessments. Ecological risk assessments can be defined as determining the probability that an adverse effect will occur, and the magnitude of such an effect, on natural ecosystems as a result of pollution or other anthropogenic (man-made) activities. As set out by guidelines by regulatory agencies such as the US Environmental Protection Agency, ecological risk assessments consist of the following components: (1) hazard definition - in which it is decided what the adverse effects might be and what chemicals might cause them; (2) exposure assessment - which uses chemical characteristics of each contaminant, fate and transport computer simulation models, and/or measured levels of contaminants in environmental media to estimate the magnitude and contamination; (3) effects assessment - which determines predicted or actual effects on living organisms in contaminated environments; and (4) risk characterization - which calculates the probability of an effect of a particular severity and duration, given the estimated magnitude of environmental contamination.

Ecological risk assessments can either be predictive or retrospective. Predictive risk assessments are used to predict ecological consequences for the release of a chemical before it enters the environment. This is done through the integration of toxicity tests, computer simulation models of environmental fate and transport, and computer simulation models of population, community, and ecosystem level contaminant effects. Retrospective risk assessments assess the magnitude of effects, and future consequences of contamination or cleanup, for sites that are already contaminated. Because of the difficulty in separating anthropogenic effects from natural variation between sites due to indirect effects and confounding factors, a 'weight of evidence' approach, using multiple lines of evidence, needs to be used in retrospective assessments. Because this is derived from methods used in epidemiology to demonstrate pathogens as the cause of disease, this approach is termed 'ecoepidemiology'. Central to this theme is demonstrating that bioindicators of exposure coincide with bioindicators of effect. Bioindicators of exposure include chemical contaminants in biological tissues and biomarkers of exposure. Bioindicators of effect include biomarkers of effects, gross injury (tumors, lesions, deformities, and disease), overt mortality (e.g., fish or bird kills), population declines or extinction, and changes in community structure or ecosystem function.

A similar application of ecotoxicological data is hazard assessment. Unlike risk assessment, hazard assessment is nonprobabilistic and relies upon indices rather than probabilities. One such index is the 'hazard quotient', which is the ratio of the expected environmental concentration (based upon field surveys or simulation models) divided by a 'benchmark' concentration. The benchmark concentration is derived from some measure of toxicity such as the LC₅₀ or no-observed-effect level. Hazard assessments are often conducted at different levels or 'tiers' of increasing complexity and specificity: if a chemical is identified as potentially hazardous by tier (the least complex and specific test), a decision is made to take action or, if more information is needed, to proceed to tier 2 tests. After tier 2 tests, a decision is made whether to take action or proceed to tier 3 tests, and so on. This process is repeated until it is decided that there is enough information to determine whether or not there is significant ecological hazard. If there is, then regulatory action is taken.

See also: Biomarkers, Environmental; Biomonitoring; Chemicals of Environmental Concern; Ecotoxicology, Aquatic; Ecotoxicology, Avian; Ecotoxicology, Genetic; Ecotoxicology, Invertebrate; Ecotoxicology, Terrestrial; Ecotoxicology, Wildlife; Environmental Processes; Environmental Toxicology.

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Ecotoxicology, Aquatic

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Ecotoxicology, the science of contaminant fate and effects in the biosphere, emerged as a distinct discipline in the 1960s. Initial ecotoxicological concepts and methods were adopted from classic toxicology, ecology, and geochemistry. Ecotoxicology is now an interdisciplinary science encompassing effects from biomolecules to the entire biosphere, and contaminant fates from chemical speciation to global cycling.

Aquatic ecotoxicology has always been a central component of ecotoxicology because many of the first pollution issues involved the hydrosphere. Early applications of aquatic ecotoxicology included identifying and quantifying point source toxicity in support of water quality regulation. Standard toxicity testing protocols were generated based primarily on effects to individuals but also included some descriptive metrics of ecological community structure. Research in this important field contributed insights needed to formulate key US laws such as the Clean Water Act (CWA) and the Toxic Substances Control Act (TSCA).

In the early 1990s, the activities of ecotoxicologists expanded to support the ecological risk assessment paradigm. Aquatic ecotoxicologists established the conceptual underpinnings for hazard identification and risk characterization, and the data for characterizing exposure and ecological effects. Such knowledge now supports regulatory activities related to federal acts such as the Marine Protection Research and Sanctuaries Act (MPRSA), the Comprehensive Environmental Response, Compensation, Liability Act (CERCLA), and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Currently, much aquatic ecotoxicological prediction is based on laboratory testing in which organisms are exposed to a contaminant for a specified time and effects are estimated for either lethal or sublethal impacts to individual organisms. Regression models are commonly applied to acute exposure data to predict effect metrics such as the LC_{50} (concentration killing 50% of exposed individuals by the end of the exposure) or EC_{50} (concentration having an effect on 50% of exposed individuals by the end of the exposure). Metrics derived from analysis of variance (ANOVA) and post-ANOVA tests are applied for more chronic exposures or subtle effects that are more difficult to model. The lowest exposure concentration with a statistically significant effect (lowest observed effect concentration or LOEC) and the highest exposure concentration with no statistically significant effect (no observed effect concentration or NOEC) are the most common such effect metrics. Notionally, the LOEC and NOEC bound an effect threshold concentration for the tested contaminant.

Laboratory experiments quantifying contaminant effects commonly involve either exposure via water or sediments; exposure via food is addressed less frequently. The most common laboratory tests expose individual organisms directly to contaminant present at a range of concentrations in water. In experiments assessing effects of sediment-associated contaminants, benthic organisms are exposed directly to sediments or nonbenthic species are exposed to sediment elutriate. Elutriate tests are designed to provide data on exposure that occurs during sediment disturbances such as that resulting from storm or dredging activities. Other types of experiments include bioaccumulation or bioavailability tests that determine the potential for contaminant accumulation in organisms. In the absence of knowledge of the effects or bioaccumulation potential for a specific chemical, the magnitude of effect or bioaccumulation is sometimes predicted with quantitative structure-activity relationship (OSAR) models that relate contaminant molecular qualities for a class of contaminants such as polychlorinated biphenyls to their bioactivity (i.e., effect or potential for bioaccumulation).

Augmenting results of laboratory experiments, mesocosms, and field studies are commonly used to study the structure and function of impacted aquatic communities. Mesocosms are experimental ponds or streams designed to simulate, in a simplified manner, aquatic ecosystems. Relative to laboratory systems, mesocosms are normally located outdoors and less controlled, but can achieve more ecological realism. Field studies include surveys and natural system manipulations. The former provide relatively inexpensive observation of the consequences of contamination to communities and, although affording the least controlled observation, are the most often used type of study. Used less frequently because of increased costs, the latter involve manipulation of an entire ecosystem such as a lake or a portion of it. Intelligent combining of conclusions from laboratory tests, mesocosms, and

field studies allows aquatic ecotoxicologists to assess hazard or risk due to contamination.

Current trends in the aquatic ecotoxicology literature suggest several possible changes in the near future. More insights about population consequences will be gained during assessments through the application of emerging molecular techniques, and of population-based metrics and methods. Conventional, laboratory-generated effect metrics such as the LC_{50} , NOEC, and LOEC will be used with more balance with metrics of community and population effects. This will be required to meet the demands of modern ecological risk assessment. To more directly address the needs of ecological risk assessment, more emphasis will also be placed on methods of quantifying the uncertainty in effect estimates.

See also: Ecotoxicology; Environmental Toxicology; Pollution, Water; Risk Assessment, Ecological.

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Ecotoxicology, Avian

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Ecotoxicology is the study of the harmful effects of chemicals on ecosystems. Avian ecotoxicology is the subset that concerns itself with the bird component of these ecosystems. Birds have long been considered an important and valued component of the natural landscape because of their visibility, broad geographical distribution, and the wide variety of ecological niches they occupy. Birds feature prominently in terrestrial ecotoxicology. The harmful effects of chemicals on birds are varied in nature. They include effects on general health, survival, and reproductive potential, generally acting directly on individuals through cellular or physiological mechanisms, or indirectly through a related component of the ecosystem, for example, affecting nesting cover or food sources. Either way, the concern is that these effects can result in repeated losses of individuals creating a population sink. If severe or widespread enough, these sinks can lead to species declines or extirpation, which, in turn, will affect other parts of the ecosystem or will reduce an ecosystem's intrinsic aesthetic, economic, or spiritual value to human beings. As with all other branches of ecotoxicology, avian ecotoxicology is an interdisciplinary endeavor that draws on such fields as analytical chemistry, toxicology, behavior, physiology, population, as well as landscape ecology.

Early Beginnings – Concerns about a 'Silent Spring'

Some of the earliest efforts at large-scale pest control with arsenicals resulted in observed bird mortality. The plight of birds entered public consciousness following the 1962 publication of *Silent Spring*. The author, Rachel Carson, foretold of a world where "...On the mornings that had once throbbed with the dawn chorus of robins, catbirds, doves, jays, wrens, and scores of other bird voices there was now no sound; only silence lay over the fields and woods and marsh..." unless the situation was redressed and the then profligate and indiscriminate use of pesticides was placed under some form of control. The spraying of ornamental trees, woodlots and forests, especially, exposed large numbers of birds to these new chemical poisons.

The single most important event that put birds in the forefront of the new science of ecotoxicology is undoubtedly the discovery that the insecticide DDT (or more precisely DDE, a breakdown product of the pesticide) caused eggshell thinning in birds. Thin eggshells are more prone to cracking, resulting in higher levels of embryo mortality, in part as a result of pathogen entry and also from excessive water loss and desiccation. Following frequent observations of broken eggs in the nests of British peregrine falcons, time series of eggshell thickness indices suggested that the problem had begun shortly after the introduction of DDT. At higher levels of DDT contamination, some eggs simply crushed under the weight of incubating birds. Correlations between eggshell measurements and residue levels, although not always perfect, added to the evidence and captive reproduction studies removed any doubts that the effect was very real.

Because of the gradual accumulation of residues as one moved to higher levels of trophic food webs, the more serious impacts of DDT were not on songbirds, as feared by Rachel Carson, but on species at the apex of either terrestrial or aquatic food webs such as birds of prey, and fish-eating species such as ospreys, pelicans, and cormorants.

Of course, reduced eggshell thickness can be the result of lower food (and hence calcium) intake in birds and this fact has undoubtedly led to some confusion in establishing clear correlations between eggshell thickness in individual eggs and their DDE content. A vast number of pesticides and environmental contaminants can cause a rapid but transient (i.e., generally less than 5 days) decline in eggshell thickness in the laboratory. These declines are

typically associated with a decrease in food intake and, indeed, a similar pattern of thinning can be elicited with a fast. Eggshell thinning has also been seen in response to a decrease in available calcium as can occur in areas impacted by acid precipitations. However, the type of thinning that is elicited by DDE in sensitive species shows a very different pattern altogether, being much more difficult to reverse in exposed birds. DDE-induced thinning is a result of a specific action of the pesticide on calcium transport and deposition in the avian shell gland.

Researchers were also able to establish the link between declines of other predatory species such as the European sparrowhawk and the use of organochlorine pesticides other than DDT. For instance, the cyclodiene insecticides aldrin, dieldrin, and heptachlor used as seed treatments caused massive mortality of both seed-eating species and their predators. All of the insecticides had the following points in common: they were highly soluble in fats and refractory to metabolism. The impacts on the predatory species typically take place in periods of food stress when fat soluble residues are released from fat stores and returned into general circulation. In a food-stressed individual, the brain remains as the most lipid rich tissue and this is where contaminants move to. Toxicity results when threshold values in brain tissue are exceeded. At sublethal levels, documented effects of cyclodiene insecticides in birds have included changes in their reproductive, social, and avoidance behaviors.

Silent Spring also raised concerns about the very high toxicity of another group of insecticides to birds. These were the organophosphorus insecticides, neurotoxic compounds that were offshoots of nerve gas research during the Second World War. Both organophosphorus compounds and another insecticide group, the methyl carbamate insecticides, work by inhibiting acetylcholinesterase, an enzyme that is vital to nerve transmission and the proper functioning of neuromuscular junctions. Unfortunately, this is a nonspecific mechanism of toxicity because acetylcholinesterase is important to both invertebrate and vertebrate life forms; also, birds are typically much more sensitive than mammals to these two chemical groups. The introduction of some organophosphorus insecticides to replace DDT and the use of phosphamidon in forestry operations, for example, resulted in massive songbird mortality in North American forests.

Pesticide Use Since Silent Spring

DDT and other organochlorine insecticides were eventually banned in the United States and in many

other countries, in part because of the evidence accumulated on birds, notably declining populations of some species. Despite these bans, birds continue to be exposed to residues of these pesticides in areas of heavy past use such as orchards and even residential lawns. Exposure results from the consumption of earthworms primarily. Deep-burrowing worm species are bringing residues back to the surface and reintroducing them into the food chain.

By the late 1960s, crude screening of pesticides for their impact on birds had begun in a number of countries. Systematic review of pesticide applications for their risk to birds began in 1972 in the United States although uniform test guidelines took another 10 years to develop. These guidelines are virtually unchanged today. They consist of a test of acute toxicity in adult birds, one of dietary toxicity in chicks, and a truncated reproductive test where eggs are collected from dosed birds and artificially incubated. Throughout the 1980s and early 1990s, a number of field trials conducted on the most toxic pesticides registered in the United States were carried out but this practice has been greatly scaled back now in favor of a new approach that emphasizes early attempts at risk mitigation, more sophisticated modeling of the laboratory tests, as well as the consideration of incident data. It is fair to say that, despite a few examples to the contrary, the consideration of birds in the official risk assessment process has not had a huge impact on registration decisions, at least in the United States. Insecticides well known for their ability to kill birds have remained on the market for decades. Several are still in general use today. Indeed, the best available scientific evidence suggests that bird mortality is frequent and largely unavoidable in our farm fields and this would be true as long as insecticides of high acute toxicity continue to be used. The Federal Insecticide Fungicide and Rodenticide Act (FIFRA), which provides the regulatory framework for pesticide use in the United States, uses a risk versus benefit approach to evaluate chemical effects, thus leaving the door wide open for bird mortality to be judged acceptable in light of the perceived economic benefits.

Also, the rapid modernization and intensification of agricultural production in all parts of the world has meant that concerns about the impact of specific pesticides on birds extend beyond national borders. The same toxic pesticide can affect a bird species on its North American breeding grounds as well as on its Latin American wintering and migratory staging grounds.

The cholinergic system targeted by both organophosphorus and carbamate insecticides is ubiquitous in animals. It is therefore not surprising that exposure to these pesticide classes has been linked to a broad range of physiological and behavioral responses in birds. The most relevant adverse effects are undoubtedly those that can result in lowered reproduction and survival. This includes an increased susceptibility to predation, a decreased tolerance to cold, reduced feeding abilities, and disrupted reproductive behaviors such as parental care.

Concerns are also on the increase with another class of pesticides: the coumarin anticoagulants. An increasing number of birds of prey are being found to have been secondarily poisoned by the newer 'single feed' anticoagulant pesticides. Also, a very high proportion of asymptomatic birds appear to be carrying liver residues of these compounds, which is cause for concern.

Finally, many concerns with pesticides currently have to do with the indirect effects of pesticides on birds. The most notable indirect effect is the loss of invertebrate food sources as a result of insecticide or herbicide use. Herbicides can affect the density of phytophagus insects by reducing the food and shelter provided by plants. The link between insect populations and avian breeding success has been documented in the United Kingdom primarily following long-term studies on the grey partridge and a few other farmland species. Very little of this type of work has been carried out in North America.

Birds as Sentinels of Environmental Quality

Although concerns about bird mortality and/or local population declines associated with a specific chemical arise from time to time, most of the past work in avian ecotoxicology has been generated, not with a concern for avian populations but rather with the view that birds could serve as sentinels of environmental quality. The rehabilitation of some species – for example, the bald eagle in the Great Lakes ecosystem – has even been enshrined in policy. Birds are also used in contaminated site remediation programs to indicate biological damage or remediation success. A few well-studied examples of anthropogenic contaminants and their impact on birds are reviewed below.

Persistent Organic Pollutants (POPs)

DDT and the cyclodiene pesticides are only a few components of a large 'soup' of persistent environmental contaminants consisting of halogenated organics – that is, molecules with a carbon skeleton (usually aromatic) deriving some or all of their toxicological activity through the insertion of chlorine, bromine, or fluorine atoms. Examples are polychlorinated biphenyls (PCBs), dioxins, furans, chlorinated benzenes and terpenes, polybrominated diphenyl ethers (PBDEs), perfluorooctane sulfonates (PFOS), etc. They originate from a combination of agricultural, manufacturing, industrial, and combustion sources. They vary in the extent to which they are easily metabolized and cleared and the extent to which they are fat soluble and accumulate in biota and birds in particular. Because birds often accumulate residues to very high levels making chemical detection easier, sentinel bird species have been used to monitor levels of POPs and assess the efficacy of controls placed on some of the contaminants. Eggs are most often used for establishing contaminant trends. The main issue confronting avian ecotoxicologists working in areas with high levels of POPs (e.g., the North American Great Lakes) has been the attribution of specific biological effects such as elevated rates of malformations or higher levels of chick mortality to specific components of the contaminant 'soup'. Because many of the POPs result in the induction of the same detoxifying enzyme, cytochrome P4501A; the potential of contaminant mixtures is often expressed as 'toxic equivalents'. Enzyme titers in the liver are commonly measured indirectly, by measuring catalytic activity either with the aryl hydrocarbon hydroxylase assay or the 7-ethoxyresorufin O-deethylase or similar assay. These are more convenient and less costly than the chemical analysis of the hundreds of congeners of PCBs, dioxins, and furans. However, this technique is not a panacea and actually has been shown not to work very well in at least one key sentinel species, the Herring Gull. There are data, from both field and laboratory, on the possible pervasive effects of some POPs, principally dioxins and some of the conformationally-similar PCBs (coplanar PCBs), on stress and immune responses, hormone levels, vitamin A storage levels, as well as porphyrin synthesis but the significance of these findings to individual well-being and survival is debated.

Hydrocarbons

Birds are exposed to hydrocarbons through a variety of routes. Oil spills, not only the large spills of crude that make the front pages of the newspapers, but the countless small spills that result from leaks, bilge cleaning, and other spills of various types of fuel oils affect birds through fouling of plumage, external coating of eggs (which results in elevated embryonic mortality), ingestion, usually during preening, or habitat degradation. Hydrocarbon ingestion is often lethal but can also have a wide number of sublethal manifestations on behavior, reproductive success, immune function, and other measures of individual health. For example, crude oil ingestion in breeding ducks suppresses follicular development, alters gonadal hormone levels, and reduces eggshell thickness as well as hatchability. The small amount of oil needed to produce these effects suggests that the impact on hatching is not from oil passing into the egg although it is unclear whether eggshell thinning alone can explain the reduced hatching success. Polycyclic aromatic hydrocarbons tend to represent the most toxic fraction of oils and fuels. Because this fraction is more volatile, hydrocarbons tend to be most toxic immediately following release. Large spills of hydrocarbons can have transient effects on local seabird populations, especially those species that try to escape by diving rather than flying away from the slick.

Lead

By far the most important source of lead contamination in birds is lead shot. This problem is especially acute in waterfowl that ingests aquatic sediments in areas of heavy shooting and in their consumers (e.g., eagles). Imbedded shot and bullet fragments also represent a source of exposure for predators and scavengers. Lead poisoning from shot affects waterbird species worldwide. Fortunately, a solution is readily available in the form of a nontoxic shot even though many jurisdictions have been very slow in implementing this change. Lead sinkers also represent a problem for loon (diver) species as well as for swans in areas where recreational fishing is practiced. Again, changing fishing weights from lead to another metal such as steel is not difficult or costly when compared to other environmental remediation measures. Lead bullets will prove more difficult to replace and there still are no readily available replacements. Lead bullet fragments in carrion are proving a major problem for the introduction of the critically endangered California condor into its former range in the western US. Other sources of lead in birds have included paints, automobile exhaust, and industrial emissions or waste. Songbirds nesting downwind from metal smelters have experienced eggshell quality problems and poor hatching success, in large part from the lead contamination. Lead is neurotoxic at very low doses and is particularly effective at disrupting neural development in developing birds.

Mercury

Mercury in its organic form also biomagnifies in food webs. The use of mercury for gold extraction has been responsible for widespread contamination of aquatic ecosystems and the practice continues unabated in many parts of the world. Other sources,

historic and present, include industrial processes, pulp bleaching, as well as combustion and release of natural mercury from flooded sediments, especially under acidic water conditions. There is good evidence that the acidification of poorly buffered lakes receiving acidic precipitation from combustion sources results in higher levels of mercury being biologically available to aquatic biota and thence to fish-eating bird species. Historically, mercury used in pesticide seed treatments caused severe mortality in seed-eating bird species and their predators. Longterm monitoring programs in seabirds suggest that mercury contamination is increasing globally. At sublethal doses, mercury has been found to affect reproduction although there appears to be wide interspecific variation in the levels that prove embryotoxic and teratogenic. Mercury is also a neurotoxicant and, even in small doses, can modify normal behaviours in bird chicks. This has been established in the laboratory through the usual tests such as visual cliffs and open fields but, at least one intriguing example has been documented in the wild: young loons on high mercury lakes spend less time on the backs of their parents.

Selenium

Selenium is an essential element but it is very embryotoxic at high doses. The classic selenium study is the work performed at the Kesterson Wildlife Refuge, an area which received drainwater that had percolated through selenium-rich soils in surrounding agricultural areas. Eggs and embryos are particularly susceptible to maternally acquired selenium. Effects include poor hatching success and poor survival of hatched young. The most dramatic effect, however, consists of very visible malformations in embryos and chicks including small or missing eyes, absence of legs or toes, incomplete beak development, development of the brain outside of the skull case, and fissures into the abdominal cavity. These were seen in wild wader species and replicated in the laboratory. The case of selenium is particularly interesting because, although it passes into the egg, it is not bioaccumulative. Selenium also offers an interesting observation on interspecies sensitivity differences. There was an \sim 10-fold difference in the threshold for embryotoxicity in two closely related species: the black-winged stilt and American avocet. Thresholds for major malformations were not as divergent: calculated EC_{10} values differed by approximately fourfold.

Endocrine Disruptors

Since the publication of the book *Our Stolen Future* by Theo Colborn and co-authors in 1996 and the

publicity that surrounded its publication, there has been renewed interest in understanding the endocrine basis of pesticide- and pollutant-induced effects on reproduction although such interactions have long been established in bird populations exposed to contaminants and pesticides. A number of pesticides and contaminants, several of which have been mentioned in earlier sections, can affect the avian endocrine system at some concentration. The field evidence to date for endocrine effects in birds is exclusively from persistent and bioaccumulative contaminants, typically in the predatory top of the food chain species. The key question that remains to be answered is whether long-term population stability is compromised by such effects. Also, we need to find out whether exposure to modern agricultural chemicals - that is, typically not persistent and not bioaccumulative - is such that some of their demonstrated endocrine modulation potential is likely to be expressed in the wild.

Tools of the Trade

Avian ecotoxicology brings to bear a number of tools to investigate effects from the molecular to the population levels. At the molecular level, current advances are similar to those being made in mammalian toxicology, namely toxicogenomics and the use of such tools as gene amplification and differential display of coding regions of the genome in response to a toxicant as well as microarrays - often those that have been developed for probing the mammalian genome. A wide array of biochemical markers and bioassays have been developed in birds to measure immune function, stress response, and general organismal health and function as well as more targeted disruptions such as cholinesterase inhibition. At the other end of the spectrum are the standard time-honored methods used by avian ecologists worldwide: behavioral assessments, censusing, markrecapture and telemetry, monitoring of reproductive success, and the placement of these measurements in a population and landscape context. More than 40 years after the publication of Silent Spring the field of avian ecotoxicology is vibrant and growing in several directions. We can undoubtedly rejoice at the fact that impacts of chemicals on birds are being studied more intensively now than when Rachel Carson wrote her seminal work, and that the field of avian ecotoxicology is attracting innovative researchers from a multitude of disciplines. We might, however, lament the fact that many of our experimental subjects are in trouble with many more bird species declining than either increasing or holding their own. We might also ask why the regulatory, political, and

social responses to avian toxicology research findings often lag so far behind our understanding of the problems.

See also: Carbamate Pesticides; DDT (Dichlorodiphenyltrichloroethane); Endocrine System; Federal Insecticide, Fungicide, and Rodenticide Act, US; Lead; Pesticides; Polybrominated Diphenyl Ethers (PBDEs); Polychlorinated Biphenyls (PCBs); Polycyclic Aromatic Hydrocarbons (PAHs); Selenium.

Further Reading

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Ecotoxicology, Genetic

Wendy L Rose and Susan L Anderson

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Introduction

Genetic ecotoxicology is a discipline that emerged from early studies in radiobiology. In the 1950s, radiation exposure was shown to alter the development, growth, and reproduction of mammals, fishes, and invertebrates. Growth retardation, suppression of cell division, and modified cell differentiation were detected in radiation-exposed organisms. Radiation exposure was also linked to gonad sterility as well as reduced fecundity, hatching success, and fertilization success in vertebrate and invertebrate species. As the field of radiobiology expanded in the late 1960s and early 1970s, scientists began to associate congenital and developmental abnormalities with chromosome damage and mutations resulting from radiation exposure. With increasing awareness of environmental pollution, research originated on the effects of toxicants, in addition to radiation, on the genetic material of aquatic and terrestrial organisms. Moreover, studies began to address the relationship between heritable genetic damage and consequences to populations and communities.

Currently, we define genetic ecotoxicology as the study of the effects of toxicants and radiation on the genetic material of aquatic and terrestrial organisms, and related population- and community-level responses. Genetic ecotoxicology can be distinguished from genetic toxicology, which primarily focuses on the individual-level effects of genotoxicants in

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Relevant Website

http://www.ourstolenfuture.org – The book Our Stolen Future deals with research on endocrine disruption. It discusses how common contaminants can affect the development of the fetus. This website is a good resource to check recent developments.

humans and model mammalian species. Genetic ecotoxicologists are interested in a range of effects including: DNA and chromosome damage, reduced developmental or reproductive success, diminished population size and distribution, age class alterations, and shifts in community structure. Genetic ecotoxicology is a complex discipline that integrates genetic toxicology, ecology, and population genetics. In the following sections, we hope to clarify the goals of this expanding field by: (1) examining the ways in which toxicant exposure may cause deleterious consequences to aquatic organisms and their populations, and (2) describing widely used methods in genetic ecotoxicology and their application to studies of toxicant effects on populations. The discussion below is limited to studies on aquatic organisms, which have been used as model species in numerous investigations of radiation- and toxicant-induced genetic damage at both the individual and population levels. At the individual level, this overview mainly concentrates on the effects of genotoxicant exposure on aquatic organisms, rather than radiation-induced responses, which are covered elsewhere in this encyclopedia.

How Toxicant Exposure Could Lead to Population-Level Consequences

To best understand the questions driving genetic ecotoxicological research, we have synthesized a model relating toxicant exposure to population-level consequences (Figure 1). Below, we discuss some of the factors and processes that may contribute to the decline of a population based on recent reviews in this field.

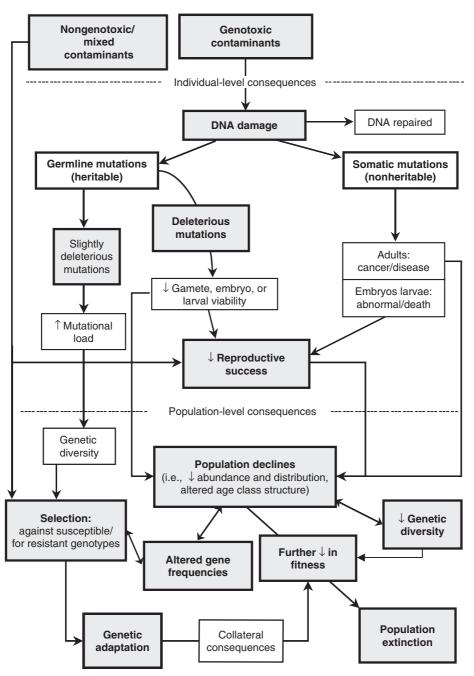


Figure 1 Model of how genotoxic and nongenotoxic contaminant exposure may lead to population-level consequences such as declines and extinction. The gray boxes indicate key steps in the pathways leading to population-level responses of contaminant exposure. Within the boxes, the up arrows suggest increases and the down arrows suggest decreases in the endpoints of toxicant exposure.

Genotoxic contaminants are chemicals that interact with and cause DNA damage such as strand breaks, adducts, pyrimidine dimers, or chromosomal damage in exposed organisms (Figure 2). Genotoxicants may cause damage by chemical interaction with the DNA molecule, interference with the structural integrity of DNA, or through indirect mechanisms including induction of oxidative stress, and inhibition of DNA repair. Most DNA damage will be repaired or may occur in introns, sections of DNA that do not code for protein structure or function. However, if DNA damage is not repaired or is misrepaired, gene expression may be compromised or mutations, alterations in DNA sequence, may arise.

The severity of DNA damage depends upon whether mutations arise in germline (heritable) or somatic (nonheritable) cells. Heritable genetic material is found within the germline cells, or sperm and eggs, while nonheritable genetic material is found within somatic cells, or all other cells in an organism. In adults,

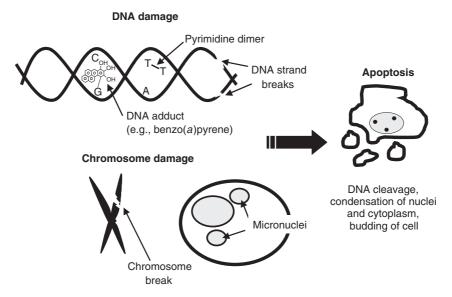


Figure 2 Genetic and cytogenetic endpoints used in genetic ecotoxicology to assess individual-level effects in toxicant-exposed organisms. The large arrow indicates that severe or misrepaired damage to DNA or chromosomes may cause a cell to undergo apoptosis or programmed cell death.

somatic mutations may cause metabolic or physiological impairment, cancer, and decreased survival in exposed individuals. If large numbers of individuals demonstrate chronic toxicities or disease such as cancer, then individual-level consequences of somatic mutations may eventually lead to population declines. However, it is more likely that individual-level effects of toxicants simply contribute to the decline of populations. More acute population-level effects may occur as a result of somatic mutations in embryos and larvae, which represent the youngest segment of a population. Specifically, the age class structure of toxicant-exposed populations may be altered, leading to shifts in reproductive cycles and reduced developmental or reproductive success. Regardless of age, somatic mutations are less severe than germline mutations, which directly affect the integrity of the germline cells, and the next generation of organisms.

Germline cells may incur deleterious or slightly deleterious mutations as a result of toxicant exposure. Germline cells that acquire deleterious mutations may undergo cell death or dysfunction resulting in impaired fertility, fertilization, embryonic development, hatching success, or larval and adult viability, all of which are indicative of diminished reproductive success. As a result, there may be a decrease in population size and ultimately, a reduction in genetic diversity, or the degree of gene variation within a population that allows it to resist harsh environmental changes or epidemics. Slightly deleterious mutations, mutations that do not directly lead to a life-threatening disease, but may contribute to an increased mutational load, also may arise in germline cells. However, few studies have demonstrated with certainty that an increased mutational load, and results thereof, may result from toxicant or radiation exposure.

Dramatic life history alterations that result from toxicant exposure could lead to population extinction through a variety of mechanisms. For example, if one life stage of an organism is more sensitive to toxicant exposure than others, reduced survival and fecundity may bring about population bottlenecks, severe reductions in population size, or alterations in age class structure. Bottlenecks may be accompanied by a decrease in genetic diversity, inbreeding depression, further diminution of fitness, and eventually population extinction. Alternative hypotheses such as decreases in population density also may explain how toxicant exposure could lead to severe population declines or extinction.

Nongenotoxic contaminants are toxic substances that do not directly alter the genetic material but may impair reproductive success or lead to selection and indirect changes in population genetic structure. The well-known dichlorodiphenyltrichloroethane (DDT), once sprayed to eradicate malaria-spreading insects, caused eggshell thinning in brown pelicans and other species and led to impaired reproductive success and widespread population size reductions. Thus, selection against susceptible or for resistant genotypes may occur in populations that have been exposed to nongenotoxic contaminants. Selection is the process that results in the survival and reproductive success of organisms or populations that are best adjusted to their environment. As a result of selection, the following may occur: (1) gene frequency alterations; (2) modifications in life history characteristics and population viability; (3) population size reductions; and (4) genetic variability decreases. In addition, genetic adaptation may have collateral consequences such as slow growth, late development, reduced longevity, and reduced fecundity.

Methods for Detecting Individual-Level Effects of Genotoxic Contaminants in Organisms

There are many well-known methods for detecting structural damage to DNA as depicted in Figure 2. Techniques vary in their selectivity for specific groups of toxicants, sensitivity for detecting low-level toxicant exposure, time requirements, cost, technical difficulty, use of radioactivity, and appropriateness for measuring DNA or chromosome damage in field-exposed organisms. Here, we discuss some of the most widely accepted methods, according to recent reviews in genetic ecotoxicology for evaluating DNA damage in toxicant- or radiation-exposed organisms.

³²P-postlabeling analysis is an effective method for the detection of DNA adducts, chemicals that are bound covalently to DNA, and implicated in chemically induced carcinogenesis. The ³²P-postlabeling technique involves the isolation and hydrolysis of DNA, labeling of normal and adducted 3'-monophosphates with ³²P, resolution of adducts from normal nucleotides by TLC, and detection of adducts by autoradiography or screen phosphor imaging. In a series of studies in the 1990s, Stein et al. used ³²Ppostlabeling analysis to demonstrate that DNA adduct levels in fishes were higher in industrial and urban areas as compared to fishes from reference or relatively noncontaminated sites, aquatic environments. For example, polycyclic aromatic hydrocarbon (PAH)-DNA adducts in oyster toadfish (Opsanus tau) liver were significantly higher from a creosote-contaminated site in the Elizabeth River, Virginia, when compared with reference sites, and levels of PAH-DNA adducts decreased with increasing distance from the creosote contamination.

The alkaline unwinding and comet assays are methods used to detect DNA strand breaks that arise as a result of exposure to genotoxicants or normal metabolic processes. DNA strand breaks may be incurred following the loss of a base in the DNA strand, leaving a transitory gap in the DNA subsequent to DNA repair. In the alkaline unwinding method, DNA is isolated and denatured at a specific temperature and pH, and the DNA strand break levels are calculated as the inverse proportion of the Hoecsht-stained double-stranded DNA. Additional modifications of this method are available for the resolution of single- versus double-stranded DNA strand breaks.

In the comet assay, cells are embedded in agarose on slides, lysed, and electrophoresed under alkaline conditions. Upon fluorescence staining and microscopy, cells with DNA damage appear as comets and have DNA strands that have migrated out of the nuclei. The distance or amount of DNA migration indicates the extent of DNA strand breakage. The comet assay has been used to evaluate DNA damage in plants, invertebrates, fishes, and amphibians from sites worldwide. For instance, the comet assay was used to demonstrate that DNA damage in mussels exposed to contaminants in San Diego Bay was significantly higher than DNA damage in reference mussels.

Severe DNA cleavage indicative of apoptosis, or programmed cell death, has been suggested as a possible biomarker of genotoxicant exposure in aquatic and terrestrial organisms. The TdT-mediated dUTP Nick-End Labeling (TUNEL) assay may be used to assess apoptosis or DNA cleavage in field-exposed organisms. Here, tissues or whole organisms are fixed, sectioned and embedded, and proteolytically digested, and fluorescein- or biotinylated-dUTPs are transferred to 3'-OH groups on cleaved DNA strands using a terminal deoxynucleotidyl transferase (TdT) enzyme. Biotinylated DNA fragments are detected with a streptavidin-HRP conjugate and substrate (DAB and hydrogen peroxide) and viewed by light microscopy. Fluorescein-bound DNA fragments are measured using fluorescent or confocal microscopy or flow cytometry.

Techniques are also available for detecting the effects of genotoxicants on chromosome integrity. Chromosomal aberrations are analyzed during metaphase and anaphase of mitosis and represent a method in which the effects of genotoxicants can be measured in tissues with a high mitotic index. For metaphase preparations, organisms are treated with colchicine, graded hypotonic solutions, and fixative. Fixed cell suspensions are spread onto slides, and stained with Giemsa and aceto-orcein. For anaphase preparations, cells are typically fixed in formalin solutions, stained with Giemsa and squashed on slides. Both metaphase and anaphase cell preparations are scored by the percentage and type of chromosomal aberrations. An integrated study of the health of Atlantic mackerel (Scomber scombrus) from the New York Bight in 1980 demonstrated that mitotic and chromosomal abnormalities were useful measures of toxicant exposure because they were associated with elevated levels of planktonic hydrocarbon and zinc, embryonic differentiation, and gross embryonic malformations.

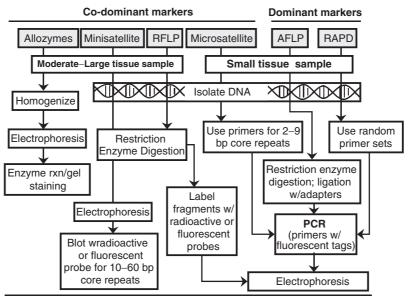
In addition to chromosomal aberrations, the micronucleus assay can be used to measure chromosome integrity. Micronuclei, small secondary nuclei or DNA aggregates, arise during cell division when chromosomes break or misalign. Resulting DNA fragments become incorporated into one of the daughter cells during cell division. In the simplified micronuclei method, dividing cells are spread and fixed on slides, stained using Wright–Giemsa or fluorescent stains, and the number of cells with micronuclei are scored.

Chromosomal damage also may be assessed in a large number of cells using flow cytometric analysis. In this method, an increase in DNA content variation in the cells of interest reflects an increase in the amount of chromosomal damage such as chromosome deletions or aneuploidy. The method involves isolation of nuclei from a cell suspension, staining of cells with a DNA stain such as propidium iodide, and measuring DNA content for cells in the G_0/G_1 phase of the cell cycle by flow cytometry. This method was used to measure DNA content variation in redbreast sunfish (Lepomis auritis) along a gradient from a toxic effluent release (East Fork Popular Creek, TN), in relationship to other biological and community level responses. DNA content variation and percentage pollutant-tolerant species was highest at the site of a toxic effluent release and decreased with increasing distance, and additional biomarker responses demonstrated similar trends.

In genetic ecotoxicology, experiments should be designed to not only measure exposure or effects, but also to discern differences among treatments due to nontoxicant-related environmental stressors, and to draw linkages between genotoxicity and fitness. To best examine mechanisms through which genotoxicity is related to effects on fitness, multiple endpoints of genotoxicity and fitness should be measured. Careful consideration should be taken to assess whether factors unrelated to toxicant exposure such as handling stress are causing modifications in the measured biological responses. Prior to field sampling, laboratory studies should be performed to evaluate tissue- and time-specific differences, species differences, and seasonal differences in the biological endpoints of interest. Finally, integration of field and laboratory experiments is also recommended; measurements evaluated in field-exposed organisms should be similarly assessed in organisms exposed to toxicants within the same media in the laboratory.

Methods for Detecting Population-Level Effects of Toxicants in Field-Exposed Organisms

There are a variety of methods that can be used to examine population genetic structure. In Figure 3, methods for evaluating population-level effects of toxicants in aquatic organisms have been categorized



Capture images and score data

Figure 3 Population genetic methods used in genetic ecotoxicology to examine toxicant effects in populations of aquatic and terrestrial organisms. Methods are categorized as either co-dominant (allozymes, minisatellites, RFLP, microsatellites) or dominant (AFLP, RAPD) markers. As shown here, techniques used to generate these markers are based on similar procedures such as PCR and electrophoresis, and similar methods of analysis. bp, base pairs.

as co-dominant or dominant markers, those in which heterozygous and homozygous dominant genotypes can or cannot be distinguished, respectively. Heterozygous genotypes have different alleles or forms of a gene at corresponding loci (e.g., positions of a gene along a chromosome) on homologous chromosomes. Homozygous genotypes have the same alleles at corresponding loci. Co-dominant markers include allozymes, microsatellites, and restriction fragment length polymorphisms (RFLPs), while dominant markers include amplified fragment length polymorphisms (AFLPs) and randomly amplified polymorphic DNAs (RAPDs). Techniques used to generate this diverse group of markers are based on similar procedures such as PCR and electrophoresis, and similar principles and methods of analysis (Figure 3).

Allozymes are co-dominant markers that have been used for several decades to assess the population genetic structure of aquatic and terrestrial populations. Allozymes are enzymes that vary in their electrophoretic mobility and are indicative of different alleles of single genetic loci. The allozyme technique uses starch or acrylamide gel electrophoresis to separate allozymes within tissue homogenates. Allozymes are visualized upon exposure to a particular enzyme catalyst or by staining gels with dyes, and scored by the number of specific allozyme types per individual.

Restriction fragment length polymorphism analysis is another technique that generates co-dominant allele data. In this method, DNA is isolated and digested with restriction enzymes, enzymes that cut DNA at specific positions within target sequences. The DNA fragments are labeled with fluorescent or radioactive tags, and electrophoresed. Images are captured using autoradiography or gel scanners and individuals scored according to the DNA fragment sizes. In the 1990s, Wirgin et al. used both allozyme assays and RFLP analysis to determine that there were genetic polymorphisms in the cytochrome P₄₅₀1A (CYP1A) gene of some Atlantic tomcod (Microgadus tomcod) from the Hudson River that were not present in tomcod from the less contaminated Saco River. The alleles were sequenced and evidence was found for selection against the Hudson River CYP1A allele, which was only detected in the heterozygous state.

In addition to selection, RFLP has also been used to demonstrate that decreases in genetic variation due to toxicant exposure may be suggestive of bottlenecks. RFLP analysis was used in the 1990s to demonstrate that copepods exposed to PAHs for several generations in the laboratory and near oil platforms experienced a reduced mtDNA composite genotype diversity compared to controls or those at reference sites.

Microsatellites are hypervariable co-dominant loci composed of arrays of 2–9 bp repeating motifs. Differences in the number of repeat motifs in an array define microsatellite polymorphisms. Method development requires the identification of microsatellite loci, and for each locus, the design of PCR primers to anneal to conserved regions flanking the microsatellite. Analysis involves PCR amplification with fluorescently labeled primers followed by electrophoresis to distinguish microsatellite alleles of different array size.

In contrast to allozyme, RFLP, and microsatellite analyses, the RAPD technique uses the random PCR amplification of DNA sequences to generate dominant allele data. Specifically, the method involves the isolation of DNA, PCR amplification of DNA sequences using a random set of short primers with fluorescent tags, and electrophoresis. The presence or absence of bands is scored upon visualization of gels. In a series of studies in the 1990s, Theodorakis et al. provided evidence of selection in radionuclideexposed mosquitofish (Gambusia affinis) from Tennessee ponds using RAPD and allozyme assays. The authors determined that genetic diversity was higher in radionuclide-exposed mosquitofish populations than in reference populations. A lower incidence of DNA strand breaks and higher fecundity were found to correlate with the RAPD banding patterns in radionuclide-exposed mosquitofish. In a later study, similar RAPD banding patterns were detected in another mosquitofish species (Gambusia holbrooki) from a separate radionuclide drainage, providing further evidence of natural selection in radionuclide-exposed mosquitofish populations.

The AFLP technique is another method in which dominant allele data are collected. Here, DNA is isolated and digested with restriction enzymes, and DNA fragments are ligated to adaptors, short double-stranded DNA sequences. Ligated fragments are then amplified with fluorescently labeled primers that recognize and bind to adaptors during PCR, and fragments are separated by electrophoresis. Visualization and scoring of gels is similar to that of RAPD analysis. In a recent study, AFLP and microsatellite markers were used to test the null hypothesis that genetic variation among Sacramento sucker (Catostomus occidentalis) populations in the California's Central Valley was due to biogeographical influences. The alternate hypothesis was that genetic variation among populations was explained by differences in long-term pesticide exposure history. Using both AFLPs and microsatellites, differences in watershed geography best explained the genetic variation among sucker populations and demonstrated the importance of testing contaminant exposure hypotheses against the null hypothesis of biogeographical and historical influences.

Regardless of the markers used, population genetic analyses are based on similar principles. First, allele scores are generated from either the presence or absence of bands (dominant markers) or sizes of bands (co-dominant markers) on gels, and a genotype is generated from the composite of alleles. Allele and genotype frequencies at single loci are calculated from the data and multi-locus genotypes are used to further characterize population structure based on population genetic models. For instance, genetic structure may be determined from allele frequencies, whereas genetic variability may be determined from mean heterozygosity and percentage polymorphism.

Meticulous experimental design and clear hypotheses are necessary to distinguish among factors that affect genetic patterns and determine potential mechanisms through which differences arose among populations. Background levels of natural genetic variation among many populations should be determined prior to testing contaminant exposure hypotheses. Chemical analyses and biological responses to toxicants should be measured at field sites to establish toxicant exposure. More than one method is recommended for the examination of population genetic structure to verify that results are accurate. A sufficient sample size should be used to detect differences among treatments, and several sites should be included for each treatment type. Finally, we stress that it is exceedingly important to rigorously test contaminant exposure hypotheses; experiments must be designed to reject the null hypothesis that genetic patterns among populations are accounted for by life history and biogeographical factors.

Conclusion

In summary, many factors contribute to the ongoing success or failure of aquatic and terrestrial populations in the face of environmental pollution. The growing field of genetic ecotoxicology offers many ways for scientists to examine the effects of toxicants and radiation on aquatic and terrestrial organisms, both at the individual and population levels. The methods described here can be used to address a diversity of environmental pollution problems such as assessing impacts of oil spills on aquatic organisms and populations, developing standards for cleanup of hazardous waste sites, evaluating the relative roles of habitat destruction and contaminant exposure in the management of endangered species, and screening industrial and nonpoint source inputs to ecosystems. Because it is difficult to identify all factors contributing to the demise of a species, we propose that careful field sampling designs and rigorous hypothesis testing, coupled with strategic selection of molecular markers and subsequent statistical analyses, will help scientists to best examine these issues.

See also: Apoptosis; Ecotoxicology, Aquatic; Benzo(*a*)pyrene; Biomarkers, Environmental; Cytochrome P-450; DDT (Dichlorodiphenyltrichloroethane); Genetic Toxicology; Polycyclic Aromatic Hydrocarbons (PAHs); Radiation Toxicology, Ionizing and Nonionizing.

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Ecotoxicology, Invertebrate

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The Background and Basic Concepts

Ecotoxicology concentrates on studies of toxic effects on organization levels higher than organisms. Ecotoxicology differs from toxicology and relies on the fundamentals of ecology, which means that ecotoxicologists study pollutants and their effects on different ecosystems. The objectives of ecotoxicology cover both fundamental and applied aspects.

Invertebrate ecotoxicology is a specialized area of ecotoxicology that deals with all aspects of ecological and toxicological effects of toxic substances on invertebrates and their consequences at the population level and above. The main objectives of invertebrate ecotoxicology are similar to those of ecotoxicology itself, related to the areas where invertebrates can be used:

- Obtaining data for risk assessment and environmental management.
- Meeting the legal requirements for the development and release of new chemicals into the environment.
- Developing empirical or theoretical principles to improve our knowledge of the behavior and effects of either natural or anthropogenic chemicals and to predict and evaluate changes caused by them in ecosystems.

There are many important factors which determine the study of invertebrates in ecotoxicology at both population and ecosystem levels. They represent more than 90% of all animal species and are much more abundant than vertebrates. They are present in nearly all types of ecosystems, from the deep oceanic bottom, through the surface water to soil and other terrestrial areas, including those with the most severe conditions for biological life. They often play a key role in different chains of the food web, determining interrelationships within the nets and participating in upward biomagnification of chemicals in the net. They are present in all heterotrophic layers, utilizing variety of food, take part in the decomposition of organic matter, transfer of biogenic substances and xenobiotics. Despite their small dimensions they exist in large quantities and represent animals that are short- or long-lived; they can also be maintained in the laboratory more easily and cheaply than vertebrates. Many species are abundant throughout the year, especially in the soil or in sediments. Invertebrates raise less ethical concern than vertebrates, especially mammals. In cases where mechanisms of toxic action of chemicals are similar to those observed in the case of vertebrates they may replace them in routine toxicity tests of novel compounds or pesticides. Invertebrates are more useful as the key animals in standardized ecotoxicological testing of soils or sediments. They should also provide a useful material for better understanding the interactions of chemicals with the biotic part of the environment and their consequences to an assessed ecosystem. Moreover, short generation time and abundance make some species useful models to study microevolution during long-term in situ investigations of populations living in stressed environments for many generations. They can develop resistance to toxic substances such as pesticides or heavy metals.

There are also several disadvantages of the use of invertebrate species in ecotoxicological investigations. Many of them are not suitable for continuous assessments of seasonal effects. Their generation time is limited to certain periods when they are active. Depending on physical conditions and food availability, they may disappear or be present as inactive forms (different diapausing stages, insect pupae, dormant eggs). In many species only adults can be used, because in case of their immature forms there are difficulties in determining their taxonomic position which cannot be overcome without sectioning, and, in turn, cannot be done prior to laboratory assays. In such a case they can serve only for studies at the community or ecosystem level where pooling of samples is acceptable, and their selection is based on similarities of their body size/biomass or feeding habits. More individuals are necessary in a sample than in the case of vertebrates; preparatory techniques are more complicated, more practice and sometimes pooling of individuals in a sample are needed. At the molecular level invertebrates may respond to the same chemical in a way similar to vertebrates. External factors cannot interfere with such responses causing sometimes insensitivity of invertebrates. On the contrary, due to lower activity of microsomal detoxification enzymes, insecticides are often more toxic to invertebrates than to vertebrates.

Population ecotoxicology of invertebrates covers broad ecological and evolutionary aspects of the chemical effects on individuals, changes in population demography and interrelations between various populations and species. Fitness can be a good measure of management of organisms with toxicants. Changes in relative fitness may lead to the evolution of resistance. The best-known example is the evolution of insect resistance to insecticides, and more recently resistance of various soil-dwelling invertebrates to heavy metals.

Invertebrates in Ecotoxicity Testing

Many ecotoxicological tests are performed by studying invertebrate biochemical fingerprint techniques for risk assessment. Invertebrates are recommended by OECD Test Guidelines to be used for assessing the effects of chemicals on the reproductive output in soil acute toxicity tests at organism, population and community levels for legislative purposes of new or existing chemicals. The OECD-recommended methods for testing physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment are also partially based on the use of invertebrates. Such tests are of little value for the whole ecosystem. They are performed in controlled laboratory conditions, while in nature organisms are exposed to a mixture of pollutants of different concentrations, which differ in bioavailability, routes of reaching the targets in the organism, and interact with a series of abiotic and biotic factors.

Invertebrate Ecotoxicology Gives Tools for Searching and Evaluating Microevolution

Generally, toxic substances are potent selection factors. Invertebrate ecotoxicology is therefore a very attractive science which helps study ecological and evolutionary phenomena directly confirming the existence of microevolutionary processes which could not be investigated in other ways. In a relatively short time many generations of short-living invertebrate species can be used to measure a selective pressure of sublethal concentrations of chemicals on genomic changes. Where tolerance limits are exceeded such changes may lead to the extinction of some populations while other populations may develop resistance through inherited adaptive biochemical/physiological/structural or behavioral features against various toxic substances. A spatial and temporal selection of genotypes with newly inherited features is good evidence of evolved tolerance to metals in populations from heavily contaminated sites. Moreover, the disappearance of newly inherited mechanisms of resistance/tolerance can be studied in populations in

conditions of ceased pressure from the chemicals. Studies of metal tolerance were recognized as the best examples of microevolution.

Metal Tolerance and Metal Adaptation in Soil-Dwelling and Aquatic Invertebrates

Exposed and unexposed populations of some terrestrial invertebrate species demonstrate divergences of physiology or tolerance to heavy metals in their food. Adaptation was proved in natural populations of such taxonomic groups as Oligochaeta, Mollusca, Crustacea (Isopoda), Myriapoda, Arachnida, Apterygota, and Insecta. Adaptations are speciesdependent and their various strategies have been identified. For example, the terrestrial isopod, Porcellio scaber, differs in life-history traits, storage, and excretion patterns. The females from the contaminated site had smaller body size and started to reproduce earlier than the isopods from the unpolluted site. They had been selected for early reproduction and allocated more energy to increase reproduction. A similar life history with an increased reproductive effort under metal stress characterizes populations of a collembolan Orchesella cincta. Both species differ in the strategy of metal bioconcentration and bioelimination. Porcellio scaber is a slow bioeliminator of metals which effectively accumulates metals mainly in metal-binding intracellular granules stored in hepatopancreas, and partly in metal-binding glycoproteins. Orchesella cincta, in contrast to the isopod, is a very quick eliminator of metals. Net assimilation rates of metals are lower in collembolans than in isopods. Genetic adaptation of O. cincta may occur within years or decades. The consequence of metal adaptation is reduced genetic variation for tolerance, which indicates additional costs of tolerance. Metal-adapted O. cincta shows increased mortality when transferred to uncontaminated conditions.

Invertebrates in Ecotoxicological Studies of Endocrine-Disrupting Substances

Endocrine disruptors are 'chemical substances that interfere with, or have adverse effects on, the production, distribution, or function of these same hormones'. These substances do not affect growth or cause increased mortality. They affect the development of organisms which is manifested in alterations of reproductive capacity or metabolic disorders of many steroid hormones. Some aquatic invertebrate species (cladocerans, mollusks) with a short reproduction time have been intensively studied as the

targets and useful models of hazard assessment for endocrine disrupting pollutants. Impaired reproduction in Daphnia magna caused by changes in endocrine metabolism as a result of alterations in biotransformation enzymes has been recently used as an early warning system of reproductive toxicity. The best-known example of an endocrine-disrupting chemical is tributyltin (TBT) - the antifouling agent used for painting boats responsible for abnormalities in the development of female reproductive organs in about 100 marine gastropods. The dogwhelk, Nucella lapillus, common on the rocky shores of northern Europe and the North Atlantic, is the best studied neogastropod species which is very sensitive to TBT (lowest-observed-effect concentration, $LOEC = 1 \text{ ng l}^{-1}$ as Sn). In TBT-affected females a phenomenon known as imposex (or pseudohermaphroditism) has been described. Inhibition of the enzyme converting testosterone to 17E estradiol -P450-dependent aromatase in TBT-exposed females increases the testosterone content which induces development of nonfunctional male characteristics vas deferens (a channel between prostate and penis) and a penis. The vas deferens occludes the genital papillae and such females become sterile by blocking the release of egg capsules. Any further development of aborted capsules might eventually kill the exposed females. Affected populations may decline or become locally extinct. The measurement of two parameters at the cellular and organism level can be used as potent early warning indices of endocrine-disrupting chemicals in marine ecosystems: changes in the vas deferens sequence (VDS) and the relative size of the female and male penises (RPS). The regulatory restrictions for the use of antifouling paints containing TBT gave positive results and since then the recovery of dogwhelk populations has been documented in many areas along European shores; nevertheless, the toxic effects of TBT appear to persist to some extent in open waters.

Biomarker Concept in Invertebrate Ecotoxicology

Invertebrates are an advantageous group for the use of biomarkers for *in situ* measurements. In a general sense, biomarkers are understood as various biochemical or physiological parameters of an organism (here an invertebrate) which can be used to demonstrate the exposure to environmental chemicals or to detect toxic effects. Biomarkers offer a quick and sensitive detection of chemical stress within the organisms and might indicate health status at the organism level of the key invertebrate species from both terrestrial and aquatic ecosystems.

When effects of pollutants are seen at the community or ecosystem levels, it may be too late to start reclamation activities, which become inefficient and extremely costly. That is why invertebrates have been effectively used as sources of biomarkers. They can be extrapolated to actual or potential changes at the population level, and considered as predictive tools to assess changes and consequences on community and ecosystem levels. Only some biomarkers identified in invertebrates are highly specific and sensitive, the majority are less specific to chemicals and then indicate the exposure or toxic effects of their mixture. In invertebrates biomarkers can be used as the diagnostic tools of their health or would give the basis for predicting the fate of stressed environment and to start remediation activities.

The use of invertebrate biomarkers as tools in assessment procedures is subject to many problems and restrictions. One of them is the lack of knowledge about normal ranges of measured parameters in a given species. Dose-response curves are known for only selected chemicals and the effects of their interactions are usually treated mechanistically. Our knowledge of how various factors - abiotic (season, temperature, pH, salinity, insulation, humidity, type of soil, or sediment), biological (developmental stage, age, sex, physiological state, reproductive state, molting, adaptation) or ecological (competition, abundance, food availability, habitat) - may influence the response of the target species is insufficient. Generally with increased hierarchical level of our study the magnitude of possible interactions increases with increased complexity and concomitantly increased unpredictability. That is why this approach is also inadequate and needs further study. Tolerance of invertebrates to various pollutants can be higher than that of vertebrates due to generally lower energy requirements, the possibility of diapausing or a good survival without food (in utmost cases encysting). In the majority of invertebrate species, a short generation time gives higher probability of genetically based adaptations.

Antioxidant systems as biomarkers of disturbances were studied by using many key species of freshwater, marine, and terrestrial ecosystems. The quality of benthic zone is validated with mussels, mostly bivalves, which are abundant, sedentary, reproduce in the same area, and give a possibility by using various life stages in long-term monitoring studies. Biomarkers demonstrating the antioxidative capacity in *Unio tumidus* were used for the survey of different aquatic ecosystems of the Mediterranean Sea along the coasts of Italy, France, and Spain. End points were activity of glutathione-related antioxidative enzymes and lipid peroxidation. Disturbances should be measured by using a set of biomarkers, not a single one. These measures can be used in an integrated approach with population and community studies of a given ecosystem. All collected data are necessary to make the diagnosis of ecosystem quality and predict scenarios for the future.

In invertebrates biomarker responses can also be linked with responses at higher levels of organization. Impairments caused by pollutants in aquatic ecosystems could be predicted studying freshwater crustaceans (*Gammarus pulex*, *Asellus aquaticus*, *Cambarus robustus*) as biomonitors. Casual links of the chemical stress responses can be established between various levels of biological organization. For example, reduced energy allocation to growth (scope for growth) in chemically stressed populations correlates well with the responses at the population level (a decrease in brood viability, reduction in offspring size).

Invertebrates in Ecotoxicity Assessment at the Population and Community Level

Population parameters cannot be generally more sensitive to chemical stress than individual parameters. Demographic approaches are practically absent in present risk assessment methodologies which are based generally on the end points for toxicity testing. Demographic methods can be studied using shortlived invertebrates existing in stressing conditions for their entire life. There is a series of potential endpoints to be used for measurements in organisms experimentally exposed to a series of concentrations of a given chemical or a mixture of chemicals. They should be egg-laying, hatching, growth, diapause, molting, rates of development, reproduction, or survival. For all these end points sensitivity to chemicals can be different. It would be perfect to use an integrative approach in which all life-history traits are integrated. Demographic techniques are applied in ecotoxicology to a variety of invertebrates species, mostly short-living freshwater species. A common base which allows comparisons between many species with different life-history traits is r – intrinsic rate of increase. Two important demographic processes determine population dynamics: natality and lethality.

Another convenient parameter in ecotoxicology is net reproduction index (R_0) which indicates how many individuals (generally females) in the next generation (N_{T+1}) can replace the parental generation (N_T): $R_0 = N_{T+1}/N_T$. The development of a population in a real time (at time intervals when important changes could happen – change in the age group or life stage, molting, maturity, production of offspring, death and so on) can be calculated on the basis of appropriate matrix equations where a columnar vector represents the age distribution at time t I multiplied by a matrix L in order to get age distribution at time T + 1. Matrix population model allows calculations of the eigenvalue of the projection matrix λ , a factor often called 'finite rate of increase' which shows whether month by month or year by year a given population decreases ($\lambda < 1$), increases ($\lambda > 1$) or remains stable ($\lambda = 1$). Such approaches in ecotoxicology can be found in relation to some soil-dwelling species, gastropods, chilopods, or insects exposed to metals or organophosphates.

Trade-offs are an essential part of the life-history theory. The effects of toxicants at the population level can be theoretically well predicted on the basis of the trade-off in the physiological allocation of energy resources which are not unlimited. In ecotoxicology this concept is used to recognize if there is any correlation between different life-history traits. There can be a compensatory reaction, as in example of metal-adapted isopods *P. scaber* where less energy is allocated to growth (individuals are smaller), more to reproduction. Other examples of trade-offs in invertebrates are negative correlations between the survival of juvenile forms and reproduction at the adult stage. Theoretically it should be clear that detoxification needs higher energy requirements. At the moment this type of trade-off is not well supported empirically and depends on the mode of action of a toxic substance.

Life-history traits are also applied to the analysis of sensitivity distribution and comparisons between populations exposed to similar chemicals as stressing factors. A convenient factor, called sublethal sensitivity index (SSI), can be used to identify populations on the basis of their reproductive sensitivity in a wide range from which they can reproduce at concentrations of a given chemical even exceeding the LC_{50} value (low SSI) to this at which reproduction ceases at concentrations much lower than an appropriate LC₅₀ value (high SSI). Sensitivity analysis changed the opinion that effects of chemicals at the population level are determined by the effects on the most sensitive life-history traits, which are generally early stages of development. In these type of studies an important contribution came both from the studies of small invertebrates like population of soil nematodes and avian populations.

The main focus of the ecological risk assessment is to minimize undesired events caused by chemicals. Species sensitivity distribution (SSD) is an example of an ecotoxicological method which is based on such events at above the no-effect level/concentration. We can assume that within a community species differ in sensitivity to various substances, and a distribution of sensitivity can be expressed by a parametric equation. If we know parameters of no-effect concentration distribution for the community, testing all species for no-effect concentration is not necessary. In practice SSDs have been positively accepted as indicators of scenario analysis of risk to concentration of toxic substances and, vice versa, leading to the protection of sensitive species.

Invertebrates as Tools in Genetic Ecotoxicity Studies

Genetic ecotoxicology focuses on studying alterations in genetic material in the biotic compartments of ecosystems. Ecotoxicology is interested in several consequences of exposure to genotoxic substances such as gamete losses, developmental abnormalities, neoplasia, lethal mutations, or changes in genetic diversity which affect Darwinian fitness (alterations in the growth rates, reproductive output, and viability of offspring). Genotoxic effects which occur in germ cells are transferred to consecutive generations resulting in alterations of gene expression in natural invertebrate populations in a way similar to that observed in vertebrates. Many different highly advanced techniques have been applied to study exposure, and sometimes effects, caused by genotoxic chemicals in invertebrates. The most common assays for screening chemicals for their DNA-damaging properties are DNA fingerprints, differential display mRNA, DNA alkaline unwinding, DNA strand breaks, DNA adducts, comet assay, micronuclei tests, c-K-ras oncogenes, and many others described in textbooks on genetics.

The evidence that chemicals induce neoplasia in invertebrates is scarce and has been demonstrated in mollusks (clams, snails, mussels) and planarians exposed to polychlorinated biphenyls and polycyclic aromatic hydrocarbons. Higher mortality of individuals with tumors is common as they could be easily hunted and are more sensitive to various pathogens.

Ecotoxicological genetics of invertebrates at the population level allows the demonstration of general relations between genetic variability and environmental pollution. The effects of mutagenic agents on DNA are various somatic disorders which might indirectly cause reproductive disorders resulting in increased mortality. Genetic diversity is gradually or rapidly lost (effects of so-called genetic bottleneck) and thus direct effects of toxic chemicals can result in extinction of the population.

In conclusion, studies of ecotoxicology of invertebrates which play a key role in different ecosystems allow better understanding of chemical-biological interactions in various stress conditions. In many cases they can substitute vertebrates in ecotoxicity testing of chemicals. They are more appropriate for integrated in situ testing, using the end points which cannot be used with vertebrates, thus their role in the assessment procedures for risk assessment should increase. In ecotoxicology studies we should accept that a high degree of internal complexity is an inherent property. Studies on invertebrates increase the possibility of parallel analysis of many variables, thus increasing the accuracy of our evaluations whether a community operates within normal range or stress can be recognized and defined.

See also: Biomarkers, Environmental; Ecotoxicology; Ecotoxicology, Aquatic; Ecotoxicology, Genetic; Environmental Hormone Disruptors.

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Ecotoxicology, Terrestrial

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Terrestrial ecotoxicology is the study of how environmental pollutants affect land-dependent organisms and their environment. It includes the subdisciplines of: wildlife toxicology (how poisons affect birds, mammals, reptiles, and amphibians), plant toxicology, invertebrate toxicology, soil invertebrate toxicology, soil microbiology (how poisons affect soil microbial functions), biogeochemistry (how pollutants move through air and soil, are biodegraded by soil organisms, or are taken up by plants), and ecology (how plants and animals interact with each other as well as with abiotic (nonliving) parts of the ecosystem).

An ecotoxicological process requires three primary elements: (1) a source (what is the pollutant and where is it coming from?), (2) a receptor (which organism is likely to be affected by the pollutant?), and (3) an exposure pathway (how does the pollutant get from the source to the receptor?). The exposure pathway element also involves quantifying how much pollutant (if any) reaches a receptor. If one of these elements is missing, it is unlikely that a pollutant will be able to affect an organism. If all three are present, an ecotoxicologist can assess the potential for adverse responses in individuals, populations, or communities.

Pollutants and Sources

All manner of pollutants are considered under the discipline of terrestrial ecotoxicology including pesticides, persistent organic pollutants, other organic substances, and metals and metalloids (e.g., selenium and arsenic). Naturally occurring toxins such as those produced by poisonous plants, snakes, or invertebrates generally are not included unless people intentionally apply them for pest control. For example, the pyrethroid pesticides are derived from the naturally occurring pyrethrin toxin that is found in chrysanthemum.

Pollutants may enter the terrestrial environment through direct application, as is the case with pesticides, fertilizers, or biosolids (sewage sludge). Improperly managed landfills and waste sites can contribute mixtures of pollutants to soil systems, through surface runoff of leachates or blowing dusts. Local areas may be contaminated by wet or dry deposition of air pollutants emitted from industrial processes or through land composting or disposal of industrial waste products. Long-range transport of volatile substances can result in contamination of soils or foliage many miles distant from the source, while automobile exhaust and other particulate emissions are generally deposited locally. Floodwaters and other sporadic or episodic surface runoff may leave behind pollutants that settle onto or bind with soils.

Receptors

Typical terrestrial receptors include soil microbes, invertebrates (e.g., beetles) including soil-dwelling invertebrates (e.g., insect larvae, worms, nematodes), plants, amphibians, reptiles, birds, and mammals. Although not shown in Figure 1, terrestrial organisms that depend upon aquatic ecosystems for some or all of their food and habitat, such as fish-eating (piscivorous) birds (e.g., cormorants, osprey, eagles) and mammals (e.g., mink, otter) are also potential receptors. Domestic animals such as livestock and pets, or agricultural crops, generally are not considered as receptors, although information about the toxic effects of chemicals in these species may be used in the absence of data about wild organisms.

Exposure Routes and Pathways

Terrestrial organisms can be exposed to pollutants in soil through one or more of six primary pathways (Figure 1): (1) direct (dermal) contact with soil, (2) ingestion of the soil itself incidental to grooming or consumption of soil-dwelling organisms, (3) inhalation of pollutants released from soil as vapors, (4) ingestion of plants, (5) ingestion of soil-dwelling organisms, or (6) ingestion of other organisms that have taken up pollutants from the soil. If pollutants in soil are leached or eroded into water sources, consumption of polluted drinking water can become an exposure pathway for terrestrial organisms. Understanding the relative importance of these exposure pathways is necessary both from a risk mitigation standpoint (to identify the exposure pathway(s) that need to be severed) and for an understanding of potential toxic effects. For example, inhalation exposure is more likely to result in pulmonary (lung) damage, while dietary ingestion will produce effects to the liver and general systemic toxicity.

Direct (dermal) exposure can be a significant exposure pathway for humans and for other terrestrial organisms. While feathers, fur, or scales protect the skin of nonhuman receptors, many substances are

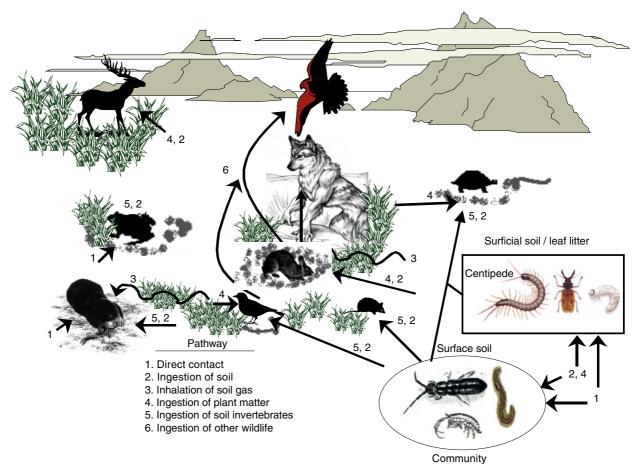


Figure 1 Exposure pathways and receptors in a typical terrestrial food web. (Reprinted with permission from Lanno R (ed.) (2003) Contaminated soils. In: *Soil–Chemical Interactions to Ecosystem Management*, 400pp. Pensacola, FL: Society of Environmental Toxicology and Chemistry; © SETAC, Pensacola, FL, USA.)

able to penetrate to the skin and be absorbed. Additionally, penetration of eggshells of birds or reptiles by pollutants, particularly petroleum oils and certain polycyclic aromatic hydrocarbons, can be a significant exposure pathway.

Plants, soft-bodied soil-dwelling organisms (e.g., earthworms), and soil microbes can be exposed through passive diffusion, and, occasionally active uptake, to pollutants that have moved into the water that fills the spaces between the soil particles (known as 'soil porewater'). Polluted soil particles can be 'splashed' on plants by rain and those that become airborne can be deposited on plants as dust. Such deposition or splashing of soil particles generally is not a significant exposure pathway for plants, as their leaves are relatively impervious to particulates. However, receptors consuming plants may be exposed to polluted particles adhering to the outside of the plant. Highly volatile substances that are released from soil as gas or are brought into an area through the air can be 'inhaled' (absorbed) by plants, resulting in significant toxic effects (ozone is an example of such a pollutant).

Hard-bodied terrestrial invertebrates (e.g., beetles, centipedes) can be exposed through incidental ingestion of pollutant-containing soil particles or through ingestion of food items (e.g., leaf litter or other soil organisms) that have taken up pollutants from soil.

Small mammals (e.g., moles, mice, and voles) and certain bird species that live or nest in subsurface burrows may inhale volatile pollutants that are evaporated out of the soil as a vapor. This pathway is likely to be significant only in those cases where poor ventilation allows vapors to collect and concentrate and where the receptor spends a lot of time (e.g., when nesting) in such a poorly ventilated space. There is, however, little information available about how to quantify this exposure pathway. Small mammals are more likely to be significantly exposed to pollutants that have been taken up in their food items (e.g., plants, soil invertebrates) or through incidental ingestion of polluted soil particles. Larger avian and mammalian receptors may be directly exposed to soil-related pollutants via incidental ingestion of soil and indirectly exposed through ingestion of contaminated food items (e.g., plants, soil invertebrates, or other animals). The dietary pathway is 'indirect', in that the larger receptor is exposed to soil-related pollutant brought to it in its food through the food web and does not need to be in the vicinity of the pollutant source. In fact, if the contaminated food item is capable of traveling, a larger receptor could be exposed quite some distance from the source.

A terrestrial food web (e.g., Figure 1) is a simplified representation of the complex interactions of below-ground processes and above-ground plants and animals. It is used to illustrate the trophic level and predator-prey relationships among selected terrestrial receptors and their potential food items. Terrestrial ecotoxicology uses information about the structure of a local food web to make predictions about how pollutants may move from soil and into plants or animals at various trophic levels, including those possibly some distance from the source of the pollutant.

Pollutants adhering to soil particles can enter a food web in three primary ways: (1) along with nutrients (nitrogen, phosphorus) and essential elements (e.g., zinc, copper, manganese), they can move from soil particles into the soil porewater; they can then be taken up from the porewater by plant roots through active or passive mechanisms and distributed throughout the plant, (2) from porewater into soildwelling receptors (e.g., earthworms) through transdermal osmosis, and (3) by being stripped from soil or organic particles as these are digested by soildwelling organisms. However, not all of a pollutant that can be measured in soil using chemical analysis methods is necessarily biologically available (bioavailable) to a receptor. For example, only about 3% of the mercury in mine tailings can be extracted with a biologically relevant mild acid (i.e., synthetic stomach acid), whereas over 90% can be recovered if concentrated nitric acid is used ('total recoverable' method). Bioavailability is reduced when the pollutant tightly adheres to soil particles, and is a function of soil pH, amount of organic carbon in the soil, relative amount of clay versus sand, and several other similar factors. The length of time the material is present in the soil can also influence bioavailability, as many chemicals can become more tightly bound with time.

Above- and below-ground plant parts, along with soil invertebrates, are the base (lowest trophic levels) of the terrestrial food web. Receptors at an intermediate trophic level can be exposed to pollutants that have moved from the soil to their food items. Examples would include foliage-feeding invertebrates, invertebrate-eating invertebrates, and birds, mammals, and other animals that feed on plants and invertebrates (e.g., amphibians and reptiles). Pollutants can reach predators at the top of the food web, such as wolves and hawks, when they feed on lower and intermediate trophic level receptors that have taken up pollutants.

Two important processes, which are different but related, govern the movement of a pollutant into and through a terrestrial food web: bioaccumulation and biomagnification. The movement of pollutants from the soil into a plant or animal at a given trophic level of a terrestrial food web is called 'bioaccumulation'. The concentration of a pollutant capable of bioaccumulation will be higher in a plant or animal than in the soil. The concentration of some pollutants increases as it is passed from one trophic level up to a higher level through the food chain. Such pollutants are said to 'biomagnify' and the process is known as 'biomagnification'. As a general rule, chemicals that biomagnify are persistent in the environment for long time periods (months to years) and have a high affinity for lipids (fats). Examples are the organochlorine pesticides such as lindane or DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane); chlorinated hydrocarbons used as fire retardants such as polychlorinated biphenyls (PCBs), or industrial pollutants such as polychlorinated dioxins. An exception is the biomagnifying organometallic compound methylmercury, which has a high affinity for tissue macromolecules and not lipids. Chemicals that degrade very quickly (e.g., many pesticides) or are soluble in water (e.g., metals) will be removed from the soil and not stored in organisms for long periods. Chemicals that biomagnify can pose a high risk to top predators (those at the highest trophic levels) in terrestrial food webs, as a very small amount in soil can magnify into a substantial, and potentially toxic, concentration by the time it reaches the top of the food chain. An excellent example of the consequences of biomagnification is the use of the insecticide DDT prior to its being banned for use in the United States in 1972. DDT is very effective in controlling mosquitoes, as well as agricultural insect pests, and was applied very widely for these and other purposes. It has relatively low mammalian toxicity and is not acutely toxic to birds. However, because DDT is very persistent (it has a half-life of ~ 30 years; 'half-life' being the time it takes for one-half of the chemical to turn into something else) and is readily stored in lipids within plants and animals, the small amounts applied to the environment biomagnified in the terrestrial food chain to levels that caused eggshell thinning in birds such as robins, peregrine falcons, and pelicans. Many species of hawks, eagles, and owls were affected to the point where their populations showed significant declines. Since its use was banned, the amount of DDT in the environment has gradually declined, eggshell thickness has returned to normal, and many of the affected bird populations have recovered.

Responses in Individuals (Toxicity)

Toxic responses of plants, soil microbes and invertebrates, and wildlife can be assessed following standard laboratory toxicity test protocols developed by the US Environmental Protection Agency (USE-PA), the American Society of Testing and Materials (ASTM), and several international organizations including the Organization for Economic Cooperation and Development (OECD), and the International Organization for Standardization (ISO). There are no standard methods available for toxicity tests with reptiles. For amphibians, tests have been standardized for exposure to the aquatic life phase (eggs and tadpoles), but not for the terrestrial adults. Typically, these tests look at acute mortality following a single high-concentration application to soil or food, or at effects on growth or reproduction when exposed to lower amounts over long time periods. Soil microbial tests look at the functions that are provided by soil microorganisms, rather than effects on the microbes themselves. These include the ability to fix atmospheric nitrogen in the soil (nitrification) and organic decomposition. Because soil microorganisms release carbon dioxide (CO_2) as an end product of their metabolism in a manner similar to higher animals, 'soil respiration' or the amount of CO₂ that comes up out of the soil is another commonly measured function. However, all of these functions are highly variable and depend a great deal on soil conditions (e.g., temperature, moisture) and so must be interpreted with caution.

When conducting laboratory toxicity tests, it can be difficult to incorporate some chemicals into the soil or animal feed, particularly if the chemicals are very insoluble. Furthermore, raising test organisms in the laboratory and conducting studies for sufficient time periods to encompass the reproductive cycle of a plant or animal can be prohibitive (consider, e.g., a life-cycle test on a long-lived tree species). For this reason, as well as for other ethical concerns, most species are not tested directly, but rather a standard set of test organisms is used from which extrapolations are made to other species. It must be recognized that such interspecific extrapolations are highly uncertain, and large safety factors frequently are applied as a result. The emerging science of 'toxicogenomics' may provide a better understanding of the response of genes to pollutant exposures and allow for more accurate interspecific extrapolations. Currently, information derived from laboratory rodents for use in human health toxicity evaluations can also be applied to mammalian wildlife, as well as studies conducted with domestic livestock. Mallard ducks and quail generally represent birds, although chicken or turkey studies conducted for agricultural purposes can also be used. The earthworm is a wellstudied representative of soft-bodied soil invertebrates, and the springtail or potworm are used as hard-bodied invertebrates. The African clawed frog and the leopard frog (native to North America) are used in amphibian studies. Plants generally have been represented by domestic species, including lettuce, rapeseed, and cucumber although the use of wild plants is becoming more common.

While laboratory studies are useful for studying toxicological responses of organisms to individual chemicals under controlled conditions, they frequently are not predictive of what occurs in natural environments. Organisms may be exposed to multiple chemicals simultaneously, and are always under stress from environmental conditions (e.g., too much or too little water, heat and cold stress, predator avoidance, etc.). Therefore, bioassays may be conducted in the field to look at potential effects under natural conditions. For plants or soil invertebrates, this might be as simple as collecting soil from a contaminated location, putting it into pots, and adding the standard test organism or unexposed specimens of the same species found at the site and then following the standard protocol. Alternatively, organisms can be observed directly in the field to look for toxic responses. These can be as simple as observing dead plants or animals in a location with high contamination, changes in the way plants look such as leaf color (chlorosis) or spotting, or through health evaluations of animals. 'Biomarkers' also may be studied, which are measurable changes in animal physiology that result from exposure to pollutants. Examples of useful field biomarkers are changes in enzymes associated by hemoglobin synthesis as a result of exposure to lead or reduced activity of the cholinesterase enzyme following exposure to certain classes of pesticides. These indicate both an effect (enzyme change) and that exposure occurred. Other biomarkers such as induction of enzymes in the liver that degrade toxins (e.g., the family of cytochrome P450 enzymes) are only indicators of exposure and not of effects. New methods are emerging from the science of toxicogenomics that may make it possible to measure biological responses to very low levels of

pollutants in the environment, although with a few exceptions the relationship between changes in gene expression and whole animal toxicological response is not yet clear.

For some chemicals, the amount of pollutant present in plant or animal tissues can be used to predict whether or not they will be affected. An organism can tolerate a certain amount of a chemical in its body above which level it is likely that adverse effects will occur. This is known as the 'critical body residue', although it sometimes is specific to certain tissues (e.g., liver or kidney). For example, it is known that a level of $6 \ \mu g \ g^{-1}$ wet weight of lead in the liver of a duck will result in toxicity, and at $15 \ \mu g \ g^{-1}$ wet weight it is highly likely that the duck will die. For other chemicals, the critical body residue is not known, and the presence of chemicals in the tissues can only be used as an indication that exposure has occurred.

Responses in Populations and Communities

Environmental pollutants primarily cause effects in individual organisms. Assessing effects at the level of the individual is appropriate (and may be legally required) for: species whose numbers are so low that they are protected by special laws (e.g., US Endangered Species Act), birds protected under international treaty (e.g., the Migratory Bird Treaty Act of North America), or other animals afforded special protection (e.g., the US Bald Eagle Act). These special instances aside, terrestrial ecotoxicologists generally are more interested in the sustainability of whole populations or in changes in community biodiversity. Population models can be used to determine whether alterations in reproductive rates, mortality rates, or length of time needed to mature resulting from exposure to environmental pollutants will significantly alter the ability of a population to sustain itself over time. Depending upon the life history of a species, effects on reproduction may outweigh direct toxic (lethal) effects, or conversely may have a negligible impact on population dynamics.

Sometimes a plant or animal species of particular interest is not affected directly by a pollutant, but indirectly as, for example, the pollutant reduces either its prey resource or its predators. Either may result in a change in the community composition of the species. In addition, changes in plant communities may result in different wildlife species using a particular area, resulting in the loss of some species and increases in others. Community diversity surveys can be performed for plants and, to a lesser extent, for soil invertebrates to assess such changes. Because wildlife obtain food and shelter from plant communities, changes in how these communities are distributed across the landscape can also significantly affect the spatial distribution of wildlife. Alterations in the distribution patterns of a wildlife species can change the probability of both its exposure to pollutants and the emergence of any subsequent population-level effects. Some areas are highly conducive to production of wildlife species, whereas others do not have the right combination of habitat requirements and cannot support significant reproductive output of wildlife species. Contamination of a high-production area can result in serious consequences for a population as a whole, whereas contamination of an area that supports little or no reproduction may only expedite the demise of an otherwise nonsustainable local population.

For some environmental pollutants there can be a range of tolerances among individuals within a species such that some respond significantly less than others. Over time, those that are not significantly affected by exposures will have the greatest ecological fitness and so produce more offspring that also have the ability to cope with such exposure. This is termed 'adaptation' of the species to the pollutant, or development of tolerance or resistance. The best examples are insects that develop resistance to pesticides as a result of repeated exposures, necessitating the development of new chemicals to which they have no experience. Additionally, pollution-induced community tolerance (known as 'PICT') has been demonstrated in soil microbial communities, where the types of species may shift to accommodate the soil pollutant but overall functioning of the microbial community remains unaffected (e.g., soil respiration or nitrification continue). Plants and animals can also develop tolerance over time and adapt to some environmental pollutants. In some instances, plants have been observed to recognize pockets of pollutants in soils and avoid exposure by growing their roots around such areas. Some plants can take up large amounts of pollutants without being affected ('hyperaccumulators'; e.g., selenium uptake by locoweed), and have been used as a bioremediation method at contaminated waste sites and mining (tailings) sites.

Risk Assessment

Terrestrial ecological risk assessments are conducted following the standard framework and guidance published by the US EPA and various states. All but the most basic ecological risk assessments require a team of specialists to gather, analyze, and interpret information about fate and transport of pollutants through the soil system, uptake by plants and soil organisms, and both direct and indirect effects to the organisms of concern. The ecotoxicological information discussed above can be assembled into a risk assessment by conducting toxicity tests or field assessments of effects and estimating expected exposure levels. Exposure and effects data are then combined to estimate the likelihood of adverse effects to individual organisms. Such an estimate can be used to directly assess toxicological risks, but assessing ecological consequences at the population or community level generally requires the use of predictive models.

See also: Ecotoxicology; Ecotoxicology, Avian; Ecotoxicology, Invertebrate; Ecotoxicology, Wildlife; Risk Assessment, Ecological.

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Ecotoxicology, Wildlife

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Wildlife toxicology is a subdiscipline of environmental toxicology and evolved during the mid-twentieth century in response to concerns regarding wildlife exposure to pollutants. The term 'wildlife' generally refers to any nondomesticated species, but often refers to animals rather than plants, and sometimes is further defined as terrestrial vertebrates (mammals, birds, amphibians, and reptiles), separating them from fish and invertebrate species. However, these categories are only a matter of semantics because all of these groups are interconnected within complex trophic groups in the environment. Due to this interconnectedness, Wildlife toxicologists must often integrate accumulation and effects of contaminants among all of these groups.

In studying the impacts of pollutants on wildlife, the wildlife toxicologist evaluates exposure and effects, and determines the mechanisms involved in the assimilation, distribution, and influence chemicals have on specific cells or biological processes that effect the individual, population, or community of organisms living in the area where a pollutant occurs. In addition to evaluating the effects of pollutants on wild species, wildlife toxicologist also have used wild species as sentinels to warn of potential adverse accumulation and distribution of chemicals in the environment. Miners taking canaries into the mines to warn of poisonous gases is a classic example of the use of wildlife as toxicological sentinels. Wildlife also serve in determining the bioavailability of environmental contaminants (i.e., is the chemical able to be absorbed into the body of an organism or is it in a particular form or bound so that it is not absorbed into the body). Because wild species are closely associated with natural environments, they integrate chemicals in their environments over time and space, thus providing a biological system for evaluating bioavailability, accumulation, and effects of single or multiple chemicals.

Effects on Wildlife

The effects of pollutants on wildlife may be either direct or indirect, and these effects are influenced by the mode of action of the specific chemical. Direct effects are those that result in death or biological damage to an exposed individual. For instance, exposure of an organism to a certain chemical may not result in direct mortality, but may affect an animals motor responses and therefore its ability to capture prey or avoid predators; which ultimately may result in the death of the individual. Direct effects also may include impaired growth or reproduction that results in declining populations. For example, symptoms in wildlife exposed to chlorinated hydrocarbons (instance e.g., dichlorodiphenyltrichloroethane (DDT)) tend to be nonspecific and include nervousness, hyperexcitability, weakness, lack of appetite, and tremors. Several laboratory studies conducted during the 1940s indicated that acute (short term) exposure to some chlorinated hydrocarbon pesticides would not cause direct mortality of wildlife; however, they may cause weakness and loss of appetite that may reduce the general health of the effected species. This in turn leads to increased susceptibility to disease or predation. It was later discovered that exposure of birds of prey to DDT, or its metabolites, resulted in thinning of egg shells, which in turn resulted in population reductions due to breaking of the eggs and death of the developing embryos.

Indirect effects would be those that have an adverse influence even though the individual may not have been directly affected by exposure to a chemical. Elimination of insects, a major food source of many mammalian and avian species, or loss of habitat (cover) due to weed control, may influence the health and welfare of wild species. As insect populations are reduced due to insecticide spraying, wildlife are unable to obtain their nutrient requirements and become malnourished. The outcome may be similar to that observed from direct effects. As the animals general health diminishes because of lack of food, it loses its ability to avoid predation, or to capture prey, or it becomes more susceptible to disease and infection. Similarly, the loss of habitat can reduce an animal's ability to escape predation, or it may reduce breeding and rearing areas, which also may result in population declines in affected species.

History

During the late 1800s and early 1900s, there was a tremendous expansion in the development and use of chemicals in industry and agriculture. At that time, it was a common practice to discharge industrial waste into rivers and streams, or dispose of them in landfills, and it was common for repeated widespread application of pesticides to agricultural land. There were few laws or regulations governing industrial or agricultural chemicals, and potential effects of these chemicals on wildlife were unknown. From 1900 through the early 1930s lead arsenic, lime-sulfur, and nicotine sulfate compounds were a few of the insecticides used in agriculture. Some of the agricultural publications during this time period reported potential adverse effects of insecticides on beneficial insects, such as honey bees, and expressed some concern about effects on other wildlife. However, there were very few studies conducted during this time period that evaluated impacts of pesticides on wildlife.

During the 1930s and the 1940s, several important chemical discoveries sparked a major shift in the types and quantities of chemicals used as agricultural pesticides. The discovery of the insecticidal properties of DDT in 1938 was paramount in these discoveries. Because of its low acute toxicity to mammalian species, perceived environmental safety, and effectiveness, DDT was considered to be an ideal chemical for widespread use in the environment and was widely used as an agricultural and public health insecticide. However, DDT, as well as other chlorinated hydrocarbon insecticides, do not easily degrade and therefore accumulate in the environment over time. In addition, repeated application of DDT resulted in the evolution of more resistant insects, which in turn resulted in the application of more DDT at higher concentrations. Results of laboratory and field studies of potential adverse effects of DDT on wildlife began to appear in the mid-1940s, and some of the first reports of mass mortality among wild species occurred during the mid-1950s. These included deaths of numerous waterfowl species utilizing lakes sprayed with DDT, or one of its daughter compounds (DDD or DDE), to control gnats.

Similar to the expansion in use of agricultural chemicals during the first half of the twentieth century, there was a tremendous industrial expansion occurring in North America and many new chemicals were being developed for industrial use. One of these chemicals was polychlorinated biphenyls (PCBs), another chlorinated hydrocarbon. PCBs were first synthesized in the late 1800s, and because of their chemical properties, were used in various industries, including the electrical industry. As with DDT, adverse environmental effects of PCBs were unknown in the early 1900s, and environmental spilling and landfill disposal of used PCBs were common. During the 1920s and the 1930s, several reports of adverse effects of PCBs to humans were published; however, no field studies of adverse effects of PCBs or other industrial chemicals to wildlife are known to have been conducted. It was not until the 1960s when mink farmers in the Great Lakes Region reported reproductive failure in mink, that the widespread environmental distribution and adverse effects of PCBs on wildlife became known. It was soon discovered that fish from the Great Lakes contained elevated PCB concentrations that caused the reproductive failure observed in mink.

Reports of adverse effects in wildlife from exposure to agricultural pesticides, like DDT, and from exposure to industrial chemicals, like PCBs, that emerged during the 1950s and the 1960s, along with publications like *Silent Spring* by Rachel Carlson in 1963, lead to the increased awareness of potential impacts of chemicals on wild species. This awareness, and the studies that it generated, resulted in the offshoot of a group of toxicologists specializing in the effects of pollutants on wildlife and the birth of wildlife toxicology.

Major Contaminants of Concern

Although any chemical can be toxic to wildlife if exposure occurs at a high enough concentration, those that are used or released in the environment are of the greatest concern. Those that have been associated with adverse effects in wildlife include: the persistent organic pollutants (POPs), cholinesterase inhibiting insecticides, and some metals. Another group of chemicals, the pharmaceuticals, also are becoming a major concern to wildlife.

Persistent Organic Pollutants

POPs are carbon-containing chemical compounds that, to a varying degree, resist photochemical, biological, and chemical degradation. Because of their persistence and high lipid solubility, POPs tend to bioaccumulate in fatty tissues. They also are semivolatile and therefore can vaporize or absorb onto atmospheric particles. This permits the global transport of these chemicals in air and water. The insecticide DDT is a classic example of a POP, and the effectiveness of DDT as an insecticide lead to the synthesis of other chlorinated hydrocarbon insecticides (other POPs) shortly after WWII. These included insecticides such as aldrin, dieldrin, chlordane, and toxaphene. In addition to insecticides, other POP pesticides were developed during the 1940s and were distributed widely in the environment; for example, hexachlorobenzene, a fungicide used on seed grains. Even though use of many of these chlorinated hydrocarbon insecticides have been banned or are strictly regulated, they are often detected in tissues of wild species.

PCBs are another well known POP that has accumulated in the environment with reported adverse effects in wildlife. Previous discharge of PCBs into streams, rivers, and lakes, disposal in landfills, and atmospheric discharge has resulted in widespread environmental distribution of PCBs. The manufacturing of PCBs also may result in the production of by-products, such as dioxins and furans, which also can be highly toxic to wildlife. Even with modern day restrictions on use, PCBs persist in the environment and are still frequently detected in tissues of wild species.

Persistent organic pollutants may persist in the environment for many years, and because they are lipid soluble, can accumulate in greater concentrations (biomagnify) in species feeding higher up the food chain. Wildlife studies conducted since the 1950s have indicated that POPs can disrupt an organism's endocrine system and are often referred to as endocrine disruptors. These studies have reported population declines, reproductive impairment, eggshell thinning, metabolic and behavioral changes, influences on sex determination, and embryonic deformities in a variety of wild species including eagles, cormorants, alligators, and mink, exposed to POPs.

Cholinesterase Inhibiting Compounds

During the 1950s, two other major groups of chemicals were developed for use as insecticides, the organophosphates (OPs) and carbamates. Although some OPs and carbamates may be more acutely toxic than the organochlorine chemicals that preceded them, they are considered a more ecologically acceptable group of chemicals because, in general, they are not as long lived in the environment, and therefore are less likely to accumulate in the environment.

Unlike the nonspecific effects and uncommon occurrence of direct mortality observed in wildlife exposed to chlorinated hydrocarbon pesticides, several studies have documented direct mortality from exposure to OP and carbamate insecticides. The method by which the OPs and carbamate insecticides affect wildlife is quite different from the method by which the chlorinated hydrocarbon insecticides effect wildlife. The OPs and carbamates inhibit cholinesterase, primarily acetylcholinesterase (AChE), which is an enzyme that functions in the breakdown of the neurotransmitter acetylcholine. Acetylcholine functions in the transmission of nerve impulses. Therefore, when AChE is inhibited by an OP or carbamate insecticide, it can no longer breakdown acetylcholine and there is continued transmission of nerve impulses that eventually leads to nerve and muscle exhaustion. The respiratory muscles are a critical muscle group that is affected, often leading to respiratory paralysis as the immediate cause of death. A major difference in the mode of action between OPs and carbamates is that the inhibition of AChE by OPs is, from a biological standpoint, irreversible, while the inhibition from exposure to carbamates is reversible in a biologically relevant time frame. There are numerous reports in the literature documenting direct adverse effects of cholinesterase inhibiting insecticides, especially regarding effects in wild avian species. In addition to direct effects, indirect effects on wildlife resulting from decreased food availability (insects) are well known. As with all insecticides used in the environment, the timing and method of application greatly influences the occurrence of potential adverse effects.

Metals

Unlike the POPs, OPs, and carbamates, which are not naturally occurring, metals are elements that do occur at low concentrations in the environment. Mining and various industrial activities may have resulted in an increase in concentration of many metals to levels that are potentially toxic. Ignorance, mismanagement, and/or accidental release have resulted in the accumulation of metals in some environments, and metals such as mercury, lead, cadmium, and selenium, have been associated with adverse effects in wildlife. Pollution by metals is usually localized in the vicinity of the polluting source; however, some metals, such as mercury, have been transported in the atmosphere or in water resulting in pollution of areas distant from the source. Wildlife are often exposed through ingestion of metals in food or from incidental ingestion of metals in contaminated soil. Major concerns with regard to wildlife include: ingestion of mercury contaminated fish; ingestion of lead from ammunition associated with waterfowl hunting and skeet shooting, or from of lead sinkers used in fishing; exposure to selenium in agricultural drainage and in the vicinity of phosphate mining; and exposure to cadmium in the vicinity of battery and electroplating plants.

Pharmaceuticals

An emerging concern in wildlife toxicology is the potential adverse effects of pharmaceutical compounds. Every day, hundreds of different pharmaceuticals, including birth control chemicals, antidepressants, and antibiotics, are ingested by humans, and growth hormones and medications are used in the animal production industry. A portion of these compounds, or their by-products, are eliminated in feces and urine, and pass either by direct discharge or via sewage systems into streams and rivers. Small quantities of these pharmaceuticals from many sources result in the potential exposure of fish, amphibians, and other aquatic wildlife to a 'cocktail' of pharmaceuticals. The effects of this exposure is just beginning to be understood, but may include

suppression of motor function, reduced or delayed growth, immunological suppression, or impaired reproduction.

Because of adverse effects observed in wildlife due to historic use of chemicals in the environment, and because of accidental spills and releases of various chemicals, laws and regulations have been developed in most countries for the regulation of chemical use. Today, the development of new chemicals intended for environmental use incorporate extensive testing procedures to evaluate potential impacts to wildlife. The intent is to avoid adverse effects to wildlife similar to those that have occurred in the past. Because of existing regulations, wildlife toxicologists today are not usually faced with high concentrations of a specific chemical in the environment, except possibly near a pollution source or as a result of a spill, but rather with exposure to low levels of multiple chemicals. Because wild species integrate contaminants over time and space, they provide a biological mechanism for evaluating the accumulation and effects of these contaminants. A major difficulty for the wildlife toxicologist is determining that an adverse effect has occurred and determining the severity of that effect. In evaluating effects in wildlife, the toxicologist must rely on environmental concentrations (exposure concentrations) or tissue concentrations that can be related to laboratory and field data associated with adverse effects, or upon morphological and physiological changes (biomarkers) that indicate that exposure has occurred and resulted in an adverse effect in the organism. This assessment of risk to wildlife will continue to be a challenge given the potential exposure of wild species to the large number of existing chemicals and to new chemicals that are continuing to be developed.

See also: Chemicals of Environmental Concern; Ecotoxicology; Environmental Toxicology; Polychlorinated Biphenyls (PCBs).

Further Reading

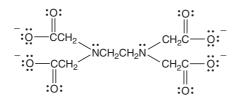
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EDTA (Ethylenediaminetetraacetic Acid)

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- CHEMICAL NAME: Ethylenediaminetetraacetic acid
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 60-00-4
- SYNONYMS: Ethylenedinitrilotetraacetic acid; Celon A; Cheelox; Edetic acid; Nullapon B Acid; Trilon BW; Versene
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organic chelating agent
- CHEMICAL FORMULA: C₁₀H₁₆N₂O₈
- CHEMICAL STRUCTURE:



Uses

EDTA is used as a food additive, in herbicides, in pharmaceuticals, and in a variety of consumer products. EDTA is used as a blood preservative by complexing free calcium ion (which promotes blood clotting). EDTA's ability to bind to lead ions makes it useful as an antidote for lead poisoning. Furthermore, EDTA is often used to treat various cardiovascular diseases.

Background Information

EDTA is a white, odorless, crystalline (sugar or sandlike) material. It has a molecular weight of 292.28 and its melting point is 240°C. It is water insoluble.

Exposure Routes and Pathways

The most probable routes of human exposure to EDTA would be ingestion and dermal contact. Workers involved in the manufacture or use of EDTA may be exposed by inhalation and dermal contact. In chelation therapy, EDTA is administered via intravenous infusion.

Toxicokinetics

EDTA is essentially not metabolized by the human body and it is rapidly excreted in the urine. About 50% of EDTA administered intravenously is excreted within 1 h and 90% within 7 h. EDTA and its metal chelates do not permeate the cellular membrane to a significant extent; thus, most of the EDTA remains in the extracellular fluid until excreted in the urine.

Mechanism of Toxicity

The principle toxicity of EDTA relates to the metal chelate, especially in lead poisoning. Lead may be released from the chelate in the kidneys, and then the lead may affect the tubules and glomeruli of the kidneys.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, more live fetuses resulted when calcium disodium EDTA was used to treat lead poisoning. However, in rats that were not lead poisoned, increases in submucous clefts, cleft palate, syndactyly, adactyly, abnormal ribs, and abnormal vertebrae occurred. Furthermore, the doses of EDTA in the study were comparable to those used in man and without noticeable changes in the mother. Since zinc calcium EDTA did not cause teratogenicity at low levels in rats, zinc calcium EDTA should be available for use in pregnancy.

Human

Cases of anuria have been reported when EDTA was administered to treat lead poisoning. Such kidney injury is reversible and is probably not due to the chelate directly, but to the reabsorption of the metal in the tubules. Of 130 children that received dimercaprol and EDTA, 3% developed acute renal failure and 13% had biochemical evidence of nephrotoxicity. However, lead poisoning can cause kidney injury without EDTA therapy. In another study, 122 patients were given EDTA and none showed posttreatment increases in plasma creatinine.

Reversible mild increases in plasma hepatic aminotransferase activities are frequently reported after use of EDTA. Furthermore, extravasation may result in development of painful calcinosis at the injection site.

In a workplace setting, the following acute health effects may occur immediately or shortly after exposure to EDTA: contact may irritate the skin causing a rash or burning feeling; contact with high concentrations may irritate the eyes; and inhalation of EDTA dust may irritate the nose and throat.

Chronic Toxicity (or Exposure)

Animal

Laboratory studies on various animal species as well as reports from veterinary practices have revealed that long-term therapy with EDTA may cause deficiencies in zinc and vitamin B_6 .

Human

Prolonged systemic therapy with EDTA has resulted in zinc and vitamin B_6 deficiencies. Furthermore, febrile reactions with headache, myalgia, nausea, vomiting, lachrymation, nasal lesions, glycosuria, hypotension, and electrocardiographic (ECG or EKG) changes have been reported.

In Vitro Toxicity Data

All known pharmacological effects of EDTA result from formation of chelates with divalent and trivalent metal ions in the body. Also, the effects on rat liver glucocorticoid receptor *in vitro* have been studied. At 4°C, 10 mmol EDTA had a stabilizing effect on unbound hepatic glucocorticoid receptors. Apparently, endogenous metal ions are involved in the processes of glucocorticoid–receptor complex stabilization and transformation. Furthermore, EDTA increases the absorption of a number of agents. This effect is nonspecific because EDTA increases the absorption of bases, acids, and neutral compounds. It appears that by chelating calcium, EDTA causes a general increase in membrane permeability.

Clinical Management

In case of contact with EDTA, the eyes should be flushed immediately with running water for at least 15 min. Affected skin should be washed with soap and water. Contaminated clothing and shoes should be removed and isolated at the site.

Exposure Standards and Guidelines

The FAO/WHO acceptable daily intake for calcium disodium edetate as a food additive is 2.5 mg kg^{-1} body weight (1.9 mg kg⁻¹ of body weight as the free acid).

See also: Food and Agriculture Organization of the United Nations; Lead.

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Education and Careers in Toxicology See Toxicology, Education and Careers.

Effluent Biomonitoring

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Introduction

The Clean Water Act mandates the reduction of water pollution sources, largely through control of point source discharges. Under the National Pollutant Discharge Elimination System (NPDES), municipal and industrial entities that discharge wastewater (e.g., sewage, pulp, and paper) into national waterways are required to obtain a permit and meet imposed effluent limitations. These limitations, designed to be protective of water uses, human health, and aquatic life, are generally expressed in terms of a numerical limitation for a specific chemical (e.g., $10 \,\mu g \, l^{-1}$ of copper). However, one shortcoming to chemical-specific requirements is that bioavailability and toxicity of multiple chemical species in complex effluents are not directly evaluated, thereby providing little information on an organism's ability to integrate the effects of prolonged exposure to multiple contaminants.

In addition to chemical-specific requirements, toxicity requirements can also be imposed to ensure that the discharge is meeting the goal of protecting aquatic biota. NPDES permit requirements for aquatic toxicity may be numerical limitations or merely biomonitoring requirements. Whole-effluent toxicity (WET) testing monitors whether the discharge is likely to be toxic to aquatic life by exposing test organisms to various concentrations of the effluent and observing their response. The principal advantage to WET testing over chemical-specific requirements is the direct and integrated biological assessment of antagonistic, synergistic, or additive effects from multiple chemical interactions. During both acute (short-term) and chronic (long-term) WET exposures, generally invertebrate and fish species are challenged with various concentrations of effluent under controlled conditions, and lethal or sublethal end points are measured. When conducting compliance biomonitoring, if toxicity is observed above a certain magnitude, defined in terms of acute or chronic 'toxic units' (TUa and TUc), duration (e.g., time-averaged in-stream concentration), and frequency (e.g., not to be exceeded more than once every 3 years), additional studies may be required to evaluate, treat, and reduce effluent toxicity. (The TUa is defined as 100/median lethal concentration (LC_{50}) , and the TUc is defined as 100/no-observedeffect concentration (NOEC), or 100/chronic value which is defined as the geometric mean of the lowestobserved-effect concentration (LOEC) and (NOEC). In addition, under the US EPA Total Maximum Daily Load (TMDL) program, limits have also been established for toxicity, expressed as WET-derived toxic units, considering both point and nonpoint sources.

This entry is intended to provide an introduction and practical analysis of the current standardized aspects of aquatic toxicity biomonitoring of industrial and municipal effluents. These tests and monitoring programs are needed to determine whether management requirements or regulatory criteria are being met as well as to assess the temporal and spatial trends in water quality.

Acute and Chronic Tests

A battery of aquatic biological tests has been developed to evaluate the toxic effects elicited from effluents. For all the types of toxicity tests, selection of exposure concentration and duration, test species and strains, and monitored water quality parameters are critical. The short- and long-term methods for evaluating the potential of toxic discharges have been well characterized.

In general, weaknesses in the results of aquatic biological tests include the following: (1) lack of ecological relevance due to laboratory culture conditions (e.g., test organisms more or less sensitive, more or less crowded or stressed, than indigenous species); (2) restriction to monitoring a single life stage of uniform size under a set of controlled conditions (vs. varying temperatures, seasons, food supply, predation, etc.); and (3) lack of direct relevance to the susceptibility and bioavailability of other life stages.

In addition, laboratory-prepared water used for diluting the effluent is relatively free of suspended solids, humic matter, and other varying components (e.g., hardness level) that may serve as nutrients, provide sorption sites, and have direct effects on the functional expression of toxicity. Thus, toxicity as expressed by the LC_{50} should not be viewed as a biological constant but as a value that varies with age and physical, chemical, and biological factors.

Acute Toxicity Tests

Acute bioassays are designed to assess the effects of toxic substances that occur within a relatively short period after exposure (48–96 h). The effluent toxicity elicited by test organisms is often fatal and rarely reversible. The relevant information to be gained from this type of test is the distribution of the exposure–response relationship and the nature and potency of the toxic effects, such as immobility, percentage mortality, and time interval to mortality.

Ideally, the exposure duration should be long enough to allow for steady-state conditions (body burden and elimination of toxics) to occur, although this is not always achieved.

The responses monitored are generally binary variables – meaning 'all or none' (e.g., mobile or immobile) – with known sample size and unknown probability, versus more graded or continuous observations.

Although crude as an end point, mortality is highly visible, clearly defined, and measurable and has been utilized as a first tier in hazard prediction. The acute procedure is often applied in situations in which the effluent is diluted at least 80 times; if there is less dilution of the effluent by the receiving water, then the 7 day chronic test is performed often.

Acute tests can be further divided with respect to flow regime: typically either static or static-renewal. During the static procedure, test organisms are exposed to the environmental sample in relatively nontoxic and nonreactive cups, glass beakers, or aquaria and the test solution is not changed. Advantages to this design include ease of operation, minimal space needed, and minimal waste generated. This system is relatively simple and cost-effective for evaluating large numbers of samples or where only limited volumes of effluent are available. However, the concentrations of the effluent components - chemical compounds and their degradation products, dissolved oxygen, metabolic by-products, and hydrogen ions - may change throughout the test due to complex biochemical events: uptake by the test organism, adsorption on the organism or on the walls of the test container, biodegradation, vaporization, and precipitation. The potential accumulation of metabolic or other wastes may lead to undue stress on the test organisms and variable results. The application of the static-renewal test may minimize these difficulties.

During the static-renewal acute test, the exposure solutions are periodically renewed (24 or 48 h) either by carefully transferring the test organisms to a freshly prepared exposure medium or by gently decanting and refilling test containers. In contrast to the static procedure, the static-renewal system is designed to mimic more natural intermittent exposure scenarios (e.g., acid rain and agrochemical applications) and mitigate the changes induced by unstable, volatile, or high oxygen-demanding effluents.

Chronic Toxicity Tests

Chronic whole-effluent toxicity tests involve exposing organisms (usually emphasizing presumably sensitive early life stages, such as neonates or newly hatched larvae) to various effluent concentrations for a period of typically 7 days, during which the exposure solutions are renewed daily. The 7 day cladoceran survival and reproduction test is one of several procedures employed for estimating the chronic toxicity of effluents. Generally, these longer term tests provide more information on effluent effects such as fecundity, growth, reproduction, life span, behavior, and mortality.

Specific exposure concentrations for effluent dilution are often evenly spaced in a linear or geometric series (e.g., 100%, 50%, 25%, 12.5%, and 6.25% effluent). Replicate exposures are required, with test vessels arranged in the incubator in a randomized block design. At the end of the test, statistical calculations of NOECs for survival and reproduction or growth are typically performed as a multiple comparison procedure of effluent-treated groups. The induced effects of effluent pollutants on growth or reproduction and on survival may or may not be equal, suggesting information on the relative sensitivities of the test organisms. The test end points and duration are assumed to demonstrate that the aggregated substances in the effluent have or have not been protective of aquatic life.

Advantages to the chronic test include its attempts to detect longer term, and perhaps more subtle, sublethal, toxic effects by studying relatively susceptible neonates or larvae and their reproduction or growth. The 7 day chronic procedure may mimic intermittent and fluctuating pollutant exposures in nature through daily renewals, thereby allowing for organismal recovery, adaptation, acclimation, or simple stressor avoidance.

In general, a greater proportion of aquatic organisms are exposed to sublethal concentrations of toxics compared to acutely lethal concentrations. There is general acceptance that chronic exposure of fish to sublethal levels of toxics makes them more prone to disease states. Although scientifically controversial, intuitively one can grasp how individual responses such as growth, reproduction rates, and survival probability, as a function of age, could relate to population level responses.

One limitation to chronic WET tests is that they do not indicate which stressor(s) is causing the observed effects. Furthermore, they are generally subject to false positives (type I error) and false negatives (type II error) because of weak correlation between WET tests results and in-stream effects. For instance, during the 7 day fathead minnow survival and growth test, growth rate end points (dry weights compared to controls) may not be a reliable indicator of latent toxic effects since there is potential for reproductive failure in successive generations even in the absence of statistically poor growth.

A related general weakness in WET testing schemes involves the natural 'variability' of effluents, and whole-effluent tests, which may be unrelated to the actual effluent toxicity but related to short-term spatial, temporal, and seasonal variation at a site. Interpretation of WET data is therefore complicated, as one may not be able to easily compare to the reference values like one can with chemical analyses. More frequent effluent testing may identify these atypical toxicity responses.

Another major disadvantage of the chronic WET tests is referred to as 'simulated toxicity', or 'pathogen interference', whereby the observed toxicity is attributable to adverse interactions from biological growth of freshwater pathogenic (e.g., *Aeromonas hydrophilia*) or sheathed (*Sphaerotilus natans*) bacteria or fungi on the test organisms and is not necessarily a manifestation of chemical constituents present in the effluent. The microbial grow-th, and resulting anomalous WET results, may be a result of contaminated culture conditions (e.g., masses of bacterial growth surrounding brood pouch eggs), contaminated sampling equipment, or microbial proliferation due to nutrients associated with the effluent or laboratory culture environment.

Statistical disadvantages in chronic testing data analyses have been presented, suggesting that NO-ECs are misleading and should be phased out of regulatory use. This research suggests that NOEC concepts do not consider basic variability and are artifacts of the test design in terms of effluent concentrations and intervals chosen. Another concern is that WET data are bounded, and it is not possible to measure effects beyond 100% effluent; thus WET data as expressed in toxic units cannot be measured as values <1, leading to difficulties in statistical analysis and data interpretation. As an alternative, regression analysis has been proposed as a more robust procedure than traditional hypothesis testing to mitigate the problems of violation of assumptions, experimental error, and variability in test protocols.

Physical, Chemical, and Biological Test Factors

Although variability is inherent in all environmental measurements, a variety of physical, chemical, and biological factors should be noted in compliance biomonitoring to enhance the consistency and defensibility of toxicity test results. These various parameters may affect the effluent toxicity to aquatic biota, and it is important that the investigator take them into account. Test conditions should mimic receiving water conditions, whenever feasible, to allow accurate assessment of the in-stream effect of an effluent. However, the use of upstream water for dilution should be avoided due to the potential variability in quality, or toxicity, over the testing period.

Physical considerations of particular importance in aquatic toxicity testing include exposure temperature, periodicity and intensity of light, test organism loading, test duration, laboratory equipment, test methods, and data recording. For instance, control and test solution volume should be sufficient to allow organisms free mobility, to provide adequate supply of dissolved oxygen (e.g., $>4 \text{ mg l}^{-1}$), and to prevent buildup of metabolic waste products such as ammonia. Chemical considerations of interest that contribute to the observation of WET toxicity include pH, alkalinity, hardness (as well as the ratios of calcium and magnesium), salinity, dissolved gases, organics, inorganics, sediments, and humic substances. For instance, the bioavailability and toxicity of metals to aquatic biota are known to differ with pH, hardness, and oxidation state. Fluctuations of pH levels of a receiving water can result in over- or under-predicted effluent toxicity. Biological factors inherent to biomonitoring include test organism age, class, size, genetic state, acclimation to culture conditions, nutritional health, parental care, and food quality and quantity. For instance, dietary factors such as algal digestibility and availability affect test organism body size, sensitivity, and performance, as evidenced in *Ceriodaphnia* reproduction tests.

Laboratory Dilution Water

Water selected for diluting the effluent should be of uniform quality, free of contaminants (e.g., benzene, pesticides), and available in large enough volumes for culture maintenance, acclimation, and testing. Based on these minimal requirements, synthetic laboratory water prepared with reagent-grade chemicals and deionized water is particularly useful for most small laboratory operations and where precise water quality parameters such as alkalinity, hardness, pH, and specific conductance should be measured to check for consistency between batches, as part of good laboratory practice. However, if a variety and numerous types of toxicity tests are performed routinely, then a high-quality surface water such as a large lake may be desirable. Generally, lake water is less prone to changes in quality (suspended solids, organic matter, and runoff contaminants) that may affect results compared to river water. A municipal water supply is least desirable for laboratory diluent due to potential interferences from the constant oxidation of residuals needed for safe and potable water.

Test Organism Selection and Culture

Invertebrates and fish are typically used in toxicity testing of effluents and are the primary focus of most historical and present methods (ASTM, US EPA, and OECD). For acute and chronic effluent testing, the most typical invertebrate species is the water flea (Daphnia magna, D. pulex, or Ceriodaphnia dubia), and the routine vertebrate test species is the fathead minnow (*Pimephales promelas*). These test species have held regulatory acceptance and meet regulatory guidelines. Another reason these test organisms have been favored is their relatively small size, lending themselves to practical considerations such as availability of the effluent and sensitivity to toxics. Aquatic criteria often require acute and chronic toxicity data on a range of organisms representing multiple trophic levels. Standardized culturing techniques are required, and should be refined, to assure the body size, performance, health, and sensitivity of test organisms. The quality and quantity of food for cultures – microalgae (*Selenastrum capricornutum*), cereal leaves, trout chow, yeast, rotifers, or brine shrimp – are critical for individual energy assimilation and balance and for precise and quantifiable data.

Good Laboratory Practice

Good laboratory practice regulations govern the planning, experimental design, and conducting of whole effluent toxicity studies and are described in federal publications and toxicity testing manuals (e.g., Code of Federal Regulations, USA). In laboratories, much cost and effort are associated with the handling of samples and information as well as the test itself. Good laboratory quality assurance begins before a sample is accepted, carries through during sample log-in and testing, and continues after completion of the analysis.

This system of quality assurance was introduced to ensure that toxicity tests are competently performed and that data are not fabricated. A sound, written quality assurance program is an essential basis for laboratory operation and is often found in the form of standard operating procedures. The quality assurance program functions not only to monitor the reliability of data recorded and reported but also to control data quality in order to meet regulatory requirements. Clearly written protocols for each procedure employed eliminate or reduce errors in laboratory operation caused by factors such as personnel (qualifications and technical competence), supplies (e.g., purity of reagents), equipment calibration and maintenance, sample storage and handling, and analytical methods. Quality control describes the set of quantifiable measures, including established protocols and standard equipment, used to define daily laboratory activity.

The good laboratory practice criteria for whole effluent toxicity tests include species acceptability, exposure system conditions, physical and chemical conditions, and statistical data analysis methods. For instance, the test acceptability criteria for the larval fathead minnow 7 day chronic tests involves having 80% or greater survival of controls and an average dry weight of surviving control fish equal to or greater than 0.25 mg.

Sample Collection and Handling

Care should be taken that the sample collected is representative and undergoes minimal changes prior to toxicity evaluation. Hence, a 24 h composite sample obtained with a refrigerated, proportional flow sampling device is a good choice. This type of sample could then be readily shipped to the contracting laboratory in a cooler packed with ice to maintain sample integrity. A 'chain-of-custody' form must accompany the shipment indicating the source and type of sample, time of collection, whether pre- or postchlorination, and the name of the individual who collected the sample.

Upon arrival, the sample should be logged in the laboratory record book indicating time of arrival, sample temperature, and pH. The sample should in all cases be analyzed for residual chlorine and, if present, oxidized with sodium thiosulfate before it is employed for toxicity evaluation. A portion of the sample should also be removed for alkalinity and hardness analyses. Often it is necessary to coarse filter the sample to remove floc or suspended debris before testing; however, this practice may reduce the sample's toxicity. The remainder of the sample should be kept at 4°C for a period not exceeding 72 h after initial sample collection. It is desirable to employ two separate 24 h composite samples for performing a 96 h acute larval fathead minnow test. This would allow renewal after 48 h exposure. In the 7 day tests with Ceriodaphnia and Pimephales, three separate 24 h composite samples should be employed for daily renewal of the various exposure solutions. Toxicity data summary sheets should include daily routine physico-chemical measurements and sample information. It is essential that good laboratory practices be used in all aspects of sample collection, treatment, and analysis to obtain quality and defensible results.

Data Analysis

Statistical methods in effluent toxicity evaluations enable the investigator to quantify the observed exposure–response relation, with reference to the desired end point. The resulting statistical confidence interval in the data may then be used to ensure test reproducibility, to compare multiple test results, and for regulatory decision-making.

Statistical methods frequently employed in effluent toxicity evaluations include point estimation technique such as probit analysis, and hypothesis testing like Dunnett's analysis of variance (ANOVA). Point estimation technique enables the investigator to derive a quantitative dose–response relationship. This method has been generally applied to statistical analyses of acute effluent monitoring data.

Hypothesis testing is generally used as a qualitative measure to evaluate whether the differences between treatments and control are statistically different, especially in chronic bioassays. However, this approach has a major limitation in determining biological end points; inasmuch as potential end points are controlled by the selected dose range, results from multiple samples cannot be compared in a quantitative manner.

Commonly used statistical software includes Tox-Stat (West, Inc., USA), SAS (SAS Institute Inc., Cary, NC, USA), and various US EPA provided programs.

Acute Methods

Many models have been used to calculate EC_{50} or LC_{50} values from acute effluent monitoring data, such as graphic interpolation, moving average, probability unit (probit), logistic unit (logit), Litchfield–Wilcoxon, and Spearman–Karber (often trimmed) methods. The challenge then arises as to which model to choose, given the toxicity data. The most obvious choice is the model that holds the most biological support. However, the answer is that there is not much biological basis for these models, and the investigator is left to choose the most appropriate statistical procedure based on the test results and standard methods.

A parametric probit method can be applied to, for example, determination of LC_{50} only when the test data contain two or more partial mortalities, and are proved to be appropriate for such method by significant chi-square tests. The probit and logit models assume that the organism tolerances have a lognormal distribution, with a typical exposure-response curve given by a sigmoidal-shaped curve. Both methods utilize transformations and curve fitting of the data and require varying observations of partial mortalities. Otherwise, distribution-free Spearman-Karber (often trimmed) method is recommended. Graphical method can be used to obtain LC_{50} values when no partial mortality was observed. The criterion for acceptable acute tests in terms of mortality in the controls is often specified to be less than 10%.

Chronic Methods

In terms of the statistical methods of the partial life cycle whole-effluent tests, survival, growth, and reproduction data from the 7 day cladoceran or fish exposure are often analyzed using hypothesis testing to determine 'acceptable' concentrations. In order to determine the appropriateness of using parametric statistical methods, the data are first tested for normality of distribution and homogeneity of variance, for which the US EPA recommends the use of Shapiro–Wilk's test and Bartlett's test, respectively. Kolmogorov test for normality and Levine's test for homogeneity can be also used for these purposes. Dunnett's ANOVA test is typically used for a parametric data set to compare the treatment mean with the control mean in order to obtain the NOEC and the LOEC for each end point. If either the test for normality or homogeneity fails, then the nonparametric Steel's Many One Rank test may be used when numbers of replicates among treatments are equal. Statistical analyses for sublethal end points such as growth and reproduction are generally performed with treatments of which mortalities are not significantly different from that of control. A distribution-free ICp analysis may be applied for point estimation of effect concentrations for sublethal end points such as IC_{25} . This nonparametric model uses smoothing of test data to fit the assumption of monotonic response.

Test Failures

Whole effluent toxicity tests may 'fail' primarily for two reasons: an aborted test due to a lack of good quality data generated (e.g., organism health or effluent sample exceeded its holding time of 72 h), or whole effluent toxicity is demonstrated to exceed permitted levels.

In terms of the second case, whole effluent toxicity may be the direct result of unidentified and persistent contaminants discharged to a wastewater treatment facility. These influent sources of test failures include refractory substances (pass through toxics) in effluent, such as floc and coagulating agents, or pesticides such as diazinon, which cause observed patterns of strong toxicity to the test organisms. Furthermore, ammonia may be present in concentrations that cause WET test failures even though the effluent discharge meets NPDES permit numerical limitations. Although the federal regulations do not define the 'failure' of an effluent toxicity test as an automatic permit violation (subject to fine or notice of violation), in practice, single-species WET tests have been treated in this manner. For those with permits, it is very important to read proposed or actual permit language for qualitative or quantitative information surrounding what constitutes a reasonable potential to approach exceedance of the limit, a pattern of toxicity, or a 'failure', which might trigger fines or additional testing. In fact, the significance of episodic exceedance of WET limits should depend on a host of additional factors, such as the receiving water conditions, mixing zone, and duration of events.

Often, regulatory requirements are such that test failures trigger additional evaluations. As part of the toxicity-based approach to effluent permitting, toxicity identification and reduction evaluations (TI/RE) schemes were implemented to identify and reduce the toxic components of complex effluents (e.g., un-ionized ammonia, hexavalent chromium, selenium, and chlorides) and assist managers in controlling toxics. The TIE process involves the stepwise chemical manipulation (pH adjustment, filtration, aeration, C18 solid-phase extraction column, EDTA chelation, and sodium thiosulfate treatments) of the effluent to identify specific compounds causing toxicity and renders them biologically unavailable.

However, before implementing potentially timeconsuming and expensive toxicity reduction studies, it is important to understand their capabilities and limitations. For instance, past protocols have had the greatest success with effluent constituents that cause acute toxicity and have questionable usefulness for chronic toxicity. In addition, no method ensures success for very complex waste mixtures (e.g., polar organic compounds), which are exceedingly difficult to analyze.

Furthermore, in the United States a TRE may vary between states. The US EPA's TRE is based on compliance with whole effluent toxicity, but a specific state's toxic reduction evaluation may address compliance with chemical-specific water quality standards and implement action-oriented programs that provide technological solutions for pollutant removal (e.g., pretreatment facilities). Thus, various toxicity reduction options may be available and should be evaluated before initiating time-consuming and expensive studies.

Alternative Test Methods

There are major efforts in support of replacing *in vivo* vertebrate tests, refining existing approaches, and reducing test organism mortality. In terms of the adequacy or inadequacy of current *in vivo* designs, questions have arisen as to their accuracy in predicting hazards to humans and aquatic life, inter- and intra-laboratory variation, and ethical concerns on how animals are used. For instance, in the United Kingdom, fish are protected under the Animal Scientific Procedures Act of 1986 as soon as they are capable of independent feeding. Furthermore, there has been a general evolution in toxicology studies from a merely descriptive science to one describing actual mechanisms of action.

The bacterial luminescence toxicity assay Microtox (*Vibrio fischeri*) has achieved international recognition for its usefulness in detecting acute and chronic aqueous toxicity that correlates with traditional invertebrate and fish species. Moreover, microalgae (*Selenastrum*), brine shrimp (*Artemia*), plants (*Lemna*), mysid shrimp (*Mysidopsis*), and fish cells (both primary and lineages) have gained acceptance as useful alternatives for measuring aquatic toxicity. In conjunction with chemical-specific analysis, a general consensus is that a battery of biological tests should be utilized to broadly characterize toxicity because each test organism and system responds and characterizes toxicity uniquely. Overall, water quality biomonitoring must harbor goals of cost-effectiveness, sensitivity, relevance, and precise results.

See also: Biomonitoring; Clean Water Act (WA), US; EDTA (Ethylenediaminetetraacetic Acid); Good Laboratory Practices (GLP); Statistics.

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Electromagnetic Fields

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This entry gives a brief introduction to the current knowledge on the potential health effects of human exposure to electromagnetic fields, in the frequency range of 0–300 GHz. The principal characteristics of these fields are that they are of relatively weak intensity, and are at frequencies well below the range of the ionizing radiation (extreme ultraviolet, X- and γ -rays). **Table 1** shows the frequency spectrum of the electromagnetic field radiation in the frequency range of 0–10²⁰ Hz. The table also includes some examples of broadly used applications, and shows the separation between nonionizing and ionizing radiation as a function of the frequency range.

The pace in the development of electromagnetic field (EMF) technologies and the ever-increasing number of commercial applications in all areas and activities of our life is breathtaking. This is particularly true of radio frequency wireless technologies and telecommunications. Nowadays, especially in urban environments, exposure to human-made EMFs is an unavoidable fact of life, and understanding its potential health effects and possible environmental impacts and risks, a priority. Public concern, and fears, about potential adverse health effects of human exposure to electromagnetic radiation from extremely low frequency (ELF) and radio frequency (RF) sources, in particular, has grown significantly in recent years. Apart from scientific uncertainties in establishing any potential health risks, which induces the public debate on the validity of limit values, this problem is also caused by the roll out of the mobile telephone networks, and the high visibility of increasing number of base stations. This number will have to rise even more in order to implement the latest developments on mobile phone technology and this expected growth makes it imperative for public authorities to ensure transparency, trust, and confidence in decision-making.

General Definitions

The EMF has two components, the electric field (E) and the magnetic field (H), which vary in time and propagate together in space. These quantities are vectors having both magnitude and direction. The electric field exists whenever an electric charge is present, and the magnetic field arises from the motion of electric charges (an electric current). Electric fields and magnetic fields are strongest close to their

Table 1	Frequency spectrum of the electromagnetic field radiation in the frequency range of 0–10 ²⁰ Hz. It includes some examples of
broadly u	sed applications and also shows the separation between nonionizing and ionizing radiation as a function of the frequency range

Radiation type	Frequency range	Application examples
Nonionizing		
Static fields	0 Hz	Static electricity
ELF (extremely low frequencies)	50 Hz to 3 kHz (1 kHz = 10^3 Hz)	Electric power transmission, domestic power supply and appliances (50–60 Hz)
VLF (very low frequencies)	3–30 kHz	TV, video display units, induction heaters
RF (radio frequencies) 30 kHz to	30 kHz to 3 MHz (1 MHz = 10^{6} Hz)	Induction heaters, electronic surveillance
300 GHz	200 kHz to 900 MHz	Radio AM, FM, and TV broadcasting
	300 MHz to 3 GHz	Mobile telephony
	900 and 1800 MHz	GSM (European standard)
	1900 MHz to 2.2 GHz (1 GHz = 10^9 Hz)	UMTS (standard for enhanced telephony services including mobile internet)
	2.5–300 GHz	Microwave ovens, civil and military radars, satellite links
Infrared Visible	300–10 ⁵ GHz 10 ⁵ –10 ⁶ GHz	Intruder detectors, remote controls
	or wavelength 0.8–0.4 μ m (1 μ m = 10 ⁻⁶ m)	Light, lasers
Ultraviolet	0.4–0.1 μm, 0.1–0.01 μm (EUV)	Sun, phototherapy
Ionizing		
X-rays	$0.03 \mu\text{m}$ to 0.3nm (soft), < 0.3 nm (hard) (1 nm = 10 ⁻⁹ m)	Radiology
γ-rays	0.03 nm and less	Nuclear physics

sources and diminish rapidly away from them. Both fields exert physical forces on electric charges, but the magnetic field only does so when the charges are in motion. Electric fields are shielded very effectively by metal conductors, and building materials and trees also provide some shielding capability. Magnetic fields, on the other hand, are not blocked by common materials such as walls of buildings.

EMFs are wave motions characterized by their frequency or wavelength, strength (intensity), or power density. Frequency is the number of variations of the field per second, and it is given in hertz (Hz) or cycles per second. The wavelength is the distance between two consecutive maximum (or minimum) amplitudes in the wave, and the product between frequency and wavelength is the velocity of propagation of the wave, which is equal to the speed of light ($\sim 300\ 000\ \mathrm{km\ s^{-1}}$ in air). The field strength is the amplitude of the wave, and it is generally expressed in volts per meter (V m⁻¹) for the electric field and in ampere per meter (A m⁻¹) for the magnetic field. The magnetic field can also be specified as magnetic flux density (**B**) expressed in tesla (T).

The electromagnetic field strength is also expressed as an equivalent power density (S), in watts per square meter (Wm^{-2}) . The quantity S is proportional to the product of the electric and magnetic field strengths ($S = E \times H$). Away from the radiating source, the far-field plane-wave model gives a good representation of the EMF propagation and the power density is the power per unit area normal to the direction of propagation with amplitude given by: $S = E^2/377 = 377 \times H^2$. Under these conditions, exposure levels and safety limits can be specified by the power density of the electromagnetic field at a given location, or by its electric field intensity. Near the source, however, the relationship is more complicated and the spatial distributions of both the electric and the magnetic fields are highly variable. In this case, it is more appropriated to specify exposure levels in terms of the specific absorption rate (SAR), which is the power per unit mass that is absorbed by the human body. Limits have been prescribed for whole-body-averaged SAR and SAR for 1 or 10 g of tissue anywhere in the body.

Exposure to Weak-Intensity Nonionizing EMF Radiation in the 0–300 GHz Frequency Range

Common sources of low-level exposure to electromagnetic fields are electric and magnetic powered transport (static fields), overhead power lines, domestic electric appliances (ELF), antitheft electronic devices, or video display units (in very low to low frequencies). Low-frequency electric and magnetic fields interact with the electric charges in the biological tissues and induce electric currents in the body. The magnitude of these currents is a function of the electric properties of the body and of the intensity of the interacting field. Exposure to low-frequency fields normally results in negligible energy absorption and no measurable temperature rise in the body.

At higher frequencies (RF, above 100 kHz), on the other hand, energy absorption and tissue heating effects may be significant. Exposure to radio waves and the resulting energy absorbed by the body depends on many factors, such as intensity of the field (which varies with the position of the device in relation to the base stations and on the position and type of antenna), field modulation and length of exposure. In addition to the 'passive environmental exposure' from telecommunications and wireless technologies, RF emissions are absorbed from handsets by the head when in use. When the tissue is subjected to an RF field, part of the field is reflected, and part penetrates the tissue. Heat is generated inside the tissues to dissipate the currents induced by the associated RF electric fields.

Possible Health Effects

The harmful effects (thermal) of exposure to high levels of EMF radiation are both proven and acknowledged. With regard to low-level exposure, however, scientific research has not been able to establish, in a consistent and consensual manner, the existence of any causal links between exposure to weak EMF radiation sources, below the accepted limits, and possible adverse health effects. Moreover, there is no established evidence of potential harm from long-term exposure to radio frequencies. The argument is different on exposure to ELF, in this case possible connection between quasistatic magnetic fields and childhood leukemia cannot be ruled out completely. According to the International Agency for Research on Cancer classification, this connection is possible but more evidence is needed ("limited and still inconclusive evidence on causation coming from serious research and supported by consistent epidemiological studies").

Nonthermal Effects of RF

Nonthermal effects may result from the possible interactions between RF fields and the various components of the biological material. Established effects include: (1) The interference of radio frequencies with cardiac pacemakers is possible, however, new models of pacemakers are currently equipped with electronic filters making them immune to fields from telephones. (2) The microwave auditory effect (or 'microwave hearing') at radar frequencies which is indeed an extremely low level thermal effect. With mobile telephones, the energy in the pulses is too weak to produce a hearing effect. (3) Indirect effects such as induced currents from touching a metallic structure exposed to an electric field.

Other nonthermal effects have also been reported. They involve possible interactions of EMF with genetic material (genotoxic and carcinogenic effects, e.g., DNA damage, chromosome aberration, gene mutation, etc.), effects on cell membranes, effects on the thermoregulatory system, behavioral disorders and various effects on physiological systems or organs, etc. They are at the center of the debate on health effects of EMF at radio frequencies but it appears to be a general agreement among experts that supporting evidence for such reported effects is still insufficient and inconclusive, or even contradictory in a number of cases. There is also a broadly shared opinion on the necessity to support further research in this area and to keep the International Commission on Non-Ionizing Radiation Protection (ICNIRP) recommendations on exposure limits under review according to the available scientific evidence.

Risk Management and Preventative Measures

Misrepresentation of scientific uncertainties in identifying and quantifying health risks – particularly in the long term – may be contributing to public fears. It may also strengthen the feeling that any risk is unacceptable and should be banned. Public perception of technological risks, and EMF in particular seems to be distorted by the lack of a common knowledge base and of straightforward contextual references, and not least by the presence of often competing and conflicting sources of information. This makes very difficult for both citizens and decision makers to understand the actual facts and to reach an accurate view of the potential risks as well as the benefits of the EMF technologies. It also makes the task of managing and communicating risk a complex process that requires full transparency and the active participation of all parties concerned.

Preventative measures may be taken in order to avoid or mitigate possible harm from uncertain risks and this will depend on the degree of importance and indeed harm of such risks. The 'prudent avoidance' concept is defined as the full set of voluntary measures that can be taken by private individuals to minimize any unnecessary and/or easily avoidable exposure. The 'precautionary approach' is, on the other hand, a risk management tool allowing decision makers to take action when scientific indications of possible 'serious and irreversible' health hazards are judged to be sufficient to establish 'reasonable doubt'. Preventative measures, however, cannot be based on a hypothetical risk.

Exposure Limits

Guidelines on maximum exposure levels and reference values established by ICNIRP (1998) are endorsed by many countries (including the United States and European Union member states) and are generally accepted and increasingly applied across the world. Exposure limits and recommendations are based on peer-review of the scientific evidence; they apply to all devices emitting EMF and are based on established health effects (below which health is not affected, even to repeated exposure). As far as the current understanding and scientific evidence goes, these exposure limits seem to provide common, minimum requirements for health protection of the general population.

Basic restrictions and maximum exposure levels recommended by ICNIRP are listed in **Tables 2** and **3**. Basic restrictions apply equally to workers and to members of the general public. As a function of the frequency, restrictions are based on the potential of low frequency electric and magnetic fields, as well as

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Fraguanay ranga	Current density	SAR (full body)	SAD	
including high safety factor				
	sinced basic restrictions to	i exposure to Entr. Specifi		current density in numan body

Frequency range	<i>Current density</i> (mA m ⁻²)	SAR (full body) (Wkg ⁻¹)	SAR		
			Head/trunk	Limbs	
0 Hz	-				
>0–1 Hz	8				
1–4 Hz	8/f				
4–1000 Hz	2				
1–100 kHz	f/500				
100 kHz to 10 MHz	f/500	0.08	2	4	
10 MHz to 10 GHz		0.08	2	4	
10–300 GHz	$(10 \mathrm{W}\mathrm{m}^{-2})$	-			

SAR: specific absorption rate (watts per kg of body-mass or tissue).

	-		
Frequency range (f)	E-field (Vm ⁻¹)	B-field (μT)	Power density (W m ⁻²)
0–1 Hz	_	$4 imes 10^4$	
1–8 Hz	10000	$4 \times 10^{4}/f^{2}$	
8–25 Hz	10 000	5000/f	
0.025–0.8 kHz	250/f	5/ <i>f</i>	
0.8–3 kHz	250/f	6.25	
3–150 kHz	87	6.25	
0.15–1 MHz	87	0.92/f	
1–10 MHz	87/f ^{1/2}	0.92/f	
10–400 MHz	28	0.092	2
400–2000 MHz	$1.375 imes f^{1/2}$	$0.0046 \times f^{1/2}$	f/200
2–300 GHz	61	0.20	10

 Table 3
 Reference exposure levels for general public, recommended by ICNIRP, expressed in electric and magnetic field intensities, and electromagnetic field power density

RF radiation, to cause illness or injury through respectively the induction of currents, or the heating of body tissues. Across all frequencies they are supported by a solid body of observations and referred studies. The starting point at RF is the observed behavioral changes in experimental animals exposed to radiation levels raising whole-body temperature in excess of 1°C; SAR of $1-4 \text{ W kg}^{-1}$ or higher is needed to cause these changes. From this evidence and with the inclusion of a safety or uncertainty factor of 10, the value of 0.4 W kg^{-1} was proposed as the exposure limit for workers under conditions of whole body exposure. An additional safety or uncertainty factor of 5 was introduced for the general public (thus 0.08 W kg^{-1}).

See also: Radiation Toxicology, Ionizing and Nonionizing.

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Emergency Response and Preparedness

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Introduction

Everyday throughout the United States millions of tons of hazardous chemicals are produced, transported, stored, used, and disposed. Citizens live and work among a wide variety of what are considered hazardous chemicals. These chemicals are transported by trucks, trains, pipelines, and ships and are used on farms and in fixed facilities such as chemical plants. Hazardous substances also are found in many consumer products and services that we use everyday, including household cleaners, paints, batteries, dry-cleaning processes, pesticides, and many others. Under normal conditions, these substances are controlled and pose no threat to human life and the environment. But when they enter the environment through an accidental release, they can adversely affect human health, contaminate the land we use, the water we drink, and the air we breathe, with potentially disastrous results. Nobody expects an emergency or disaster in their community or at their workplace. However, the simple truth is that emergencies and disaster happen everyday, at anytime, and in any location. For example, during 2003, there were 15 100 releases of hazmat while in transportation, of which 465 were considered serious. On average, there are more than 41 incidents each day in the United States. The estimated monetary damage from these incidents is exceedingly high.

Since September 11, 2001, emergency response and preparedness for hazardous materials is shifting from hazmat releases being the result of accidents to hazmat releases being used as weapons of mass destruction. Current discussions of hazmat have identified a real potential for hazmat to be used as weapons of mass effect, which takes into account the potential effect on the public consciousness.

The following entry outlines current best practices to prepare for and respond to a release of hazmat, irrespective of whether the release is an act of terrorism or an accident. The basic tenets of response to both are consistent. These basic tenets include the following: the hazards are the same, the equipment employable for material detection and monitoring will still work, the modeling software of plume dispersion remains valid, and the personal protective equipment (PPE) will continue to function as designed. Two important distinctions need to be made. If the release is an intentional act, responders should be aware of the possibility of secondary devices that could be targeted at emergency responders. In addition, if the release is an intentional act, a crime scene will be established to gather and process evidence. The crime scene and secondary devices will not be discussed in this article.

Emergency Preparedness

Emergency Response Plan

An emergency response plan should be developed in order to delineate how a facility responds to an emergency. The first aspect of the plan is an inventory of the chemical, physical, and biological hazards associated with the facility. The list could include the storage, use, or transportation of hazardous materials, hazardous wastes, and hazardous substances. The Occupational Safety and Health Administration (OSHA), the Environmental Protection Agency (EPA), Transport Canada, and the Federal Emergency Management Agency (FEMA) provide regulations and guidance in developing emergency response plans.

OSHA requires that the plan be developed and implemented for anticipated emergencies. The plan must be written and available for OSHA's inspection. However, if the facility's procedures are to evacuate and have no employee assistance during the emergencies, the facility is exempt from having a written plan.

The written plan should include the following: (1) pre-emergency planning and coordination with outside parties; (2) personnel roles, lines of authority, training, and communication; (3) incident command; (4) emergency recognition and prevention; (5) safe distances and places of refuge; (6) site security and control; (7) evacuation routes and procedures; (8) decontamination; (9) emergency medical treatment and first aid; (10) emergency alerting and response procedure; (11) critique of response and follow-up; (12) PPE and emergency equipment; (13) coordination with local fire and police personnel and the Local Emergency Planning Committee; and (14) postincident remediation and recovery.

When developing the emergency response it is imperative to determine the capabilities of the local civil responders, including whether they are full or part-time, have hazardous material training and to what level, and have knowledge of facilities hazards and processes, and what response equipment is available. Upon completion of the emergency response plan, the plan should be evaluated through training including full-scale or tabletop exercises.

Creating the Emergency Response Team

The emergency response plan will establish the need for an emergency response team. The emergency response team can either be staffed by in-house employees or by outside contractors, or a combination of both. When outside contractors are chosen to provide a hazardous materials emergency response, they should be evaluated for their training, regulatory compliance, capabilities, equipment and response times. In order to allow the contractor to provide the best service, they should be provided with the emergency response plan along with a list of the chemical, biological, and physical hazards, including their physical state, temperature, associated process, or package. In-house responders can be fulltime, part-time, or additional-duty responders. The emergency response plan will establish the responder's required level of training and expectations.

A medical surveillance program must be developed that complies with OSHA's regulation (CFR 1910.120 (q) (9)), which requires physical exams to be offered when an employee becomes a responder. Physical exams are given yearly, sometimes after exposure or upon termination from the team.

If it is anticipated that responders will need the use of respirators while responding, a respiratory protection program must be developed in compliance with OSHA's regulations (29 CFR 1910.134).

Training the Emergency Response Team

When establishing a hazardous materials response team, OSHA's regulation for hazardous waste operations and emergency response operations and emergency response, also called HAZWOPER, must be followed. Under Title 29 CFR 1910.120, OSHA includes hazardous waste operations and emergency response, which have separate training requirements.

OSHA defines hazardous waste operations as facilities that conduct treatment, storage, and disposal of hazardous wastes, cleanup sites required or recognized by federal, state, and local governments, and cleanup operations at uncontrolled hazardous waste sites. Workers at these defined facilities or locations are either general workers or occasional workers. General workers, such as equipment operators, general laborers, and supervisors, must have 40 h of initial training with 3 days of supervised field experience. Workers who are at the site only occasionally for a specific task, such as groundwater monitoring and land surveying, must have 24 h of initial training and 1 day of supervised field experience. In addition to the initial training, general and occasional workers must be provided with an annual 8 h refresher class. Initial and refresher training should be on aspects of site safety and health plan, hazards present at the site, PPE needed, and work practice that can minimize risks from hazards.

OSHA (1910.120 (q)) defines an 'emergency response to a hazardous substance release' as employees engaged in emergency response no matter where it occurs. OSHA separates individuals who respond to these incidents into six levels, each having its own training requirement. OSHA's responder levels are First Responder – Awareness Level; First Responder – Operations Level; Hazardous Material Technician; Hazardous Materials Specialist; Incident Commander; and Skilled Support Personnel.

A responder at the First Responder – Awareness Level is in the position to witness or discover a release. They can only initiate an emergency response sequence. They cannot take offensive or defensive actions. Their training consists of the following: (1) to identify the hazardous substances and risks associated with the materials; (2) to anticipate the potential outcome of an incident; (3) to recognize the presence of a hazardous substance; (4) to understand their role as being trained to the awareness level; (5) to understand site security and the Emergency Response Guidebook; and (6) to know when additional resources are needed.

A responder at the First Responder – Operations Level can respond to the initial release to protect nearby persons, property, or the environment. They can take defensive actions, without trying to stop the release. They can also contain the release from a safe distance. They should have had at least 8 h of training or have had sufficient experience to objectively demonstrate competency at the First Responder -Operation Level. The employer shall certify that the First Responder – Operations has the following: (1) knowledge of basic hazards and risk assessment; (2) proper PPE selection; (3) understanding of basic hazardous material terms; (4) understanding of basic control, containment, and confinement; (5) knowledge of basic decontamination procedures; and (6) understanding of relevant standard operating and termination procedures.

A Hazardous Materials Technician can take more aggressive action toward hazardous materials incidents than an operations level first responder. They can plug, patch, and stop a release. Their training is of at least 24 h, equal to that of the first responder at the operation level; in addition, the technician must have competency and the employer shall certify that competency in the following areas: (1) function of the Incident Command System (ICS); (2) proper PPE selection; (3) hazard and risk assessment techniques; (4) advanced control, containment, and confinement operations; (5) decontamination procedures – or lack of decontamination; (6) termination procedures; and (7) basics of chemical and toxicological terminology and behavior.

A Hazardous Materials Specialist can respond to and support the hazardous materials technicians. They may have direct or specific knowledge of various substances and can act as a liaison between the federal, state, and local governments. Their training is of at least 24 h, equal to that of the Technician; in addition, the hazardous materials specialists must have competency and the employer shall certify that competency in the following areas: (1) proper PPE selection; (2) specialized control, containment, and confinement operations; (3) decontamination procedure; (4) ability to develop site safety and health plan; and (5) understanding of chemical, radiological, and toxicological terminology and behavior. The On-Scene Incident Commander (IC) assumes control of the incident. Their training is of at least 24 h, equal to that of the first responder at the operation level; in addition, they must have competency and the employer shall certify that competency in the following areas: (1) knowledge and ability to implement the employer's ICS and emergency response plan; (2) knowledge and understanding of the hazards and risks associated with workers working in chemical protective clothing; (3) knowledge and understanding of the state, federal, or regional emergency response plan; and (4) knowledge and understanding of the importance of decontamination procedures.

Skilled support personnel are proficient in the operation of certain equipment, such as earth-moving or heavy-lifting, which is needed temporarily to provide immediate emergency support which cannot reasonably be provided in a timely fashion by an employer's own employee or a contractor. Skilled support personnel are not required to be trained. However, they must receive an initial briefing at the site on the proper use, function, and limitation of PPE, the chemical and physical hazards involved, and the duties to be performed. All other appropriate safety and health precautions that are provided to the employer's own employees should be used to ensure the safety and health of these personnel.

All hazardous material responders shall receive annual refresher training of sufficient content and duration to maintain their competency or demonstrate competency to the employer yearly.

Trainers who instruct any of the responder levels shall have satisfactorily completed a training course for teaching the subjects they are expected to teach, such as at the US National Fire Academy, and possess training and/or academic credentials, instruction experience necessary to demonstrate competent instructional skills, and good command of the subject matter of the course they are to teach.

Equipping the Emergency Response Team

In order to provide the proper equipment to the emergency response team, a complete review of the chemical and physical hazards and the function to be performed by the hazardous materials responder should be done. This review will allow the selection of the proper PPE and response equipment. OSHA, in 29 CFR 1910.120 appendix A, describes four basic levels of protection for the hazardous materials emergency responder:

• Level A: Positive-pressure self-contained breathing apparatus (SCBA) or supplied air, totally encapsulated suit – gas tight, inner and outer gloves, chemical-resistant boots with protective toe, and hard hat.

- *Level B*: Positive-pressure SCBA or supplied air, hooded coveralls or two-piece splash clothing, inner and outer gloves, chemical-resistant boots with protective toe, and hard hat.
- *Level* C: Full-face or half-face air-purifying respirator, splash clothing or chemical-resistant coveralls, inner and outer gloves, chemical-resistant boots with protective toe, and hard hat.
- *Level D*: Coveralls, gloves, boots with protective toe, safety glasses or splash goggles, and hard hat.

When selecting the type and manufacture of the PPE, the purchaser should understand the functions being preformed by the responder and the chemical and physical hazards associated with the operation. They must also know how the operation and chemical will effect the degradation of the suit, gloves, and boots and the tactility and dexterity needed by the responder. Especially when Levels A and B equipment is in use, it is important not to overlook nonchemical hazards, such as heat stress, cold stress, slip, trip and falls, moving equipment, and lifting.

ICS and Structure

The ICS was developed in the 1970s after southern California wildfires caused the destruction of 600 000 acres and 772 structures and 6 fatalities. Congress funded a study to analyze the problems and found a lack of common organization, poor on-scene and interagency communications, inadequate joint planning, lack of valid and timely intelligence, inadequate resource management, and limited prediction capabilities. The ICS was developed as a tool for command, control, and coordination of resources at the scene of an emergency. Incident command consists of procedures for organizing personnel, facilities, equipment, and communication.

One of the most important aspects of incident command is the span of control. In an effort to minimize overtasking and to help ensure good decision making, planning, and execution, no person within the incident command has more than seven direct reports, with the optimum number being five. The IC is responsible for all aspects of the emergency. This includes, but is not limited to, hazard mitigation, remediation, safety, evacuations, and restoration. A public information officer provides press releases and briefings in order to provide information on the incident to the public and to minimize misinformation. A safety officer is responsible for the safety of the incident and always has the authority to shut down the site due to safety issues. The liaison has the responsibility to work with other agencies working outside the incident command. Operations are responsible for the mitigation of the incident. Teams, sections, or groups that could work under operations are entry, decontamination, environmental remediation, and fire suppression. Planning has the responsibility of developing the operations for the response. The planning group looks hours or days in the future and provides operations with their work plan. Logistics is responsible for obtaining all of the materials needed for the response and staging the resource(s) upon their arrival. Upon receiving the operation plan from the planning section, logistics ensures that the right materials are available to support the response. Finance is responsible to know what has been spent and what will be spent and to start paying for the response.

The incident command should not be a means to obtain control or authority for other agencies or departments, a way to subvert the normal chain of command within a department or agency, too big and cumbersome to be used in small everyday events, or restricted to use by government and departments.

Incident Analysis and Initial Response

Accidents involving hazardous materials must be evaluated and approached with great care. Absence of visible warning labels, placards, etc., does not guarantee that the material is harmless. An incident may present such a high degree of hazard that the only safe course is to evacuate or shelter in place. If you are the first on scene, your first step is to call for help, and make appropriate notifications to local, state, and federal emergency response personnel. Provide as many details as possible, such as name, location, and telephone number, location of the incident, type of vehicle or container involved, wind direction and speed, identification of any injuries, presence of smoke, fire, or fumes, presence of marking, labels, or placards, carrier or facility name, etc. After the initial report, attempt to ensure that all unnecessary people are cleared from the site. Do not smoke, use flares, shut off engine(s), and resist the urge to 'run' to the accident site and rescue injured personnel until after the materials are identified and the nature and severity of the hazard is assessed.

Remain a safe distance upwind. Use binoculars to survey the area. Make notes such as location of the injured, the surrounding hazards, location of threatened people, markings, labels, or placards, note the number and types of vehicles, the containers involved, and any visible damage and/or leakage. Note the accessibility to the site and the possible escape routes along with the weather conditions and any notable topographical features such as water bodies.

To be able to make rapid and sound decisions for appropriate resource allocation, there must be an understanding of the hazards present, location of the incident, availability of equipment, training and capabilities of the personnel, and potential for the incident to 'grow'. To illustrate the interdependencies of these criteria we can look at a couple of scenarios involving the same material and quantity released. In scenario 1, you have a 1000 gal diesel fuel spill in the parking lot of a truck stop, the parking lot is asphalt, and the parking lot runoff is to a storm water collection pond. For scenario 2, you have all the same incident facts, except that the parking lot runoff is to a stream, livestock use the stream for water, and 1000 ft downstream the water utility has an intake for the potable water system. It is evident that analvsis of a response to these scenarios would not be the same. However, the initial incident report from the truck stop or truck driver, may only be of a 1000 gal spill and the location of the incident. It is through planning, training, and communication that the additional information that is pivotal to making an appropriate and adequate analysis will be gained.

The IC must analyze the incident based on the facts and be prepared to change the response tactics and allocated resources as the situation reports mandate. The IC is a decision maker and a delegator. To be effective the IC must assign sector officer responsibilities to qualified staff, encourage ideas and opinions to be voiced through the command structure, insist on key stake-holder participation, seek the input of experts and practitioners alike, continually re-evaluate the tactics and strategy, and develop alternate plans.

An incident creates a dynamic environment, the decisions made in the initial analysis and the corresponding responses are critical to a desirable outcome. The continued thoughtful consideration of the interdependencies between the hazards, location, equipment, personnel, and cascading effects will make for sound decision making and appropriate resource allocation.

Response Actions

The method of accident mitigation is directly dependent on the type of material involved and the interaction of that material in the environment. The chemicals must be identified in order to develop a prudent response. Failure to correctly identify a material prior to mitigation may result in injury or exacerbating the incident. To help in the initial stages of an incident, Ludwig Benner developed the DECIDE Process to guide responders through this stage as follows:

DECIDE Process.

D – Determine if there is a hazard present by looking for placards, signs, labels, or shipping papers. E – Estimate likely harm without intervention: Identify the possible damage if the incident is allowed to run its course without intervention. There may be less danger to personnel, equipment, and the environment by choosing this option.

C – Choose response objectives: Select the harm you want to prevent (the exposures you want to protect against) before you act.

I – Identify action options: With your objective in mind, identify the options available to accomplish it. You must consider your practical options before you act.

D – Execute the best option: When you have multiple options, you should pick the option that provides a solution with the greatest gain and the least loss.

E – Evaluate progress: Once you have decided on a course of action, you must constantly monitor your progress.

If at all possible, the leak should be contained with dams, dikes, or secondary containment. When applicable the leak may be capped or the container can be patched. In order to minimize secondary contamination, responders' personal protective clothing should be decontaminated prior to doffing. Decontamination is also conducted on response tools and equipment.

Chemistry and Toxicology

Chemistry

When responding to hazmat incidents the general public and first responders often have difficulty in accurately determining the exact chemical(s) released. Confusion occurs because chemicals are often identified by product or trade names, placards, labels, or identification numbers, or have different synonyms. Thus, one must first ensure the exact chemical identification (ID). Product or chemical ID can best be determined by referencing the chemical's Chemical Abstract Services number (CAS#).

After identifying the exact chemical(s) for the incident, the responder needs to obtain critical information about the chemical's physical/chemical properties in order to determine the chemical's principle hazards and environmental fate (e.g., how will it behave and move in the environment).

- 1. Physical state at 20°C (68°F) is used to determine the nature of the chemical, in other words, if it is a solid, liquid, or gas at a defined, typically ambient temperature. Changing temperature may alter the physical state.
- 2. Boiling point is the temperature at which a liquid changes to a gas under standard atmospheric pressure and is used to determine if the chemical will become a gas during an incident.
- 3. Vapor pressure is a measure of the relative volatility of a chemical. A chemical with a high vapor pressure 'gives off' more vapors than a substance with a low vapor pressure at the same temperature and thus would require consideration as gas as well as a liquid or solid in a spill situation.
- 4. Vapor density is used to determine if a gas is heavier than air and thus will accumulate in low spots. A vapor density of less than 1 indicates that the vapor will be buoyant and rise in air, and vice versa. Heavy vapors present a particular hazard in the way they accumulate. If toxic, they may poison workers or responders; if nontoxic, they may displace air and cause suffocation; if flammable, they represent a fire or explosion hazard. Examples of gases heavier than air include chlorine, hydrogen sulfide, and sulfur dioxide.
- 5. Water solubility is used to determine if the chemical will mix with water, which is important in the handling and recovery of spilled material.
- 6. Specific gravity (SG) of a liquid is used to determine whether a spilled material that is insoluble will float or sink. Materials heavier than water have SGs greater than 1 and materials lighter than water have SGs less than 1.
- 7. Flashpoint is the lowest temperature at which a chemical gives off enough vapor to form an ignitable mixture with air near the surface of the liquid when exposed to an ignition source. Flash point values are used to rate the flammability or combustibility of a substance.
- 8. Autoignition temperature is the temperature at which ignition occurs without an ignition source and the material continues to burn without further heat input.
- 9. Flammable or explosive limits are the upper and lower vapor concentrations at which a mixture will burn or explode. LEL is the lower explosive limit and UEL is the upper explosive limit.
- 10. Odor data or odor threshold values are used to help determine the warning properties of the chemical (e.g., if it can be smelled at concentrations below health guidelines or standards).

Consideration should also be given to chemical incompatibilities (whether it will react with other chemicals), combustion or decomposition products (hazardous gases), and impurities or other chemicals or additives in the product.

Toxicology

Regardless of the specialization within toxicology, or the types of toxicities of major interest to the toxicologist, essentially every toxicologist performs one or both of the two basic functions of toxicology, which are (1) to examine the nature of the adverse effects produced by a chemical or physical agent (hazard identification function) and (2) to assess the probability of these toxicities occurring under specific conditions of exposure (risk assessment function). Ultimately, the goal and basic purpose of toxicology is to understand the toxic properties of a chemical so that these adverse effects can be prevented by developing appropriate handling or exposure guidelines.

Toxicologists typically divide the exposure of animals or humans to chemicals into four categories: acute, subacute, subchronic, and chronic. Of these four types of exposure, the general public, emergency responders, or nearby workers typically involve a situation in which potential exposure exists on an acute $(\langle 24 h \rangle)$ or subacute (days to months) basis. Chronic (months to years) exposure scenarios are unlikely because the majority of the release of hazardous materials is typically contained, neutralized, or remediated in a relatively short period of time. For most hazardous materials, the toxic effects that follow a single exposure are quite different from those produced by repeated exposures. For example, the primary acute toxic manifestation of benzene exposure is central nervous system (CNS) depression, but repeated exposures over a long period of time can cause leukemia. Acute exposure to hazardous materials that are rapidly absorbed into the body in most cases is likely to produce immediate toxic effects. Conversely, in chronic exposure scenarios, some immediate effects may occur after each dose received in addition to the long-term, low-level, or chronic effects of the toxic substance. Thus, emergency responders must understand the specific type of exposure scenario they are responding to in order to identify the potential type of toxicity a chemical may cause in humans.

The major routes (pathways) by which hazardous materials solids, liquids, gases, or vapors gain access to the body are the gastrointestinal tract (ingestion), lungs (inhalation), skin (topical, percutaneous, or dermal), and other parental (other than intestinal

canal) routes. Toxic agents generally produce the greatest effect in the shortest period of time when given directly into the bloodstream. However, rarely, if ever, does this route of exposure occur during incidents involving hazardous materials. During a hazmat incident, toxic agents most frequently result from breathing contaminated air (inhalation) and/or direct and prolonged contact of the skin with the substance (dermal exposure). Comparison of the lethal dose of a hazardous material often provides useful information about its extent of absorption. For example, if the lethal dose for an agent by the oral or dermal route is similar to the lethal dose after an intravenous dose, the assumption is that the toxic agent is absorbed easily and rapidly into the body. Thus, emergency responders should evaluate the LD₅₀ data (lethal dose to 50% of the test organisms) for an agent using available information such as Material Safety Data Sheets, toxicology literature, or the DOT guide book to help understand the most important routes of exposure they want to protect against. Emergency responders should also look for the concentration of the hazardous material they are dealing with and determine whether the hazardous material is a single chemical or a mixture that contains one or more chemicals at various concentrations.

The pathway by which a substance enters the body determines how much of it enters (rate and extent of absorption) and which organs are initially exposed to the largest concentration of the substance. For example, the water and lipid solubility characteristics of a chemical affect its absorption across the lungs (after inhalation), the skin (after dermal application), or the gastrointestinal tract (after oral ingestion), and the effect differs for each organ. Furthermore, the rate and site of absorption (organ) may in turn determine the rates of metabolism and excretion. So, changing the route of exposure may alter the dose required to produce toxicity, and it may also alter the organ toxicity that is observed.

Some toxic effects of hazardous materials are reversible, and others are irreversible. If a chemical produces injury to a tissue or cell, the ability of that tissue to regenerate largely determines whether the effect is reversible or irreversible. For example, most toxic injuries to a tissue such as the liver, which has a high ability to regenerate, are reversible whereas the injury to the CNS is usually irreversible because the cells of the CNS cannot divide and be replaced.

The general site in the body for toxic action of a hazardous material can be local or systemic. Local effects refer to those that occur at the site of first contact between the hazardous material and the biological system. For example, chlorine gas reacts

with lung tissue at the site of contact, causing damage and swelling of that tissue, and depending upon dose may be fatal even though very little of the chemical is absorbed into the bloodstream. Systemic effects of a hazardous material require absorption into the blood, then distribution of the agent from its entry point to a distant site in the body where adverse effects are produced. Most chemicals (except for highly reactive chemicals) cause systemic effects. Further, most hazardous materials that cause systemic effects do not cause a similar degree of toxicity in all organs. These agents typically elicit a toxic response in only one or two organs. These organs or sites are referred to by toxicologists as the target organs of toxicity. The CNS is the most frequently affected by toxicity from hazardous materials followed by the circulatory system, the blood and hematopoietic system, visceral organs such as the liver, kidney, and lung, and the skin. Muscle and bone are the least frequent target tissues for hazardous materials.

Exposure Limits Used by Emergency Responders

Perhaps the greatest danger faced by the public, workers, or emergency responders is inhalation of gases and vapors caused by the release of hazardous materials to the environment. One of the key tasks in responding to an incident involves identifying, measuring, notifying, evacuating, sheltering, or otherwise protecting populations that may be exposed to such gases or vapors. To accomplish this task, the emergency responder must identify, first, what chemical or chemicals are a human health concern from the spilled product and, second, what are the acceptable levels of these chemicals for workers, the public, etc. Thus, the emergency responder must have a working knowledge of the source and nature of available exposure limits for airborne contaminants as well as an understanding of the strengths and limitations of these exposure limits for their intended use. The following are some recognized sources for toxicology data for airborne contaminants:

- 1. American Conference of Governmental Industrial Hygienists (ACGIH), threshold limit values (TLVs) time-weighted average (TWA)
- 2. OSHA, permissible exposure limits (PELs) TWA.
- 3. National Institute for Occupation Safety and Health Administration (NIOSH), immediately dangerous to life or health (IDLH) levels.
- 4. American Industrial Hygiene Association (AIHA), workplace environmental exposure limits (WEELs).

- 5. AIHA emergency response planning guidelines (ERPGs) and temporary emergency exposure limits (TEELs).
- 6. National Academy of Sciences (NAS)/National Research Council (NRC) emergency exposure guidance levels (EEGLs) and short-term public emergency guidance levels (SPEGLs).
- 7. EPA National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances.

The ACGIH, through its TLV committee, reviews available data on chemicals to establish exposure limits for employees working in the presence of airborne chemicals/substances. The committee publishes a list of several hundred compounds and recommended exposure limits in a booklet entitled Threshold Limit Values and Biological Exposure Indices. The primary purpose of the exposure limits is to protect healthy workers in chronic exposure situations. Even though these limits are intended to prevent toxicity from chronic exposures, they nonetheless provide valuable guideposts for identifying exposure limits that will usually be decidedly safe for short-term acute exposures. Exposure limits established and published by ACGIH are of several different types:

- *TLV TWA*: The TWA concentration for a normal 8 h workday and a 40 h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effects.
- *TLV STEL*: The TWA concentration to which workers should not be exposed for longer than 15 min and which should not be repeated more than 4 times per day, with at least 60 min between successive exposures. This limit supplements the TLV TWA where there are recognized acute effects from a substance whose toxic effects are primarily of a chronic nature. STELs are recommended only where toxic effects have been reported from high short-term exposures in either animals or humans.
- *TLV-Ceiling (TLV-C)*: The concentration in air that should not be exceeded at any point of the workplace exposure. Ceiling limits may supplement other limits or stand alone.

In addition, the ACGIH occasionally enters the notation 'skin' after listed substances. This notation indicates the potential for absorption of the chemicals through the skin, eyes, or other mucous membranes and that such exposure may contribute to the overall exposure. For emergency responders, this notation indicates the need for special protective measures. The OSHA sets safe and healthy workplace standards. When OSHA was formed, they adopted the then current ACGIH TLV – TWAs and TLV-Cs as occupational exposure limits and made them federal standards. However, instead of calling them TLV – TWAs, OSHA called them PELs. OSHA has both TWA and ceiling values for various chemicals. PELs are listed in Title 29 of the Code of Federal Regulations (CFR), Part 1910, Subpart Z, General Industry Standards for Toxic and Hazardous Substances. Emergency responders should understand that ACGIH and OSHA values are not always the same for each chemical.

NIOSH defines IDLH levels as the maximum airborne contaminant concentration "from which one could escape within 30 minutes without any escape-impairing symptoms or any irreversible health effects." Since, IDLH levels are only intended for emergency situations, their concentration for a particular chemical is considerably higher than OSHA and ACGIH values.

AIHA established a committee to develop WEELs for chemicals which have no current exposure guidelines established by other organizations. There are two WEEL limits for most materials. The first is an 8 h TWA value similar to ACGIH TLV – TWA values. The second, which is only available for a limited number of cases, is a short-term TWA for exposure of either 1 or 15 min duration.

AIHA ERPG values were developed for selected chemicals by a task force made up of several major chemical companies. The results of the task force for ERPG values have three limits for each material:

- *ERPG-3*: The maximum airborne concentration below which, it is believed, nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects.
- *ERPG-2*: The maximum airborne concentration below which, it is believed, nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible adverse or other serious health effects or symptoms which could impair an individual's ability to take protective action.
- *ERPG-1*: The maximum airborne concentration below which, it is believed, nearly all individuals could be exposed for up to 1 h without experiencing or developing health effects more severe than sensory perception or mild irritation, if relevant.

In addition, TEELs are provided for 676 chemicals. The TEEL is an interim, temporary, or equivalent exposure limit for which official ERPGs have not yet been developed.

NAS/NRC has published a list of EEGLs and SPEGLs as guidance in advance planning for the management of emergencies.

SPEGLS are concentrations whose occurrence is expected to be rare in the lifetime of any one individual. These values "reflect an acceptance of the statistical likelihood of a nonincapacitating reversible effect in an exposed population while avoiding significant decrements in performance." They are considered concentrations acceptable for public exposure during emergencies.

EEGLs differ from SPEGLs in that they are intended to apply to defined occupational groups.

The National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances is developing AEGLs on an ongoing basis to assist federal and state agencies and private sector organizations with their need for short-term hazardous chemical exposure information. AEGLs represent short-term threshold or ceiling exposure values intended for the protection of the general public, including susceptible or sensitive individuals, but not hypersusceptible or hypersensitive individuals. AEGLS represent biological reference values for this defined human population and consist of three biological end points for each of four different exposure periods of 30 min, and 1, 4, and 8 h.

- AEGL-1 is the airborne concentration (expressed as ppm or mg m⁻³) of a substance at or above which it is predicted that the general population, including susceptible but excluding hypersusceptible individuals, could experience notable discomfort. Airborne concentrations below AEGL-1 represent exposure levels that could produce mild odor, taste, or other sensory irritations.
- AEGL-2 is the airborne concentration (expressed as ppm or mg m⁻³) of a substance at or above which it is predicted that the general population, including susceptible but excluding hypersusceptible individuals, could experience irreversible or other serious long-lasting effects or impaired ability to escape. Airborne concentrations below AEGL-2 but at or above AEGL-1 represent exposure levels that may cause notable discomfort.
- AEGL-3 is the airborne concentration (expressed as ppm or mgm⁻³) of a substance at or above which it is predicted that the general population, including susceptible but excluding hypersusceptible individuals, could experience life-threatening effects or death. Airborne concentrations below AEGL-3 but at or above AEGL-2 represent exposure levels that may cause irreversible or other

serious, long-lasting effects or impaired ability to escape.

In addition to the above emergency response toxicity values for various hazardous materials, various states have developed their own values for acute and chronic situations.

Monitoring and Detecting Hazardous Substances

Detecting and quantifying gases, vapors, aerosols, and particulates during a chemical emergency is essential to protect both response workers and members of the community. Proper air monitoring/ sampling can be used to determine a multitude of key questions during a chemical incident including: (1) Can workers safely perform their necessary tasks? (2) Does PPE need to be upgraded or can it be downgraded? (3) Do evacuation zones need to be expanded or can they be reduced?

There are essentially two types of methods used to detect and quantify hazardous chemicals - air monitoring and air sampling. Air monitoring involves the use of direct reading instruments or real-time instruments. These instruments can be used to obtain nearly instantaneous concentrations of a hazardous substance in the field. Direct reading instruments generally have a short response time from seconds to minutes, depending on the type of instrument. Results are usually displayed on an LCD screen or a color change can be compared with a calibrated scale as in the case of colorimetric detector tubes. Many direct reading instruments are capable of data logging, which alleviates the need for the user to hand log every reading and allows more detailed statistical analyses downstream. There are many different subclasses of real-time instrumentation such as photoionization detectors (PIDs), flame ionization detectors, combustible gas instruments, infrared spectrophotometers, ultraviolet (UV) spectrophotometers, electrochemical sensors, and colorimetric devices. Direct-reading instruments are powerful tools in the field because important decisions can be made in a relatively short time frame.

Direct-reading instruments can be used to monitor a specific chemical or can be used to monitor multiple chemicals. All direct-reading instruments are designed to operate in a specific detection range, which depends on the manufacturer and the properties of the chemical. All direct-reading instruments should be calibrated prior to performing any type of analysis regardless of the circumstances in which they will be used. It is also important to pay close attention to the particular manufacturer's technical notes regarding the instrument. For example, every PID operates on the same fundamental principle; however, correction factors for a specific chemical could vary greatly between instruments by different manufacturers.

As opposed to air monitoring, air sampling involves collecting an air sample for subsequent analysis by a laboratory using one or multiple methods such as ion chromatography, gas chromatography, or high-performance liquid chromatography. Air sample collection can be performed using several methods and mediums. These mediums include sorbent tubes, filter cassettes, SummaTM canisters or mini cans, and impingers. The method used to collect an air sample depends on the properties of the chemical being studied and the goal of the study. With the exception of the SummaTM canister, a sampling pump is used to pull air through a medium, such as a sorbent tube, at a determined flow rate. The sampling pump is calibrated both before and after the sampling event and an average flow rate is obtained. The flow rate along with the total sampling time is used to determine the volume of air that has passed through the medium and thus a concentration can be determined.

SummaTM canisters are evacuated cylinders that allow the attachment of regulators that collect air over a preset time period. Common regulators are instantaneous (grab), 8 h, and 12–8 h regulators.

Industrial Hygiene Issues

Emergency personnel responding to an incident are generally focused on a variety of concerns such as controlling a leaking or venting container, evacuating the area, or assessing the situation. When performing these activities, they are often exposed to a variety of hazards. These hazards can be classified as chemical, physical, radiological, or biological.

Hazards associated with exposure to chemicals are usually the primary concern. However, other hazards may be more of a concern than the potential effects from short-term exposure to a chemical. Physical hazards include thermal stress, noise, fire and explosion, fatigue, and general safety concerns such as slips, trips, and falls, fall protection, and moving vehicles. Thermal stress (heat more so than cold) is almost always a concern for responders, particularly when working in protective clothing designed to shield them from skin contact with chemicals. In warm weather, moderate work activity in protective clothing can cause a rapid increase in the core temperature. In cold weather, the protective clothing often traps heat and insulates the responder from the cold. If the protective clothing is removed outdoors, perspiration can lower the core temperature and lead to hypothermia and frostbite. Noise from heavy equipment and remediation activities can exceed occupational levels. When working around chemicals, there are always concerns about fire and explosion. Responders are typically confronted with walking on uneven, wet, and slippery terrain. This coupled with carrying equipment and wearing protective clothing increases the likelihood of a fall. It is common for responders to work at elevated heights or below grade tasks. These conditions increase the chances of injuries to personnel or from falling objects. During most incidents the amount of vehicle and equipment traffic increases and responders must always be concerned about these potential hazards. Additionally, responders often work long hours without adequate rest between shifts and fatigue has the potential to magnify each of these potential hazards.

Air monitoring should be performed on hazmat personnel to ensure that workers are not overexposed to chemicals and to comply with applicable regulations such as the OSHA standard. Note that some chemicals have substance-specific standards that need to be followed during an incident. The OSHA has established substance-specific standards for 30 chemicals that they have identified as unique and in need of specific guidance. Employees potentially exposed to a chemical with a specific standard must be monitored and protected in accordance with that chemical's specific standard. A substancespecific standard may require integrated air monitoring for 8h TWA exposure, STEL monitoring, real-time air monitoring, or various forms of biological monitoring.

Regulations regarding the use and transport of ionizing radioactive material help to minimize the likelihood of encountering radioactive materials during an incident. However, responders should be familiar with the radiation symbol and ask if there is any potential for exposure to ionizing radioactive materials. A more likely hazard is exposure to nonionizing radiation, in particular UV radiation from the sun that can cause sunburn to unprotected skin and eye damage in a short amount of time. Other nonionizing radiation activities that responders may encounter include welding and the use of devices that use lasers.

Because of recent events, the awareness of responders to biological hazards associated with weapons of mass destruction has increased. These biological agents are certainly a matter of concern, but other biological hazards exist that responders are more likely to encounter. These include poison plants, biting and stinging insects, reptiles, and infections of cuts and scrapes. Responders who administer first aid should be trained in universal precautions for protection against blood-borne pathogens. Additionally, responders should practice good personal hygiene to protect themselves from exposure to infectious agents in water and on surfaces typically transmitted by hand-to-mouth contact.

Overview of Consequence Analyses for Emergency Planning and Response

Mathematical models provide emergency planners and emergency responders a tool to effectively evaluate hazards, plan for emergencies, and respond appropriately to incidents. With constant improvement in computer speed and network access speed, this field is one which has been experiencing tremendous growth over the last 5 years, and one which will likely continue to morph rapidly for years to come.

Consequence analyses for emergency planning generally take the form of risk assessments. Risk assessments utilize time-averaged data or probability curves to determine the likelihood that a situation poses a hazard. For example, to determine the hazard posed by a chemical facility to the surrounding area, one would compile meteorological data for the area, assess the chemical hazards stored at the facility, locate sensitive population zones, identify potential evacuation routes, and so on - defining as many variables as possible based on available data. Variables that fluctuate over time, such as wind speed or direction, can be defined by time-averaging data, or by generating a probability distribution, and utilizing values from the distribution proportionally over a number of model runs. Monte Carlo model simulations combine probability distributions of multiple variables and account for all combinations of those variables by running multiple scenarios, and essentially averaging the result.

Due to the wide range of variables present in emergency planning, computer models used for emergency planning require a great deal of time for development of input parameters. Examples of EPAapproved models which can be used for long-term planning are the Industrial Source Complex (ISC), CAL-PUFF, SCREEN3, ROADS, and MOBILE5. Each of these models is designed to address specific nuances of potential hazards. For example, it is possible to model the dispersion of biological agents (particulate matter) with ISC, while some other models do not have that capability.

Consequence analyses for emergency response address specific issues resulting from an incident. This type of consequence analysis includes what-if scenarios (e.g., what if a railcar experiences a boiling liquid expanding vapor explosion due to an impinging fire) and hazard assessment during the early minutes of an incident. The goal of emergency response modeling is to aid the first responder in critical decision making, such as when to shut down roads or stand down evacuations.

Unlike modeling for emergency planning, emergency response modeling utilizes on-site information specific to an ongoing incident. On-site information such as maps, meteorological data, topographical data, and others must be readily available to the model, and easy to input. Time is of the essence when modeling during an emergency. Models that are used for this purpose include ALOHA, DEGADIS, and SLAB (dense gas models), SAFER STARTM (for mobile sources), SAFER REAL-TIMETM (for fixed facilities), SEVEX, and others.

Establishing and Managing Hazard Zones

In establishing a hazardous materials emergency response, three hazard zones should be established, namely, the exclusion, contamination reduction, and support zones. In the exclusion zone, a high level of contamination is present and overexposure, without the use of PPE, is likely. Therefore, PPE is typically required. Personnel, with the exception of skilled support personnel, must be trained. The exclusion zone may be activity-specific for operations that can generate high levels of contaminants.

The contamination reduction zone is the area between the highly contaminated area and the noncontaminated area. Reasonably, the decontamination area is often located in the contamination reduction zone. PPE in the contamination reduction zone is usually one level of protection below that of the worker in the exclusion zone. Because airborne levels within the contamination reduction zone can be unpredictable and can change quickly, evacuations within this area is recommended.

The support zone is where the majority of the incident command structure is located. No exposures are likely in the support zone. No training or PPE is required in the support zone.

The zones are determined by using air monitoring and by identifying the type of operations to be conducted. Natural barriers, such as roads, building, and trees, are a good way to define where the zones should be located. If natural barriers are not available, barrier tape can be used to demarcate the different zones.

Triage and Medical Case Management

Sorting and prioritizing patients for treatment is referred to as triage. Triage establishes the organizational framework to provide the greatest good for the greatest number of casualties. The word triage comes from the French word 'trier' meaning to sort or to cull. Triage is not a static process but one that occurs at every level of medical care and preferably several times. The first triage performed will be at the scene of the incident, the second upon arrival at a medical facility and subsequently with each patient encounter in the medical treatment process.

In order for healthcare providers to triage chemically exposed casualties effectively and appropriately, they must be provided with accurate and timely information about the chemical release. This information should include a description of the chemical of concern including synonyms, physical properties, and applicable exposure standards. In addition, healthcare providers will need pertinent toxicological information on the potential routes of exposure involved, target organ systems, dose–response relationships, potential exposure sequelae, the risk, if any, of secondary exposure to healthcare staff and facilities, and recommended medical procedures to be followed for exposed individuals.

An important component of responding to a significant chemical incident is to prepare and provide a Public Health Statement/Patient Information Sheet to the healthcare facility. The healthcare provider can then disseminate this objective toxicological information to all treated individuals and the community at large to both educate and allay fears within the local population.

Healthcare facilities need to expect that in case of a chemical event, many people are likely to selfevacuate and present to medical facilities. In order to maintain some control, it is recommended that all access and egress at treatment facilities be controlled and monitored to prevent contamination of noncontaminated individuals and facility areas. It is helpful to have law enforcement involved to provide security and crowd control.

Site Remediation

Site remediation is the environmental cleanup of the site after the emergency has been completed. Gross contamination should be removed and properly disposed in accordance with applicable state and federal regulations. Cleanup standards are negotiated with the applicable state or federal agencies.

Postresponse Recovery

Before an incident is stood down and people return to their homes and businesses, incident command needs to be prepared to address questions such as: What was the chemical(s)? How can it affect my health? Is the air safe? Is my drinking water safe? Is my food safe (there may have been a power outage)? What about food left out on the table? Do I need to clean my house? What about my pet? Is it okay to turn on my air-conditioner and return to normal household activities? This information can be provided in a handout or at a public meeting. Also, consider providing a list of contacts and phone numbers for people to call in case they have questions regarding their health and property. After the emergency incident has been mitigated and people have returned to their homes, it is important also to address medical concerns and public relations, including risk communication. The command's public information team should continue to provide information to the public on the risks associated with the type and quantity of the material released during the incident, the status of the environmental remediation, and the amount of material still in the environment. Following the above guidelines can help restore the community

to normal operations with minimal disruption to people's lives.

See also: Chemical Hazard Communication and Material Safety Data Sheets; Environmental Protection Agency, US; Flavor and Extract Manufacturers Association; Hazard Identification; Hazardous Waste; Occupational Safety and Health Administration.

Relevant Websites

- http://www.bt.cdc.gov Emergency Preparedness and Response (from the US Centers for Disease Control and Prevention).
- http://www.epa.gov Emergency Response (from the US Environmental Protection Agency).
- http://hazmat.dot.gov Emergency Response Guidebook (from the Office of Hazardous Materials Safety, US Department of Transportation).
- http://bookstore.gpo.gov Emergency Response Publications (from the US Government Printing Office).
- http://www.fema.gov Federal Emergency Management Agency.
- http://www.csb.gov US Chemical Safety and Hazard Investigation Board.

Endocrine System

Karen Chou

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Overview of the Endocrine System

The life of a multicell organism requires coordination between organs and tissues. The endocrine system, composed of ductless secretory organs and structures, maintains this coordination by producing hormones in response to physiological and environmental changes. Hormones are discharged into the blood and lymphatic system, and transported to other parts of the body, including other hormone-secreting organs, in order to elicit characteristic cellular responses in target organs. The major endocrine organs include the hypothalamus, pituitary, thyroid, parathyroid, pancreas, adrenal, ovary, and testis. Other endocrine organs and tissues include the placenta, liver, kidneys, and cells throughout the gastrointestinal tract (Figure 1).

Hormones can be glycoproteins, polypeptides, peptides, steroids, or modified amino acids. They function as messengers traveling through the bloodstream to target tissues and organs, where they bind to surface or nuclear receptors and regulate gene expression, ion channels, or enzyme activities. The major target organs and tissues include mammary glands, reproductive organs, bone, muscle, and the nervous system.

Endocrine organs function to maintain a relative balance of cellular parameters in the body through the hormones they produce. Hormones are produced in response to changes in the external environment and the internal physiological status. Examples of external signals include light cycles, temperature, nutrient availability, and toxicants. Disease, growth, and reproduction are examples of internal factors that accompany changes in hormonal balance. The intensity of the endocrine effect on the target organ depends on the amount of hormones produced and the binding property, amount, and response of receptors in the target cells.

Target cells differ from nontarget cells by the presence of hormone-specific cell receptors. A receptor, therefore, is a signal discriminator capable of binding to a specific hormone and translating its message into specific cellular responses. These responses regulate protein phosphorylation, cell growth, gene expression, enzyme activity, nutrient metabolism, mineral release/retention, and cell death. Hormones also modulate responses of the immune and central nervous systems.

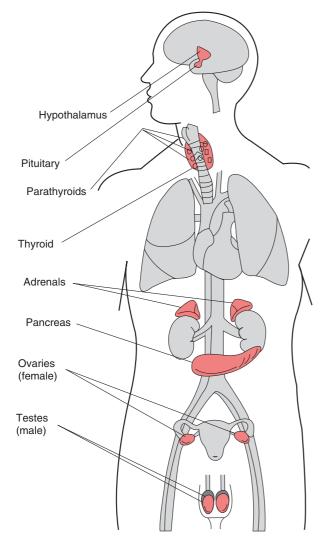


Figure 1 Major endocrine organs in the body.

Maintenance of Homeostasis

Hormones are responsible for maintaining physiological and cellular homeostasis that are essential for reproduction, growth/development and or neurobehavioral functions. The production of any hormone in the endocrine system is the result of an entire chain of events involving precisely choreographed interactions of many other endocrine organs. For example, the initiation of testosterone and estrogen production can be traced to the release of gonadotropin releasing hormone (GnRH) from the hypothalamus in the brain. GnRH stimulates the pituitary gland to produce luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn act on the testis and ovary to stimulate testosterone and estrogen production, respectively.

When there has been a sufficient cellular response in the target cell, negative feedback, a control mechanism, relates this information back to the organ that

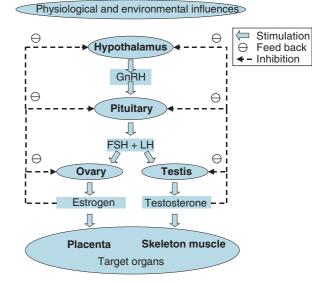


Figure 2 Examples of feedback control mechanisms in hormone homeostasis.

produced the hormone, inhibiting further hormone production (Figure 2). This mechanism prevents overstimulation and modulates the stability of the cellular status. For example, in the case of testicular function, sufficient amounts of testosterone in the blood will send feedback signals to the hypothalamus and the pituitary gland. This will reduce the production of GnRH, LH, and FSH, thus decreasing the signals for further testosterone production and release.

Mechanisms of Endocrine Disorders

Substances with the ability to modulate the endocrine system do not necessary pose any health risk for humans and other organisms. In fact, humans and animals are constantly exposed to substances in food and other environmental media that interact with the endocrine system. In general, due to precise yet adaptable control mechanisms, and the intertwined nature of the hormonal balance, moderate amounts of chemical effects on hormones seldom compromise normal physiological functions. Fluctuations of hormone concentration and receptor activities, by design, absorb environmental and physiological challenges in order to maintain functional equilibrium in the body. Only when the equilibrium control mechanisms are overwhelmed do deleterious effects occur.

Another consequence of the interdependent nature of the endocrine system is that manifestation of an endocrine disorder is virtually always associated with changes in synthesis or concentration of multiple hormones. For example, in 2,3,7,8-tetrachlorodibenzo*p*-dioxin (TCDD)-treated rats, the decrease in thyroid hormone 3,5,3',5'-tetraiodothyroxine (T₄) is always associated with an increase in the blood concentration of thyroid stimulating hormone (TSH), which is secreted by the pituitary gland in response to the low blood T₄.

Altered hormone concentrations in response to chemical exposure could be an adaptation response. It alone, therefore, is not a sufficient indicator of toxicity. Endocrine toxicity is characterized by disease conditions in the host in addition to hormonal changes.

Endocrine Disruptors

Dysfunction of the endocrine system could be due to either hyperfunction (excessive hormone production or responses) or hypofunction (insufficient hormone production or responses). Environmental chemicals that have the potential to perturb the endocrine system are known as endocrine or, synonymously, hormone disruptors.

The term endocrine disruptors was first used by Theo Colborn and Peter Thomas in 1992. In 1996, the US Environmental Protection Agency (EPA) convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee to make recommendations to EPA concerning endocrine disruptors. The term 'endocrine disruptors' has been used interchangeably with hormonally active agents and endocrine modulators. As the term is used now, endocrine disruptors include any substance that affects the synthesis, secretion, transport, binding, action, inactivation, or elimination of natural hormones in the body.

Excessive amounts of hormones in circulation may be due to overproduction of hormones in the endocrine organs, rapid release of hormones from storage, decreased hormone metabolism, or altered rate of clearance and excretion of hormones. On the other hand, cell injury in hormone-producing tissues, inhibition of synthetic enzymes, and induction of metabolic enzymes are causes of hormone deficiency.

Examples of Alterations in Endocrine System

Hormone Synthesis

Hormone synthesis can be altered by changes in the size and population of hormone producing cells, modification of the activity of hormone synthesizing enzymes, lack of precursors, or interference with enzyme cofactors such as divalent cations. For example, the fungicide fenarimol inhibits the enzyme aromatase, which converts testosterone to estrogen. Exposures to acrylamide monomer induce changes in the volume of cellular components in the thyroid gland, thus resulting in an increase in the thyroid hormone thyroxine (T4) and a decrease in TSH in blood.

Cadmium decreases testosterone production by preventing the synthesis of cholesterol, a precursor of all steroid hormones. Other chemicals that interfere with steroid hormone synthesis include aminoglutethimide, cyanoketone, and ketoconazole. Copper chelating compounds, such as dithiocarbamates, metam sodium, and carbon disulfide, suppress the conversion of dopamine to norepinephrine and subsequently to epinephrine.

Storage and Release

In addition to synthesis, the release of hormones from the storage compartment in cells also controls the amount of hormones in circulation. Reserpine and amphetamine are examples of compounds that affect hormone storage in granular vesicles. Compounds that activate LH receptors could potentially cause hypersecretion of testosterone from the Leydig cells (site of testosterone synthesis in the testes). On the other hand, direct cell injury of secretory cells may cause hyposecretion.

Carrier Proteins

Most lipid-soluble hormones in the blood are bound to specialized carrier proteins. The availability of hormones for physiological functions depends on the total concentration of the hormone as well as the amount of hormone existing in the free state; protein-bound hormones are not readily available for receptor binding. While lack of carrier proteins could impair the transport of hormones to target organs, excessive amounts may decrease the availability of free hormones.

Some estrogenic compounds (compounds with estrogen activity) are known to increase the amount of testosterone–estrogen-binding globulin (TEBG), a sex hormone carrier protein, while high doses of androgens and glucocorticoids may decrease the TEBG concentration in plasma. Salicylates and diphenylhydantoin have been shown to cause changes in thyroxine-binding globulin, a thyroid hormone carrier protein, thus modifying the amount of free circulating thyroxine.

Receptor and Ligand Interactions

Endocrine disruptors often are structural analogs of endogenous hormones (hormones produced naturally in the host). Hormone analogs may act like the endogenous hormone if the analog–receptor complex in the target cell mimics the function of the hormone–receptor complex. Hydroxy metabolites of both o,p'-DDT and methoxychlor bind to estrogen receptors and cause estrogenic effects in birds and reptiles. Alkyl phenols, the biodegradation products of alkyl phenol ethoxylates, bind to estrogen receptors in fish and human cells *in vitro* and induce estrogenic effects in rats. Some hydroxy metabolites of polychlorinated biphenyls (PCBs) are also estrogenic. The receptor-binding property of diethylstilbestrol (DES), a synthetic estrogen, is implicated in the detrimental effects on the reproductive system of men and women exposed to DES *in utero*.

Hormone analogs can also act like antagonists, if they compete with endogenous hormones at the receptor binding site, but elicit no cellular response. An example of such an estrogen antagonist is tamoxifen, which binds competitively to the estrogen receptor and alters the effectiveness of the hormone–receptor complex in regulating gene expression. Vinclozolin, a dicarboximide fungicide, is an androgen antagonist; its metabolite blocks the androgen receptor. Similarly, the major DDT metabolite, 1,1-dichloro-2,2bis(*p*-chlorophenyl)ethylene(p,p'-DDE), acts as an antiandrogen in rats, causing abnormalities in male sexual development.

Many endocrine disruptors can bind to more than one type of receptor. For example, *o,p*-DDT and chlordecone bind to both estrogen and progesterone receptors, while nonylphenol and EPTE, a metabolite of methoxychlor, bind with the same affinity to estrogen, progesterone, and androgen receptors. In addition, environmental estrogenic chemicals, as well as endogeneous estrogens, can exert rapid actions on nonestrogen receptors on cell membranes, such as receptors shared by neural transmitters, dopamine, epinephrine, and norepinephrine.

Endocrine disruptors may also interfere with hormone function by altering the nature of the receptors or interfering with interactions between the hormone-receptor complex and genes or other cellular components. For example, TCDD and some of the PCBs that resemble the chemical structure of TCDD act as antiestrogens by decreasing the sensitivity of estrogen receptors to estrogen. In such cases, despite adequate estrogen production, organisms respond as if in an estrogen-deficient condition. In addition, under normal conditions, the hormone-receptor complex elicits cellular responses by regulating gene expression. Another type of endocrine disruptor interferes with the interaction between this hormonereceptor complex and DNA.

Metabolism and Clearance

Chemicals that cause either induction (increased synthesis) or degradation of hormones are also potential disruptors of the endocrine system. Several liver enzymes, including cytochrome P450 enzymes, inducible by drugs and environmental pollutants, are involved in hormone clearance. For example, DDT and similar compounds are potent inducers of cytochrome P450-dependent monooxygenases, an enzyme system that degrades endogenous androgens. These compounds, therefore, potentially have antiandrogenic activity. Likewise, lindane has been reported to decrease the amount of circulating estrogen by increasing estrogen clearance.

It has also been hypothesized that long-term hormone imbalance can induce cancers in endocrinesensitive organs, such as gonads, adrenals, thyroid, prostate, and breast. Lipid-soluble compounds are of special concern because they are retained in the body, therefore causing a long-term effect. Some of these chemicals are known cytochrome P450 enzyme inducers, such as PCBs, DDT, and butylated hydroxytoluene, and have been implicated in cancers in the adrenals, uterus, and thyroid.

UDP-glucuronosyltransferase, an enzyme that conjugates UDP-glucuronic acid with T_4 and other steroid hormones, can be induced by TCDD via an aryl hydrocarbon receptor-dependent mechanism. In rats, TCDD exposure has been shown to increase the rate of removal of T_4 from the blood.

Structurally similar compounds can also compete with endogenous hormones for the binding sites of metabolic enzymes and make the enzyme unavailable for normal hormone degradation. This would lead to a decreased clearance rate and prolong the half-life of circulating endogenous hormones.

Cell Differentiation

Other hypotheses suggest that *in utero* and neonatal exposure to estrogenic compounds may affect cell differentiation and alter cellular responses to sex hormones later in life, resulting in cancer. This mechanism is probably involved in the development of vaginal cancer in women whose mothers were given large doses of diethylstilbestrol during pregnancy. In this case, the manifestation of vaginal cancer was not evident until years after exposure. Chronic low-dose exposure to other endocrine disruptors has been associated with thyroid, testicular, and mammary tumors in human populations. The cause-and-effect relationship for most of the associations, however, is yet to be confirmed at the biochemical and molecular levels.

Endocrine Organs as Targets of Radiation

Endocrine organs can also become the target of physical agents, such as radiation. This is especially

true if a radioactive compound is actively taken up through normal mechanisms and concentrated in the organ. For example, the inorganic iodine in the body is largely taken up by the thyroid in connection with the synthesis of thyroid hormone. More than 20% of iodine in the body is found in the thyroid, an organ which weighs less than 0.005% of the total body. Almost 10 years after the radioactive fallout from the 1986 Chernobyl nuclear power plant explosion, a more than 10-fold increase in the incidence of childhood thyroid cancer in Belarus, the Ukraine, and the Russian Federation was observed. These countries received most of the radioactive fallout. Although many toxic and radioactive compounds were released through the explosion, the geographical distribution of the cancers most closely matches the pattern of fallout from the radioactive iodine. It is also evident through this accident that children are much more susceptible to thyroid cancer caused by radioactive iodine than adults.

Radiation can also cause male infertility, impotency, decreases in sperm fertilizing ability, and damage to the process of sperm formation. These disorders have been observed in victims of the Chernobyl accident as well as in patients treated with radiotherapy. In laboratory rats, the damage to sperm formation can be alleviated by treatment with testosterone and estrogen, indicating an etiology of sex hormone imbalance.

Multiple Targets of Endocrine Disruptors

The health effect of endocrine disruptors is further complicated by the fact that an endocrine disruptor or a family of endocrine disruptors may have multiple mechanisms of actions. For example, PCBs may mimic estrogen, prevent binding of thyroid hormone to thyroid binding globulin, and accelerate the metabolism and excretion of several steroid hormones.

See also: Androgens; Diethylstilbestrol; Environmental Hormone Disruptors; Radiation Toxicology, Ionizing and Nonionizing; Reproductive System, Female; Reproductive System, Male; Toxicity Testing, Reproductive.

Further Reading

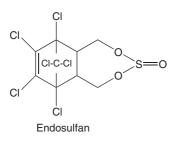
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Endosulfan

Elmar Udarbe Zamora

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- CHEMICAL ABSTRACT SERVICE REGISTRY NUMBER: CAS 115-29-7
- Synonyms: α- and β-Endosulfan; Thiodan[®] (Australia)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organochlorine insecticide
- CHEMICAL FORMULA: C₉H₆C₁₆O₃S
- CHEMICAL STRUCTURE:



Uses

Endosulfan is a cream to brown-colored solid that may exist in the form of crystals or flakes. Endosulfan is used as an insecticide for food crops such as grains, tea, fruits, and vegetables as well as on cotton and tobacco. It is also used as a wood preservative.

Exposure Routes and Pathways

Humans can be exposed through ingestion, inhalation, and dermal contact. Consuming contaminated food and water, direct skin contact with contaminated soil, smoking cigarettes made from tobacco with endosulfan residues and inhalation of the vapors during its manufacture and spray applications are possible avenues of exposure.

Toxicokinetics

In animals, $\sim 80\%$ is absorbed through the oral route and 20% through the dermal route. Three

weeks after repeated (21 days) dosing in mice, the highest concentrations were found in the liver and spleen. Little residue was found in the kidneys and fat, and there was no accumulation of radiolabeled endosulfan residues. Microsomal enzymes degrade it to polar and nonpolar metabolites including endosulfan sulfate, diol, α -hydroxy endoether, and endolactone. Excretion occurs through both urine and feces, but fecal elimination is predominant. The diol appears to be eliminated primarily via urine. Although a minor elimination route, lactating women can excrete endosulfan through breast milk.

Mechanism of Toxicity

The toxic effects of endosulfan during acute exposure are primarily due to its effects on the central nervous system. In animals, it has been shown to affect membrane permeability and glucose metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs given 200–500 mg kg⁻¹ orally exhibited salivation, vomiting and generalized tonic and clonic convulsions. In general, piloerection, salivation, hyperactivity, respiratory distress, diarrhea, tremors, hunching, and convulsions can be elicited. Respiratory arrest and renal failure can occur with high exposures. A decrease in red blood cell count and hemoglobin content were also observed. The oral LD₅₀ in rats is 18–220 and 74 mg kg⁻¹ through the dermal route. The LC₅₀ for rats exposed for 4 h through inhalation is $0.34-0.76 \text{ mg l}^{-1}$.

Human

Endosulfan can be lethal if large amounts are inhaled, swallowed or absorbed through skin. Contact may cause burns to skin and eyes. Depression, disorientation, headache, vomiting, dizziness, and tremors are the first signs seen 20 min to 12 h after ingestion of endosulfan. Respiratory arrest and renal failure appear to contribute to death.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure can potentially damage kidneys, testes and liver. It may also compromise immunological mechanisms against disease and infection. It is not genotoxic and no toxic effects in the reproductive system were seen in animals. It also does not disrupt the endocrine system.

Human

There are no data to verify if the above chronic effects seen in animals are also seen in humans. No epidemiological studies of cancer have been conducted.

In Vitro Toxicity Data

Endosulfan was not mutagenic in the Ames test or mammalian mutagenic assays and was negative in clastogenesis and micronucleus assays *in vitro* and *in vivo*.

Clinical Management

In case of inhalation, the victim should be moved to fresh air and emergency medical care called. If not breathing, artificial respiration should be administered. In case of direct contact, the skin and eyes should be flushed with running water for at least 15 min to remove the chemical as soon as possible. Contaminated clothing and shoes should be removed and isolated at the site. Normal body temperature should be maintained and the victim kept quiet. Vital signs should be monitored as the onset of toxic effects of endosulfan might be delayed.

Environmental Fate

Endosulfan enters the air, soil, and water during its manufacture and during field spray applications. During spraying, endosulfan may travel over long distances before reaching the crops, water, and soil. It takes a few weeks for endosulfan on the crops to be degraded. For residues in soil particles, it may take years for complete degradation.

Ecotoxicology

Endosulfan can accumulate in bodies of animals that live in contaminated water. It is highly toxic to many aquatic fishes. Male, 3–4-month-old Mallard ducks showed tremors, high carriage, wings crossed at the back and tail pointed down. In August 1995, more than 240 000 fish in Alabama, United States, were killed due to a run-off from cotton fields contaminated with endosulfan.

Other Hazards

Endosulfan is a combustible fluid and should be kept away from sources of ignition. It should be kept in a cool, well ventilated area and not exposed in the sun for prolonged periods. It is incompatible with strong oxidizing agents like chlorine and permanganate. Chemicals like sulfurous oxides and chlorine compounds may be produced after decomposition. Protective clothing should be worn during handling and manufacture.

Exposure Standards and Guidelines

The Environmental Protection Agency sets upper limits of not more than 74 ppb in waterways and not more than 0.1–2 ppm on raw agricultural products.

The Food and Drug Administration sets that not more than 24 ppm should be present on dried tea.

The time-weighted average set by the American Conference of Governmental Industrial Hygienists for endosulfan is 0.1 mg m^{-3} .

The Australian and New Zealand Environment and Conservation Council sets the water quality guideline not to exceed $0.01 \,\mu g l^{-1}$ in unfiltered water. (Note: Yes, originally, the limit was set at $0.1 \,\mu g \, l^{-1}$. In 1997, they changed it to $0.001 \,\mu g \, l^{-1}$ but detection is only up to $0.01 \,\mu g \, l^{-1}$ so it has been expressed as is.)

See also: Pesticides.

Further Reading

Agency for Toxic Substances and Disease Registry (AT-SDR) (2000) Toxicological Profile for Endosulfan. Update. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Relevant Websites

http://www.epa.gov – United States Environmental Protection Agency.

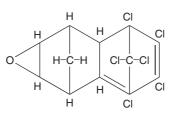
http://www.macquarie2100.org.au - Macquarie Valley Landcare Group, Inc.

Endrin

Benny L Blaylock

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 72-20-8
- SYNONYMS: 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-*endo*,*endo*-5,8dimethanonaphthalene; Endrex; Hexadrin
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organochlorine
- CHEMICAL STRUCTURE:



Uses

Endrin is used as an insecticide. The use of endrin has been significantly restricted in the United States and several other countries. There are currently no Environmental Protection Agency registrations for endrin.

Exposure Routes and Pathways

The most important exposure routes for endrin are oral and dermal.

Toxicokinetics

Endrin is absorbed through the gastrointestinal tract, respiratory tract, and through intact skin.

Endrin is metabolized by liver microsomal enzymes. In all species, oxidation of the methylene bridge in endrin to *syn*-, but mostly *anti*-12-hydroxyendrin occurs, followed by dehydrogenation to 12ketoendrin, a more toxic metabolite than the parent compound. Hydroxylated metabolites are conjugated as glucuronides and sulfates.

Unlike other organochlorine insecticides, endrin has not been found in fat samples taken from general surveys of exposed humans. In addition, it has not been found in the blood of endrin workers with the exception of recent gross accidental exposure.

The parent compound and its metabolites have been identified in both feces and urine.

Mechanism of Toxicity

Like other cyclodienes, endrin resembles picrotoxin, an antagonist of a postsynaptic receptor for the inhibitory neurotransmitter *y*-aminobutyric acid (GABA). The binding of GABA to this receptor, called the GABA_A receptor, stimulates influx of Cl⁻ ions, which hyperpolarizes the cell and makes it more resistant to depolarization. Thus, these insecticides promote excitotoxicity by blocking the stimulation of Cl⁻ influx by GABA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity with endrin is similar to that of other organochlorine cyclodiene insecticides. The oral LD_{50} in rats is 7–43 mg kg⁻¹ while in mice it is 1370 µg kg⁻¹. Animal studies confirm most health effects are to the central nervous system (CNS).

Human

Endrin is more highly toxic than other organochlorine insecticides. The major target is the CNS. Major symptomatology is exemplified by rapid onset of violent epileptiform convulsions in severe poisoning cases. The onset may occur as rapidly as 0.5 h or delayed as much as 10 h after ingestion of contaminated food. Other symptoms include headaches, dizziness, nervousness, confusion, nausea, and vomiting.

Chronic Toxicity (or Exposure)

Animal

Groups of three to seven dogs per sex were fed diets containing 0.1, 0.5, 1.0, 2.0, or 4.0 ppm endrin for 2 years. Dogs receiving 2 or 4 ppm experienced occasional convulsions, slightly increased relative liver weights, and mild histopathological effects in the liver (slight vacuolization of hepatic cells). No adverse effects on these parameters or on growth, food consumption, behavior, serum chemistry, urine chemistry or histological appearance of major organs occurred at 1 ppm (no-observed-effect level) or less. The 2 ppm level is the lowest-observed-adverse-effect level.

Animal studies have also shown exposure to endrin can cause birth defects, especially abnormal bone formation.

Human

Endrin is not classifiable as to its carcinogenicity to humans by International Agency for Research on Cancer. No long-term health effects have been noted in workers who have been exposed to endrin by inhalation or skin contact.

Clinical Management

Management of endrin poisoning is symptomatic. Diazepam or phenobarbital is used to control convulsions. In severe cases, mechanically assisted breathing may be necessary as well as administration of succinyl-choline for muscle relaxation and control. Activated charcoal as a slurry has been reported to absorb endrin and increase its rate of excretion after oral exposure. Emesis is not recommended due to potential CNS depression or seizures.

Environmental Fate

Endrin is very persistent, but it is known to photodegrade to delta-ketoendrin (half-life 7 days). Endrin released to soils will persist for extremely long periods of time (up to 14 years or more). Biodegradation may be enhanced somewhat in flooded soils or under anaerobic conditions. Its low water solubility and strong adsorption to soil makes leaching into groundwater unlikely. However, the detection of endrin in certain groundwater samples suggests that leaching may be possible in some soils. Endrin's low vapor pressure suggests only limited evaporation from soil. However, several studies have suggested that moderate to extensive loss of endrin from soils and crops was due to evaporation. Runoff from rain or irrigation of particle-associated endrin will carry particle-associated endrin to water systems.

Endrin released to water systems will not hydrolyze or biodegrade. It will be subject to photoisomerization to ketoendrin. It will extensively sorb to sediment. Evaporation from water will not be significant.

The fate of endrin in the atmosphere is unknown, but it probably will be primarily associated with particulate matter and be removed mainly by rainout and dry deposition.

Ecotoxicology

Endrin is very toxic to fish, aquatic invertebrates, and phytoplankton (96 h LC₅₀ for fish, aquatic invertebrates, and phytoplankton mostly below $1 \mu g l^{-1}$). Fish kills were observed in areas of agricultural runoff and industrial discharge; and declining populations of brown pelicans (in Louisiana, USA) and sandwich terns (in the Netherlands) have been attributed to exposure to endrin in combination with other halogenated chemicals.

Endrin has been detected in rainwater from Creek Lake in northern Saskatchewan, and has been reported in a fresh water lake in the Canadian Arctic. It has been found to bioaccumulate in species from algae, pouch snail, flathead minnow, rainbow trout, Virginia oyster, and sheepshead minnow.

Other Hazards

Endrin is slightly corrosive to metals. As a solid it is not combustible; however, it may be dissolved in a flammable solvent. Toxic hydrogen chloride and phosgene may be generated when a solution of endrin burns. Endrin is incompatible with strong oxidizers, strong acids, and parathion.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.0002 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Maximum contaminant level (MCL) is $0.002 \text{ mg} \text{ l}^{-1}$.

- Reference dose is $0.025 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Permissible exposure level is 0.1 mg m^{-3} (8 h).

See also: Cyclodienes; Dieldrin; Organochlorine Insecticides.

Relevant Websites

http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Endrin.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Endrin.

Environmental Advocacy in the United States

Peter Montague and Maria B Pellerano

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In the United States, environmental advocacy groups make up what is known as the 'environmental movement'. The history of this large, diverse social movement can best be understood by analogy to a river created by the confluence of about two dozen rivulets and tributaries. The main stems derive from two distinct nineteenth-century social movements, one promoting the conservation of natural resources, the other public health.

Ancient Sources

Of course even the nineteenth-century conservation and public health movements had ancient sources. Around 530 AD, the Roman emperor Justinian codified the legal basis for natural resource protection – the idea that air, water, oceans, wildlife, and more are owned by all of us together and none of us individually, and that the sovereign has a duty to protect and conserve these resources for present and future generations. The code of Justinian eventually led to the modern 'public trust doctrine' of environmental management and protection, described below.

Roman practices also foreshadowed the main ideas of the modern public health movement. For example, the architect Vitruvius (like the Greek physician Hippocrates before him) understood that human health is dependent upon the natural environment, so he taught his students to site and orient buildings to take advantage of fresh air and sunlight. The Romans also understood that individual health was dependent upon interventions at the scale of the whole population, not merely the individual. So, for example, the Roman aqueducts were built by the state to deliver clean water to entire cities. Public baths made cleanliness possible for everyone. Romans also separated incompatible land uses (without calling the practice 'zoning') to protect public health. For example, the Roman boarium (the cattle market) was separated from the forum (the center of public life) for health reasons. And finally, the Romans were keenly aware that certain occupations, such as silver mining, were dangerous and unhealthful, and those occupations were restricted mainly to slaves.

A fundamental question for people in every culture is the relationship of humans to the rest of the natural world. Are humans part of nature or are they separate from it and superior to it?

From the philosophies of Aristotle, Plato, and Socrates, the Romans inherited a strongly anthropocentric perspective – that nature exists solely to serve human purposes. This view was reinforced in the fourth-century AD when Rome adopted Christianity as its state religion. At that time, the dominant interpretation of the Biblical story of creation held that humans are separate from the natural world and were intended to dominate and exploit it. As time passed, this European view evolved further until the natural world was considered defective and incomplete until humans had 'developed' and 'improved' it – terms still widely used today. Although religious leaders have reinterpreted the relevant Biblical passages in modern times, it would be difficult to overstate the influence of these early, strongly anthropocentric views upon European and American life, thought, and policy.

The Conservation Movement

When Europeans first arrived in North America, they encountered indigenous people who viewed nature as a community to which humans and all other living things belonged and upon which all humans depended. The native people modified the environment with fire and took from it the plants and animals they needed, but with considerable restraint. We now know that in prehistoric times, large mammals had been driven to extinction at least partly by human agency, but for the most part the indigenous people of North America lived within nature's limits.

For the first 200 years of European advance into North America, natural resources seemed limitless. When one local environment was exhausted by logging or farming, there was always new land to be developed by moving westward. However, by the mid-nineteenth century, despoliation of the landscape in the United States had become evident on a grand scale, as documented by George Perkins Marsh in Man and Nature (1864). The movement to preserve natural landscapes in the United States has been traced back to a proposal in 1832 by artist George Catlin to protect the great Midwestern prairies and their inhabitants, buffalo, and indigenous people, from extinction by encompassing them in a large national park. That particular park never materialized and both the buffalo and the native people were subsequently decimated, but the idea of conservation for sustainable yield slowly took hold.

During the period 1830–1930, a national conservation ethic developed, aiming to preserve land and use it wisely for human purposes ranging from logging and hunting to spiritual regeneration. Particularly during the progressive era (roughly 1900– 20), scientific management of water, soil, trees, and minerals came to be accepted as a reasonable goal, though by no means a universal practice.

During the same period, another view of nature began to emerge as well – the natural world valued for its inherent beauty, as a place for solace and regeneration of the human spirit. Henry Thoreau had articulated this perspective when he lived in the woods near Walden Pond, 1845–47. John Muir, who founded the Sierra Club in 1892, became a leading proponent of this view. He said, "Everybody needs beauty as well as bread, places to play in and pray in, where nature may heal and cheer and give strength to body and soul alike." As time passed, the Thoreau– Muir tributary of the environmental movement gained a tinge of misanthropy, which naturally limited its political appeal.

Hunters and Fishers

Despite the slow spread of a conservation ethic, by the last quarter of the nineteenth century, the nation's fish and bird populations had declined dramatically. Wearing bird plumage had became fashionable in the United States and Europe, resulting in the slaughter of millions of egrets and other wading birds. The Passenger Pigeon, which had numbered in the billions, was hunted to extinction, followed by the Carolina Parakeet, the Ivory Billed Woodpecker, Bachmans Warbler, and the Heath Hen.

Habitats for native fish were being decimated by deforestation, farming, urbanization, dams, and pollution. By the 1870s, even the Great Lakes (containing one-fifth of the world's fresh surface water) were experiencing severe declines in native fish such as trout and salmon, from habitat destruction and overfishing. In 1871, Congress created the US Fisheries Commission to oversee the nation's fisheries interests, and the following year President Ulysses S. Grant designated the first national park, Yellowstone, in Montana and Wyoming.

Forest and Stream, a weekly founded in 1873, quickly became the premier publication serving recreational hunters and fishers. In 1880, George Bird Grinnell assumed the editorship and began campaigning for the protection of natural resources and habitat for wild game. He became a leading advocate of 'sustainable yield' timbering and led a campaign for the protection of Yellowstone National Park from commercial exploitation. When one of his readers, Theodore Roosevelt, became president of the United States in 1901, many of Grinnell's ideas were incorporated into federal conservation programs.

In 1886, Grinnell founded the first Audubon Society for the Protection of Birds, named after the American naturalist and artist, John James Audubon (1785–1851). Within 10 years Audubon Societies arose in many states and in 1901 a loose national federation of Audubon societies formed. In 1903, the Audubon societies provided support for Theodore Roosevelt as he set aside the first national wildlife refuge for the protection of birds and other wildlife. In 1913 and again in 1918, Congress passed laws, and signed treaties with Canada, protecting migratory birds from wanton slaughter. In 1874, the US Fisheries Commission issued a report advocating the artificial cultivation of particular fish species. By 1875, several states had developed fish hatcheries, intending to supplement fish populations by artificially stocking streams. This practice is now routine, creating a large commercially successful recreational fishing industry but masking the fact that many of the nation's freshwaters can no longer support large populations of native fish. For example, between 1966 and 1998, 745 million fish were released into the Great Lakes from hatcheries.

Throughout the twentieth century, fishers and hunters organized to protect the habitat of wild game. In 1922, sportsmen formed the Izaak Walton League (named after the seventeenth-century author of *The Complete Angler*) to protect the nation's rivers and streams from industrial dumping, sewage discharges, and soil erosion.

In 1936, President Franklin Roosevelt convened a North American Wildlife Conference to promote conservation, and from that emerged the General Wildlife Federation (later renamed the National Wildlife Federation) which grew to 4 million members composed of hunters and others interested in maintaining populations of wild species. The creation of other wildlife organizations followed - the Wildlife Society (1937), Ducks Unlimited (1937), and Defenders of Wildlife (1947). Still human encroachment into wild habitats continued to displace wild creatures, whose populations continued to decline. Of course the problem was not limited to the United States. The Nature Conservancy was formed in 1951 to take ownership of exemplary endangered ecosystems, to prevent their destruction. In 1961, an international group of scientists, conservationists, and political and business leaders formed the World Wildlife Fund (WWF), which is now a leading advocate for the control of persistent toxic pollutants worldwide.

In recent years, many groups formed to protect wildlife have been emphasizing the connection between human health and environmental deterioration – a connection readily apparent in fish. In many waters of the United States today, fish become moderately toxic as they grow to edible size. Many states now publish book-length lists, suggesting limits on consumption of particular species taken from particular waters. The need to artificially stock fishing waters, and the need to warn the public to curb consumption to avoid toxic exposures, indicate that the status of wildlife in the United States remains precarious and deeply troubled – a view confirmed by numerous reports on loss of biodiversity, poor water quality, and declining ecosystem health published throughout the 1990s.

The Public Health Movement

The public health movement to protect people arose in England during the nineteenth century in response to exceedingly high rates of death and disease amidst appalling environmental conditions in cities and factories (dark satanic mills) as society was reorganized along industrial lines.

Edwin Chadwick and the Poor Law Commission in England in the 1840s documented the fact that disease has both environmental and social determinants. In Chadwick's view, filthy air, water, and soil caused disease (by creating a 'miasma'), but so did poverty. Chadwick's three-volume report in 1842, Survey into the Sanitary Condition of the Labouring Classes in Great Britain, offered copious detail demonstrating how environmental and social conditions contribute to disease. Disease arose from filth, Chadwick had no doubt, and it was to be engineered out of existence. As Chadwick saw it, maintaining public health by preventing disease was chiefly an engineering problem, not a medical problem. Chadwick and his colleagues explicitly recognized the limitations of the medical model (one doctor, one patient) as a response to disease. The medical model aimed to cure disease, but the public health model aimed to prevent it through proper engineering of water supply, drainage, improved sewerage, and waste removal. Society had to create the conditions that made health possible, and a clean environment was crucial.

Spurred by Chadwick's efforts, in 1848 the English Parliament enacted the first Public Health Act and the Nuisances Removal and Diseases Prevention Act. These laws established prevention of disease – employing interventions by public authorities at the scale of the population, not just the individual – as the central tenets of the public health approach.

Long before medical science confirmed (between 1865 and 1890) that germs can cause illness, sovereign powers were imposing preventive measures to protect public health. Mandatory quarantining of plague victims had been practiced in Europe since the fourteenth century. In 1878, the US Congress enacted federal quarantine legislation to forcibly separate the sick from the healthy, to stem cholera, yellow fever, typhoid fever, and other epidemics of contagion. Individual liberties were sacrificed to achieve public health goals.

Vaccination to prevent smallpox was widely adopted throughout Europe in the first quarter of the nineteenth century and by 1905 compulsory vaccination was upheld by the US Supreme Court as a valid exercise of a state's 'police power' to protect public health.

Other preventive measures came into widespread use by the beginning of the twentieth century. As early as the 1840s, Dr. Oliver Wendell Holmes (father of the Supreme Court justice) advocated that physicians should wash their hands between patient visits. His ideas were considered extreme and slightly mad at the time, but eventually hand washing became routine throughout the world of medical practice and beyond. Today many people are required by law to wash their hands as a condition of employment because frequent hand washing is still considered the single most effective way to prevent the spread of communicable disease.

By the early twentieth century, it was widely understood and agreed that individuals had to yield some of their personal liberty in order to protect public health. As much as citizens of the United States once enjoyed spitting on the sidewalk, it was generally outlawed at the beginning of this century to reduce the spread of tuberculosis. It was not long before the propertied classes were expected to support public water, sewer, and solid waste disposal systems to protect public health. Landlords are today required by law to provide adequate space, light, and air in their rental properties (a triumph for Vitruvius) and property owners must take specific steps to prevent fires. In 1926, the US Supreme Court authorized municipal zoning commissions to limit the uses to which privately owned land could be put, to prevent, for example, industrial and residential uses side by side. These and many other restrictions on personal liberty were instituted by governments to protect public health, for the public good.

Today there is wide agreement that (1) prevention is the first principle of public health; (2) public health requires community action to create conditions that prevent disease and other threats to the health and welfare of individuals and the larger community; and (3) an environment that is free from harm is the starting point of good public health practice.

Since at least the time of Sir Francis Bacon (1561– 1626) and Rene Descartes (1596–1650), Europeans have believed that a mathematically based scientific knowledge of the material world is possible, that such knowledge would permit the conquest of nature, and that this conquest was the very definition of progress. The accelerating industrial revolution, based on replacement of human and animal labor by fossil fuels, seemed to prove them right. By 1930, the standard of living of average US citizens would have been unimaginable to people 100 years earlier.

The Main Stems Converging: 1950–70

By the middle of the twentieth century, it seemed as if the conquest of nature was nearly complete. Leading the way in taming nature was the chemical industry, which had learned to manipulate raw materials like coal and petroleum to create an astonishing array of useful molecules that seemed superior to anything that nature had created. Cheap fertilizer was making possible increased crop yields and abundant food. Chemical pesticides were reportedly vanquishing insect pests. An array of antioxidants, emulsifiers, thickeners, dyes, sweeteners, preservatives, and bleaching agents had made processed foods widely available. Synthetic fibers like rayon and nylon, along with synthetic dyes, were making fabrics cheaper, more colorful, and longer-lasting. Automobiles powered by low-cost leaded gasoline, constructed of special steel alloys, with tires of synthetic rubber and windshields of safety glass, gave mobility to millions.

With the help of vaccines, X-rays, radioactive isotopes, antibiotics, synthetic hormones, and vitamins, medical science seemed on the verge of eradicating most, if not all, human ailments. Like yellow fever and cholera before them, polio and tuberculosis were being vanquished. Atomic energy promised to provide cheap electricity to serve civilization's rapidly growing need for power. One corporation's slogan celebrated "Better things for better living through chemistry," and another's said, "Progress is our most important product." Belief in the inevitability of universal progress has perhaps never been stronger than it was in 1950.

But progress as conceived in 1950 depended upon technologies that turned out to have a powerful dark side. Public health hazards from radioactive fallout, pesticides, energy and transportation systems, artificial food additives, and toxic household chemicals all began to attract public attention as post-World War II optimism gave way to the 1960s.

- Atomic weapons tests in the South Pacific in 1946 exposed 40 000 US Navy personnel to radioactivity and an Army doctor's diary describing the incident hit the best seller list in 1948. In April, 1953, Geiger counters in the City of Troy, New York, recorded substantial radioactive fallout from tests that had been conducted in Nevada 36 h earlier. News reports of the incident provoked widespread fear and concern.
- In 1948 in Donora, Pennsylvania and again in London, England, in 1952, air pollution killed and injured large numbers of people 14 000 injured in Donora and 4000 killed during one weekend in London.
- By the mid-1950s, public health officials were concerned about the toxicity of various modern products. Alarmed by a rise in reported household poisonings, in 1957 the American Public Health Association passed a resolution calling for better

labeling and 'uniform control of hazardous substances', meaning household chemicals.

- Between 1945 and 1966, the US Department of Agriculture licenced ~60 000 individual pesticides at a time when the agency had only one toxicologist on staff, whose job it was to make safety evaluations and judgments based on available health studies (to the extent that any existed) for each of the 60 000 products.
- In 1957, a committee within the American Association for the Advancement of Science wrote, "We are now in the midst of a new and unprecedented scientific revolution which promises to bring about profound changes in the condition of human life. The forces and processes now coming under human control are beginning to match in size and intensity those of nature itself, and our total environment is now subject to human influence. In this situation it becomes imperative to determine that these new powers shall be used for the maximum human good, for, if the benefits to be derived from them are great, the possibility of harm is correspondingly serious."
- In 1958, Rachel Carson a trained biologist with a literary flair began writing *Silent Spring*, drawing parallels between the hazards of radioactive fallout and chemical pesticides.
- Just before Thanksgiving in 1959 the federal government issued a public warning, urging people not to eat cranberries, which had been found to be contaminated with amitrole, an herbicide thought to cause cancer in laboratory animals. This created widespread fear and awareness of cancer-causing chemicals in the nation's food.

During the 1960s, there seemed no end to the bad news. In 1961, newspapers featured photographs of entire rivers covered with foam from detergents. Anyone could see that something was amiss.

In June, 1962, chapters from Silent Spring began to appear in The New Yorker magazine and soon thereafter became a best-selling book. Many would identify this as the single most important event in the history of the modern environmental movement. In Silent Spring, Rachel Carson offered a powerful indictment of what she called 'man's war against nature'. "...[C]hemicals are the sinister and littlerecognized partners of radiation in changing the very nature of the world," she wrote. "Can anyone believe it is possible to lay down such a barrage of poisons on the surface of the Earth without making it unfit for all life?" Ms Carson raised the specter of chemical and radioactive technologies causing vast and lasting damage to the natural environment and to humans.

At the time, Ms. Carson was excoriated by representatives of chemical corporations, who accused her of being ignorant and hysterical. However, subsequent studies showed that she was correct on all essentials, and that she had underestimated the severity and magnitude of many of the problems she described.

Meanwhile, starting in the late 1950s in St. Louis, Missouri, a group of independent scientists organized by Barry Commoner took it upon themselves to begin studying radioactive fallout and then other dangerous technologies.

In the early 1960s, with colleagues around the country (such as the newly formed Physicians for Social Responsibility in Boston), the St. Louis group collected thousands of baby teeth and demonstrated that radioactive strontium-90 was building up in children as a consequence of testing nuclear weapons above-ground. Partly based on this 'baby tooth survey', in 1963 President Kennedy signed a treaty with the Soviet Union banning the testing of nuclear weapons in the atmosphere and the oceans.

The original Greater St. Louis Citizens' Committee for Nuclear Information soon expanded into a nationwide network that became known as the 'scientific information movement', guided by the idea that scientists have an ethical duty to help the public understand the technical aspects of public issues because an informed electorate is essential for democratic self-governance. They believed scientists have a duty to serve the public good, in return for which society supports the scientific enterprise through universities, government research, and the vast infrastructure of public services (libraries, courts, universities, communication networks, patent offices, standards for weights and measures, standards for accounting, and so forth) that make possible the corporate research and development enterprise. By 1968, the St. Louis group had renamed itself the Committee for Environmental Information.

Throughout the 1960s, the scientific information movement brought a public health perspective to environmental problems – human-centered, prevention-oriented, espousing population-scale interventions by the state (a ban on above-ground testing of nuclear weapons, e.g., to eliminate radioactive fallout), with no reluctance to consider the hazards of the workplace and urban environments. Here we find the beginnings of the modern environmental movement.

As the 1960s unfolded, other serious threats to public health and the workforce were revealed – toxic lead in paint and gasoline, asbestos in building insulation, food contaminated with mercury from pesticides and industrial products.

Inheritable genetic damage from radioactivity had been discovered in 1927, but during the 1960s scientists revealed that common air pollutants could alter genes, as well as cause cancer. It was becoming apparent that advanced technologies were capable of harming future generations.

"Pollution now is one of the most pervasive problems of our society," wrote President Lyndon Johnson in a report titled *Restoring the Quality of Our Environment*, published by the White House in November 1965.

That same report concluded that, "The pollution from internal combustion engines is so serious and growing so fast, that an alternative nonpolluting means of powering automobiles, buses and trucks is likely to become a national necessity." It did become a national necessity, but one that remained unmet almost 40 years later.

That same year – 1965 – Ralph Nader published Unsafe at Any Speed – charging that the US automobile industry was knowingly selling unnecessarily dangerous cars to an unsuspecting public. General Motors Corporation (GM) hired a detective to shadow Mr Nader, who then sued GM, winning a monetary settlement. Mr Nader invested the proceeds to form the Center for Study of Responsive Law and the consumer safety branch of the environmental movement was born.

In 1967, the Environmental Defense Fund (EDF, now simply Environmental Defense) was created by a group of attorneys and scientists to bring lawsuits against polluters and to educate lawyers about environmental issues. EDF was instrumental in persuading the federal government to ban DDT in 1972. In 1970 the Natural Resources Defense Council (NRDC) was formed to watchdog federal pollution-control agencies and, when necessary, to take the government to court to enforce the law. The Sierra Club Legal Defense Fund formed in 1971 (with no formal connection to the Sierra Club) to litigate on behalf of the environment; in 1997 the organization changed its name to Earthjustice.

Throughout the 1970s and 1980s, environmental litigation provided a powerful tool for environmental protection, until the federal bench and the appellate courts became less sympathetic to the environment. Since the early 1990s, environmental litigation has become more difficult for plaintiffs than it once was, and less successful at protecting the environment.

This legal tributary of the environmental movement spawned some important new theories of law that have begun to influence decisions in state courts. In 1970, Christopher Stone published *Should Trees Have Standing? Toward Legal Rights for Natural Objects*, and in 1971 Joseph Sax published *Defending* the Environment: A Strategy for Citizen Action. Stone planted the idea that perhaps nonhuman species deserved their day in court just as humans did, and Sax argued that the sovereign state had a legal duty to protect air, water, soil and more, even if it meant limiting some of the prerogatives of private property. Today this ancient 'public trust doctrine' – traceable to the code of the Roman emperor, Justinian – is evolving into an important new principle of environmental protection, and the rights of nonhuman species are the subject of intense debate.

In 1968, Ann and Paul Ehrlich published *The Population Bomb*, warning of dire threats to the future of all living things because of growing human encroachment into all of nature's domains. The book led to the creation of an organization called Zero Population Growth (ZPG), which in 2002 renamed itself The Population Connection. They offer evidence that every environmental problem would be easier to solve if the human population were smaller and growing more slowly than it is.

That same year -1968 – the first humans circled the moon in a spacecraft and brought back dramatic photographs of 'spaceship earth' – a small blue ball suspended in the vast blackness of space. These photos would forever change the way humans view their home.

In 1969 – the year Greenpeace was founded – the federal government issued the 'Mrak Report' (named for its senior author, Dr. Emil Mrak) which confirmed many of the dangers from pesticides described 7 years earlier by Rachel Carson. That same year the Cuyahoga River caught fire in Ohio, and a huge oil spill occurred off the coast of affluent Santa Barbara, CA, soiling the beaches of southern California.

To many, it seemed as though frail nature was under heavy assault by humans using powerful technologies in pursuit of narrow economic purposes. To many, it seemed that the future itself was endangered.

As a consequence, people began to react and to mobilize. For example, the Sierra Club grew from 15 000 members in 1960 to 113 000 in 1970, and 350 000 in 1983.

In the final years of the 1960s, three other specific responses developed:

• Ralph Nader expanded the new consumer safety movement (whose origins could be traced back to the founding of the National Safety Council in 1913 and Consumers Union in 1936), which came to be known as the 'public interest research movement'.

Nader hired college students (quickly dubbed 'Nader's raiders' by the media) during the summer

of 1970 to pore through the records of the federal agencies charged with protecting air, water, and food. During the following 3 years Nader issued a series of book-length studies offering evidence that government regulators were failing to protect public health and safety, the workforce, and the natural environment. From Nader's efforts there emerged a network of college-based organizations called public interest research groups (PIRGs). Akin to the earlier 'scientific information movement', PIRGs study public problems, issue reports, and advocate particular solutions.

• Labor activist Tony Mazzocchi of the Oil, Chemical and Atomic Workers organized a series of public forums, giving workers a platform for testifying about hazardous conditions in the industrial workplace. Mazzocchi compiled a formal record of the forums, to pressure Congress to enact the Occupational Safety and Health Act (OSHA). In 1970, OSHA became the first federal law aimed at protecting the health of the nation's workforce.

Starting in 1972, a national network of 'COSH' groups developed nationwide – Committees/Coalitions on Occupational Safety and Health. Currently there are 22 COSH groups across the country – private, nonprofit coalitions of labor unions, health and technical professionals, and others interested in promoting and advocating for worker health and safety.

• A coalition of activists planned a series of events across the country to be held April 22, 1970 – the first Earth Day celebration.

The Modern Environmental Movement Becomes Visible

The 1970s

Starting in the mid-1950s, the civil rights movement used the nonviolent tactics of boycotts, protest marches, and sit-ins at racially segregated businesses. By 1964 the movement had successfully ended legal discrimination against African-Americans. Soon teach-ins, sit-ins, and then large-scale protest marches against the Vietnam war further revealed the power of nonviolent direct action in ways unheard of since the labor movement's sit-down strikes of the 1930s.

Modeling itself on these contemporary protest movements, the activist movement for environmental protection broke onto the national scene on April 22, 1970 – Earth Day – with hundreds of teach-ins across the country aimed at creating awareness of environmental destruction. An advertisement in the *New York Times* on January 18, 1970, explained that, "Earth Day is a commitment to make life better, not just bigger and faster; to provide real rather than rhetorical solutions. It is a day to re-examine the ethic of individual progress at mankind's expense. It is a day to challenge the corporate and government leaders who promise change, but who shortchange the necessary programs. It is a day for looking beyond tomorrow. April 22 seeks a future worth living. April 22 seeks a future."

The following year Barry Commoner's best-selling book, *The Closing Circle*, helped millions of newly awakened readers understand something about how ecosystems work, and how certain modern technologies are disrupting them with far-reaching consequences for the future of humankind.

Although initially an upper middle-class phenomenon (like the conservation movement before it), the burgeoning 'environmental movement' began to appeal to a broader spectrum of people as the mass media told the general public that its health and wellbeing were threatened by pesticides, food dyes and other additives, dangerous household products, and the discharge of industrial toxicants into air and water. This came as no surprise to people of color or the poor, who had borne the brunt of pollution as long as anyone could remember.

Two months after Earth Day, 1970, President Richard Nixon issued an executive order creating the US Environmental Protection Agency (EPA) to administer the nation's environmental laws and programs. The President adopted an apocalyptic tone characteristic of the time when he said, "The 1970s must be the years when America pays its debt to the past by reclaiming the purity of its air, its waters, and our living environment...it is literally now or never."

President Nixon appointed William Ruckelshaus as first administrator of EPA. In an interview years later, Mr. Ruckelshaus explained the engineering perspective he brought to the job:

I thought that pollution could be solved by mild coercion. Once the federal government set some standards and began to enforce them, people would fall in line and the problem would essentially disappear. I thought we knew what the bad pollutants were, knew at what levels they caused adverse health and environmental effects, and knew the technology needed to combat them. Finally, I thought all of this could be done at a reasonable cost within a reasonable time.

"I was there about 3 months when I began to question every single one of the assumptions I had entered the agency with," Mr. Ruckelshaus said.

Between 1969 and 1990, Congress enacted (or strengthened by amendment) a series of laws aimed at protecting and restoring the quality of the environment: The National Environmental Policy Act (1969); the Clean Air Act (1970); the Water Pollution Control Act ('Clean Water Act', 1972); the Federal Environmental Pesticide Control Act (1972); the Coastal Zone Management Act (1972); the Endangered Species Act (1973); the Safe Drinking Water Act (1974); the Resource Conservation and Recovery Act (RCRA) (1976) - to manage solid and hazardous wastes; The Toxic Substances Control Act (1976); The Surface Mining Control and Reclamation Act (1977); The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) ('Superfund', 1980) creating a tax on the chemical and petroleum industries to pay for cleanup and restoration of chemically contaminated sites; and the Fish and Wildlife Conservation Act (1980). Several of these laws were subsequently amended and strengthened during the 1980s and 1990s.

From the outset, EPA was charged with administering and enforcing parts or all of these laws, but Congress rarely, if ever, allocated sufficient funds for the agency to do a thorough job.

Furthermore, from the outset, EPA personnel defined natural resource damage, and environmental contamination, as science and engineering problems, rather than problems of human behavior.

As a result of inadequate resources and the belief that applied engineering held the key, and seeking to establish a rational and consistent basis for making decisions, EPA personnel soon adopted a judgmentbased technique that often relied heavily on mathematical models for calculating risks: quantitative risk assessment. Somewhat later the assessment and management of risks were conceptually separated, though the two activities have always remained interdependent.

The concepts of risk assessment had been developed by the US Food and Drug Administration (FDA) in the late 1930s to establish allowable levels for food additives, to give consumers 'reasonable certainty of no harm when used as intended'.

In its simplest form, risk assessment asks, 'How much exposure can we allow without causing irreparable harm?' Harm to whom? Initially, to a 'maximally exposed individual' and more recently to a 'sensitive' and maximally exposed individual. For example, to keep chemical contamination to 'acceptable' levels in a river, EPA would define 'acceptable risk' to a maximally exposed individual. Acceptable risk would be defined as an exposure to a contaminant below its threshold for causing damage or, more often, a one-in-a-million risk of getting cancer from a lifetime of exposure. With acceptable risk defined, the agency would issue permits to each discharger of contamination along the length of the river, specifying numerical limits on each discharge. The goal was to keep the total discharge into the river from exceeding the acceptable risk for the maximally exposed individual. To a large extent, this approach still prevails today.

This technique has been applied to hundreds of thousands of air and water discharge permits over the past 30 years. In addition, risk assessment has become the standard way of setting safe limits for pesticides in food; determining 'how clean is clean' in the remediation of contaminated sites; judging how much contaminated fish it is safe to eat; decreeing how many logging roads can be cut into a national forest without decimating the bear population, and on and on. Today, in the United States, nearly all resource and contamination issues are decided based, to one degree or another, on risk assessments.

Because data on the hazards of individual chemicals were often incomplete, from the beginning, risk assessment was made manageable by adopting many simplifying assumptions. Initially the maximally exposed individual was assumed to be an 'average' person in good health and the only harm considered was physical manifestation of disease, such as liver necrosis or cancer - ignoring the possibility of behavioral disorders, or of harm to the immune system, the nervous system, the endocrine system, the reproductive system, the metabolic system, or the genes. Another simplifying assumption was that everyone was exposed to one chemical at a time even though in the real world everyone is exposed to low levels of a panoply of exotic chemicals - pharmaceutical products, household cleaners and disinfectants, second-hand smoke, automotive exhausts, food additives, low levels of industrial compounds in drinking water, and so on. In reaching the final number that represents 'acceptable risk', imponderables are typically taken into account by applying imprecise 'safety factors' - more recently called 'uncertainty factors' - which are usually multipliers of 10.

To remedy these limitations of risk assessment, as time has passed government risk assessors have increasingly tried to take into account some of the missing elements. However, limitations on the available data have always forced risk assessors to rely on assumptions, the use of somewhat arbitrary uncertainty factors, and judgments. As a result, despite many advances in the science of toxicology during recent decades, conclusions about risk can still vary dramatically depending upon who is doing the risk assessment. As William Ruckelshaus said in 1984, "We should remember that risk assessment data can be like the captured spy: If you torture it long enough, it will tell you anything you want to know." Peer review of risk assessments by all stakeholders can reduce the range of disagreement; nevertheless, despite substantial effort and constant improvements in risk assessments, the goal of a rational and reproducible technique for making decisions has eluded decision-makers. As William Ruckelshaus said in 1983, "No amount of data is a substitute for judgment."

Furthermore, because it is a mathematical technique based largely on scientific information and technical judgments, risk assessment tends to mystify the general public. Thus reliance on risk assessment for decision-making has had the effect of discouraging members of the public from participating in decisions affecting their lives, thus weakening democratic institutions that depend on citizen interest and participation.

In addition, as time passed, it became clear that assessing the risks to a maximally exposed individual - even a maximally exposed and sensitive individual - had the unintended consequence of overlooking millions of small discharges that posed acceptable risks to the hypothetical individual but which, taken together, contaminated the entire planet with low levels of industrial toxicants, pesticides, pharmaceuticals, and personal care products. In recent years, government scientists have measured low levels of hundreds of synthetic chemical compounds in the nation's rivers and streams, and even in drinking water: pain killers, antibiotics, dry cleaning fluid, solvents, degreasers, plasticizers, antimicrobials, flame retardants, tranquilizers, contraceptives, antidepressants, perfumes, deodorants, chemotherapeutic compounds, and so on, all of which largely escaped official notice as they leaked into the natural environment.

Despite the limitations of risk assessment as a decision-making technique, in 1983 the National Academy of Sciences (NAS) codified methods for risk assessments conducted by federal agencies. With this stamp of approval from the nation's most prestigious scientific organization, risk assessment spread quickly throughout federal, state and even municipal governments.

The 'Anti-Nuke' Movement As government was responding to environmental threats by passing laws, developing bureaucracies and refining techniques for assessing risks, citizens developed expertise on their own and began to organize around particular problems. The bombing of Hiroshima and Nagasaki in 1945 and radioactive fallout from atmospheric testing in the 1950s provoked a response initially among scientists. The Federation of American Scientists was formed in 1945; the *Bulletin of the Atomic Scientists* began publishing in 1949. As we have seen, the 'scientific information movement' developed in the late 1950s, intending to inform the citizenry.

The early history of atomic weapons and fallout left a residue of fear and distrust of nuclear technologies. The so-called 'anti-nuke' movement developed from this residue. Civilian nuclear power plants use a controlled nuclear fission reaction to boil water to turn a steam turbine to make electricity. The corporate sector was initially reluctant to finance nuclear power plants when President Eisenhower praised them in 1953. However after Congress enacted the Price-Anderson Act in 1957, to limit corporate liability in case of mishap, development of the technology proceeded apace. The first nuclear power plant built without direct government funding went on-line in late 1959.

From the beginning, four separate issues fanned citizen opposition to nuclear power: (1) the possibility that the nuclear fuel might heat up excessively, leading to a 'meltdown' that might release large quantities of radioactivity into the environment; (2) concern about small, continuing releases of radioactivity into local air and water; (3) the difficult technical problem of safely disposing of radioactive wastes that will remain dangerous for 240 000 years, far longer than homo sapiens has walked the earth; and (4) the possibility that radioactive materials from a nuclear power plant might some day be fashioned into a crude but effective atomic bomb or a 'dirty bomb' composed of radioactive materials wrapped around a core of dynamite. After more than 40 years of experience with nuclear power plants, the last two problems have not been solved, and the first two are still the subjects of intense scientific debate.

The Union of Concerned Scientists was formed in 1969. In 1970, nuclear scientists John Gofman and Arthur Tamplin published their estimate that civilian nuclear power plants could cause 24000 cancer deaths per year in the United States. This ignited citizen opposition to expansion of nuclear power technology and by 1975 the Clamshell Alliance in New England and the Abalone Alliance on the west coast had grown into large coalitions of citizens aiming to stop nuclear power. Their slogan was simple: 'No nukes!' and eventually they achieved their goal in the United States, where there are currently 103 nuclear power plants operating, but the industry ceased to expand in the mid-1970s. After the Three Mile Island nuclear plant in Pennsylvania suffered a partial fuel meltdown in 1979, investors shied away from new nuclear power stations. The Chernobyl disaster in 1986, which produced measurable radioactive fallout across large areas of Europe, only made matters worse for the nuclear power industry. In the early years of the twenty-first century, significant efforts have been made by the US government to revive the industry.

The rise of the civilian nuclear power industry served to spread concern about radioactivity to many communities that might not have otherwise given it a second thought. Proposals to truck many tons of radioactive waste across the nation's highways had similar effect.

Other proposals to develop new nuclear technologies provoked opposition as well. Although it is now legal to irradiate certain foods in the United States, including meat, to kill microorganisms and lengthen the 'shelf life' in stores, irradiated food drew significant opposition from the environmental movement, and growth of the industry has been slow.

The 1980s

The 'Toxics' Movement In 1978, families living in Love Canal, NY, near Buffalo, discovered an unusual number of serious health problems among their children, which they attributed to toxic wastes that they found oozing into their basements and onto the local school playground. They learned that the original canal had been dug for barge transportation, but it eventually had been filled with 20 000 tons of hazardous wastes and covered with a thin layer of soil. Over the next 2 years President Jimmy Carter declared Love Canal a federal emergency and the government helped many families relocate. Subsequent study confirmed that children of families living closest to the canal tended to weigh less than average at birth, and to suffer from various health problems.

One of the leaders of the Love Canal protest was a housewife named Lois Gibbs, who subsequently moved to northern Virginia to establish the Citizens Clearinghouse for Hazardous Waste (since renamed the Center for Health, Environment and Justice), to advise other communities afflicted by toxic wastes. Over the next decade, the dimensions of the chemical waste problem began to emerge and thousands of local groups formed to advocate for the cleanup of contaminated local lands. This 'toxics movement' eventually encompassed many thousands of local groups in all 50 states.

By the late 1980s, EPA acknowledged the existence of 32 000 locations contaminated with toxic chemicals but even then EPA had no formal process for discovering new sites. Congress's Office of Technology Assessment (OTA) in 1989 estimated that the total number of contaminated sites in the United States might run as high as 439 000, including contaminated military properties, mine wastes, leaking underground storage tanks, pesticide-contaminated lands, contaminated nonmilitary federal properties, underground injection wells, abandoned municipal gas manufacturing facilities, and wood-preserving plants.

In 1991, the National Academy of Sciences studied the health effects attributable to toxic waste sites and concluded, "[W]e find that the health of some members of the public is in danger," but "We are currently unable to answer the question of the overall impact on public health of hazardous wastes." The Academy pointed out that "Millions of tons of hazardous materials are slowly migrating into groundwater in areas where they could pose problems in the future, even though current risks could be negligible." The Academy concluded, "...the committee does find sufficient evidence that hazardous wastes have produced health effects in some populations. We are concerned that populations may be at risk that have not been adequately identified, because of the inadequate program of site identification and assessment."

Environmental Justice Meanwhile, in 1982, a pivotal new branch of the modern environmental movement emerged in Warren County, NC, in response to a proposed toxic waste landfill in a predominantly African-American community. Over 500 people were arrested during a series of protests and the term 'environmental racism' came into the language. This was the beginning of the 'environmental justice' (EJ) movement. In 1983, the US General Accounting Office (GAO) published a study showing that waste dumps in the southeastern US were mainly located in communities where the population was predominantly African-American or of low income. In 1987, the United Church of Christ published a study confirming that the pattern revealed in the 1983 GAO study was evident nationwide.

As the 1980s evolved, environmental justice groups developed in many different racial and ethnic communities: African-Americans, Hispanics, Asian-Pacific groups, and the indigenous people of North America. By 1991, the EJ movement had a clear national identify and philosophy, expressed in the 'Principles of Environmental Justice' adopted at the First National People of Color Environmental Leadership Summit in Washington, DC, which was attended by more than a thousand community activists.

It would be difficult to overstate the importance of the combined effects of the toxics and EJ movements. Together, they redirected the environmental movement in the United States. The movement that had emerged in 1970 – a combination of conservation organizations, plus the new litigators – tended to view issues from the traditional conservation perspective, and it was largely staffed and supported by the middle and upper-middle classes. The toxics and EJ movements introduced a public health perspective, a working class perspective, and the perspectives of people of color and people with lowincome, thus creating the diverse blend of viewpoints and interests that defines the environmental movement today.

- Toxics and EJ groups permanently expanded the definition of 'the environment' to include not just wild lands and animals but all the places were people live, work, play, pray and learn. Now for the first time, the 'environmental movement' would focus attention on the cities and to a lesser extent the workplaces where most Americans spend their lives. This new perspective meant that the environmental movement could now appeal to huge numbers of people previously overlooked by the earlier focus on wilderness and endangered species.
- They emphasized the cumulative impacts of pollutants on communities – the combined effects of all sources of contamination, not just one particular pollutant or facility. For many years, risk decisions had been made in an artificial vacuum, considering one oil refinery, or one cement kiln, or one hazardous waste incinerator, ignoring all other sources of contamination in the general vicinity. This narrow perspective had burdened certain communities with numerous sources of contamination, each of which individually was deemed 'acceptable' yet in the aggregate created a patently unhealthful environment.
- They emphasized the social determinants of disease – the health consequences of poverty, stress, and the social isolation created by artificial hierarchies based on race, income, wealth, and class – combined with poor nutrition, insufficient recreational opportunities, inadequate health care, and exposure to toxicants. They thus broke down the barrier – which had been created in the original National Environmental Policy Act of 1969 – between environmental issues and socioeconomic issues.
- They emphasized the importance of respect for cultural traditions, local knowledge, and the historical integrity of community and place. The emphasis on risk assessment as a decision-making technique had inadvertently created a great divide between 'experts' who had 'useful' knowledge, and ordinary people who 'only' had common sense, historical understanding of their communities,

strong preferences for how things should be, and a well-developed sense of right-and-wrong, fair play, and justice.

- They emphasized the essential importance of democratic participation: a key question in any decision affecting the environment or public health is, who gets to decide? And they emphasized that local communities have the preeminent right of self-determination, to decide what's best: "We speak for ourselves," they said.
- They emphasized that a clean and healthful environment is a basic human right under international law, as well as a civil right under US law.
- They emphasized leadership by women. Most community-based toxics and EJ groups were and are led by women. Perhaps this reflects somewhat the influence of the women's movement that energized women throughout the 1970s to seek equal pay for equal work and to demand other rights and opportunities that had traditionally been denied to them by a society organized along patriarchal lines.
- They emphasized that environmental issues are mainly about justice, fairness, ethical choices, and acceptable behavior, not just acceptable risk. The earlier narrow focus on science and engineering was expanded to give explicit recognition to the importance of ethics and values in decisions. In 2001, the European Union expressed the contemporary view of the proper role of science in environmental protection when it said, "science should be on tap, not on top."

In 1994, President Bill Clinton issued Executive Order 12898, requiring all federal agencies to 'make achieving environmental justice part of their mission'. The federal government's definition of environmental justice has two parts: equal protection from environmental and health hazards, and equal access to the decision-making processes that create a healthy environment in which to live, learn, and work. EJ is about fair treatment, and about expanding democracy to include everyone who is affected by a decision.

US EPA says equal access to decisions means (1) potentially affected community residents have an appropriate opportunity to participate in decisions about a proposed activity that will affect their environment and/or health; (2) the public's contribution can influence the regulatory agency's decision; (3) the concerns of all participants involved will be given serious consideration in the decision-making process; and (4) the decision-makers will seek out and facilitate the involvement of those who will likely be affected.

Toxics Use Reduction and Prevention By the mid-1980s, it was apparent to many people that certain toxic chemicals could not be managed safely and needed to be phased out or 'sunsetted'. In 1987, attorney Sanford Lewis proposed legislation to reduce the use of toxic chemicals in Massachusetts and 2 years later the state legislature passed the Toxics Use Reduction Act (TURA). The Act created the Toxics Use Reduction Institute (TURI) to help Massachusetts firms reduce their use of toxic materials. Today, compared to 1990. Massachusetts firms subject to reporting under TURA are generating 58% less waste per unit of product and have reduced on-site releases of federally reportable toxic chemicals by 90%. In addition, since 1990, quantities of chemicals shipped in product have been reduced by 47% (per unit of product shipped).

Since that time, many other states have created 'pollution prevention' programs of one kind or another, some voluntary, some mandatory. The mandatory programs, which represent a traditional public health approach, have achieved greater success than voluntary efforts.

Despite the growing emphasis on prevention and the avoidance of harmful substances, the US chemical industry currently introduces about 1700 new chemical compounds into commercial use each year, all largely untested for their effects on human health or the environment. In 2003, the European Union's proposal to require pre-market safety testing of chemicals was vigorously opposed by many national governments, including the United States, and by the chemical industry worldwide.

Agriculture Industrial agriculture got an early start in the United States. To avoid the laborious task of manuring soils to supply nutrients, inorganic fertilizers, such as superphosphates, came into use as early as the 1840s. However, a countercurrent quickly developed, the 'humus farming' movement focused on maintaining the humus content of agricultural soils. For the next 150 years, industrial agriculture would expand dramatically, but so would countercurrents stressing the need to maintain a holistic view of farm ecology – the complex relationships between plants, animals, soils, water, and human communities.

Chemical pesticides, such as Paris Green, were introduced for insect control starting in the 1870s. In the 1930s, federal farm policies began rewarding farmers who could increase their per-acre crop yield and, to that end, the US Department of Agriculture aggressively promoted the use of inorganic fertilizers and pesticides, and the development of the rural infrastructure (transportation, communication) needed to support large-scale industrialized farming. However, as some of the unintended ill consequences of industrial farming technologies came to light, the principles of 'organic' farming became more widely known and practiced. Furthermore, an anti-pesticide movement developed in the 1960s after *Silent Spring* sounded the alarm about long-lived chlorinated compounds.

The chemical industry responded by developing new pesticidal products that did not persist so long in the environment, but were more toxic. Most pesticides do not reach the target organism but enter the environment where they may cause direct and indirect effects in nontarget species.

The Northwest Coalition for Alternatives to Pesticides (NCAP) opened in Eugene, Oregon in 1977, the National Coalition Against the Misuse of Pesticides (since renamed Beyond Pesticides) formed in Washington, DC in 1981, and the Pesticide Action Network North America (PANNA) was organized in San Francisco in 1982 as part of an international network.

As time passed, the organic farming movement shifted into a 'sustainable agriculture' movement with three goals: farming practices compatible with natural systems, using organic fertilizers and few or no chemical pesticides; achieving food security, emphasizing locally grown foods; and maintaining rural economies that could sustain, and be sustained by, relatively small-scale farms. Wendell Berry's *The Unsettling of America* (1978), Wes Jackson's *New Roots of Agriculture* (1980), and the National Academy of Science's *Alternative Agriculture* (1989) offered an ecological and social critique of industrial agriculture and showed that viable alternative models already existed.

The technology-driven corporate industrial model of farming remains dominant today, but energy, chemicals and large-scale equipment have proved expensive to supply and consequently the farm economy has been badly depressed in recent years. Net farm income in 2003 was lower than it had been in 1929.

In recent years, genetic engineering techniques have been used to create proprietary plant cultivars with desirable new characteristics. However, it is not clear that this new technology can substantially reduce industrial agriculture's negative ecological impacts or solve its pressing problems of economic viability.

In the 1990s, the use of genetically modified organisms (GMOs) in agriculture provoked strong controversy on every continent. The expanding uses of biotechnology, and most recently nanotechnology, raise a fundamental question: Does history indicate that humans can gain the knowledge and the wisdom needed to rearrange the genetic and atom-scale building blocks of nature without causing widespread unintended harm?

Multiple Chemical Sensitivity As the toxics movement expanded during the 1980s, large numbers of people recognized that chemicals encountered in their daily lives negatively affected their health in one way or another.

In various large surveys 15-30% of Americans (40–80 million people) report that they are unusually sensitive or allergic to certain common chemicals such as detergents, perfumes, solvents, pesticides, pharmaceuticals, foods, or even the smell of dry-cleaned clothing. An estimated 5% (14 million people) have been diagnosed by a physician as being especially sensitive. Many people react so strongly that they can become disabled from very low exposures to common substances. Typical symptoms include prolonged fatigue, memory difficulties, dizziness, lightheadedness, loss of concentration, depression, feeling spacey or groggy, loss of motivation, feeling tense or nervous, shortness of breath, irritability, muscle aches, joint pain, headaches, head fullness or pressure, chest pains, difficulty focusing eyes, nausea, and more. This group of symptoms is known as environmental illness or, more commonly, multiple chemical sensitivity (MCS), meaning 'sensitivity to many chemicals'.

Because MCS does not fit any of the three currently accepted mechanisms of disease – infectious, immune system, or cancer – traditional medicine has not yet satisfactorily explained MCS, and so has often labeled it 'psychogenic', meaning originating in the mind. This has left MCS sufferers in limbo. Told they are crazy, or imagining their disease, or making it up, they find themselves passed from physician to physician without satisfactory answers and often without relief from their very real distress. (Some MCS sufferers do have psychological symptoms, but that does not necessarily mean their disease originated in their minds.) Forty percent of MCS sufferers report having seen more than 10 medical practitioners.

MCS came to the attention of mainstream science and medicine forcibly in 1987 when US EPA installed 27 000 square yards of new carpeting and painted and remodeled office space at its Waterside Mall headquarters in Washington, DC. Some 200 agency employees developed symptoms associated with 'sick building syndrome' (physiologic response to exotic chemicals in new construction materials) – and several dozen EPA employees later reported developing MCS. The National Research Council has now accepted that 'sick building syndrome' is a real phenomenon, producing MCS-like symptoms. In the mid-1980s, Mary Lamielle founded the National Center for Environmental Health Strategies in Voorhees, NJ, and emerged as a leading spokesperson for people suffering from MCS, and the related disorders, chronic fatigue syndrome (CFS), and fibromyalgia (FM). In 1990, Congress acknowledged all three syndromes in the Americans with Disabilities Act (ADA).

In recent years, some of the symptoms of MCS have been reported to afflict two new populations – military veterans exhibiting 'Gulf War syndrome' and women having silicone breast implants for esthetic reasons or for breast reconstruction after cancer surgery.

The 1990s

The Endocrine Disruptor Hypothesis During the 1990s, new scientific and medical studies gave people surprising new perspectives on environment and health problems.

In 1991, Dr. Theo Colborn and Dr. John Peterson Myers invited an international group of scientists to meet and share notes on their own research. The meeting produced a consensus that some industrial chemicals, under some circumstances, can interfere with the endocrine system of laboratory animals, wildlife, and perhaps humans. The endocrine system includes a complex of organs and tissues whose actions are coordinated by chemical signals provided by hormones, neurotransmitters, growth factors, cytokines, and so on. Chemical signaling systems control reproduction, growth, development and behavior in plants, mammals, birds, fish, amphibians and reptiles, strongly influencing the immune, nervous, and reproductive systems. Signaling systems assert control before birth, hatching or sprouting and retain control throughout the remainder of life.

The influence of chemicals on the endocrine system had been the subject of a federally sponsored research conference in 1979, but the issue remained unrecognized by the environmental movement until 1991. Since 1991, the 'endocrine disrupter hypothesis' has profoundly influenced the direction that the environmental movement has taken.

In 1996, the book *Our Stolen Future*, by Colborn, Myers, and Dianne Dumanoski, popularized the endocrine disrupter hypothesis by framing it as a kind of scientific detective story. Critics of the book argued that it distorted the underlying science, but like *Silent Spring* before it, subsequent research has shown it to be correct on all essentials and to have underestimated the severity and magnitude of the problem. Since 1991, a decade of intensive study of biological signaling systems has revealed (among other things):

- The effects of exposure to signal-disrupting industrial chemicals can vary significantly, depending upon the timing of exposure. The development of an organism may be highly sensitive to disruption by exogenous chemicals during a particular stage of growth, yet be relatively immune to disruption during a different period of time. Therefore, the apparent toxicity or biological effectiveness of a chemical can vary significantly, depending upon the timing of exposure.
- Prenatal exposures appear to be particularly important, perhaps because cell replication and differentiation are occurring most rapidly during this stage of life.
- Individual industrial chemicals present at insignificantly low levels can, under some circumstances, combine together to produce significant effects on signaling systems.
- Low-level exposure to a particular chemical can sometimes produce effects quite different from those caused by higher doses of the same chemical. These differences can include positive effects at low doses (hormesis) and harmful effects at higher doses, as in the case of the essential mineral, chromium. Or, in the case of some chemicals that interfere with biochemical signaling systems, harm can occur at low doses but not at higher doses, exhibiting an inverted U-shaped dose-response curve. The need for low-dose testing is becoming apparent, but in the recent past it has been considered prohibitively expensive in many instances.

These basic findings have resulted in new ways of looking at old problems, including:

- Because very few chemicals have been tested for these recently discovered effects, risk assessments now seem to rest on assumptions that are more uncertain than previously realized. If timing of exposure is sometimes crucial, if complex mixtures must be taken into consideration, and if low levels of exposure can sometimes produce greater interference in signaling systems then higher levels of exposure, then the underlying assumptions of many risk assessments completed to date need to be reexamined.
- The increasing incidence of many kinds of birth defects suggested that prenatal exposures may determine the course of life. Studies of signal-disrupting chemicals have provided evidence

that this is the case, which means that personal liberty and lifetime opportunities may be truncated or constrained by chemical exposures before birth. This kind of 'chemical trespass' in the absence of informed consent raises fundamental questions of ethics and human rights. From a public health perspective, it indicates that women who are pregnant, or who may become pregnant – which in principle includes almost all premenopausal women – should be protected from exposure to even low levels of exotic chemicals, to eliminate these potential sources of harm to their offspring.

• As the 1990s unfolded, the environmental movement and US EPA developed a focus on children, for several reasons. Compared to adults, children are undergoing more rapid cell division with more opportunities for interference by signaldisruptors; children breathe more air and ingest more water and food, per unit of body weight; children put their hands in their mouths more than adults do; children absorb chemicals through their digestive tract differently, and detoxify absorbed chemicals differently; children spend more time close to the ground where they may encounter toxicants in dust, soil and carpets as well as pesticide vapors; growth and development create 'windows of vulnerability' during which chemical exposures may cause permanent, irreversible damage; and because they are exposed to toxicants at an earlier age, children have more time to develop environmentally triggered diseases with long latency periods, such as cancer. Children are also among the most dependent members of society and therefore need special protection.

The increasing incidence of autism and attention deficit hyperactivity disorder (ADHD) among children has led researchers to uncover evidence that industrial chemicals may be contributing to these and other intellectual deficits and behavioral problems including aggression and violence. In an 'information age' that emphasizes the importance of intelligence and intellectual skills in nearly every sphere of life, diminishing children's intellectual and emotional capacities by chemical trespass without informed consent is widely regarded as unethical and unacceptable.

At this writing, the endocrine disrupter hypothesis has become very widely accepted for testing. Numerous scientific studies are now published each month, many of them providing evidence that some industrial chemicals, under some circumstances, can interfere with biological signaling systems, producing a wide range of ill effects in many different species, including humans. Environment and Disease By the 1990s, readers of US government journals (e.g., Environmental Health Perspectives, and Morbidity and Mortality Weekly *Reports*), were familiar with evidence that the incidence of many chronic diseases was increasing. Furthermore, a growing body of literature was revealing suggestive links between chemical exposures and familiar problems such as asthma, diabetes, lupus erythematosus, birth defects, infertility and other reproductive disorders, learning and behavior problems, Parkinson's disease, and several cancers (e.g., brain, female breast, prostate, testicular, and childhood leukemia), among others. The rise in childhood cancers seemed particularly worrisome because children's lifestyles had not changed dramatically during the previous 30 years, so researchers sought explanations in the environment, as noted above.

Connections between chemicals and human disease have always been difficult to establish conclusively because of routine exposures to mixtures of chemicals, the absence of an unexposed population to serve as a control, and sometimes long delays between the time of exposure and the manifestation of harm, delays sometimes spanning more than one generation. Many argued that the resulting scientific uncertainty provides grounds for continuing along our present path undeterred. Sir Austin Bradford Hill responded to such an argument in 1965 when he said, "All scientific work is incomplete – whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us as freedom to ignore the knowledge that we already have, or to postpone the action that it appears to demand at a given time."

Many survivors of serious diseases have traditionally formed support groups to share information and experiences among themselves. However, since the early 1990s, these groups have become more politically active, joining the toxics and environmental justice movements. In 2002, the Collaborative for Health and the Environment (CHE) was formed to give voice to the concerns of disease survivors, to disseminate information linking chemical exposures to various illnesses, and to urge a precautionary, preventive approach to chemical exposures.

Global-Scale Environmental Harm

In the searingly hot summer of 1988, global warming finally came to people's attention in a dramatic way when a respected government scientist told Congress that he believed humans were partially responsible for increasing the average temperature of the planet.

Carbon dioxide in the atmosphere had risen 30% during the previous 200 years, from the burning of coal and petroleum. Since 1896 scientists had periodically reminded us that increasing the carbon dioxide level in the atmosphere would eventually warm the planet by a mechanism known as the 'greenhouse effect'. Just as a glass roof warms a greenhouse by trapping solar energy, carbon dioxide in the atmosphere acts like a glass roof over the earth, trapping the sun's energy and warming the planet. Since the 1970s, scientists had wondered whether the earth's noticeable warming trend was caused by humans or was a natural fluctuation in temperature. In the summer of 1988, the public began to be told that global warming was partly caused by humans, though another 7 years would pass before the Intergovernmental Panel on Climate Change (IPCC), which includes 2500 atmospheric scientists, would officially take that position.

In addition to global warming, the 'hole' in the Earth's stratospheric ozone shield made headlines each spring starting in 1985. Human dislocation of the atmosphere revealed just how powerful human technologies had become – and how poorly understood they were. For thousands of years the Earth had seemed enormous and humans had seemed puny though clever. Now suddenly the Earth was starting to look small and humans, armed with modern technologies, were beginning to look like clumsy behemoths flying blind.

By the end of the 1980s, it was becoming evident to many people that governments were not succeeding in bringing destructive technologies under rational control. This led to a focus on another institution – the modern corporation.

Focus on the Corporation

Starting in the early 1970s, the consumer movement set out to modify corporate behavior through economic pressure. In 1973, the Interfaith Center for Corporate Responsibility brought together faithbased institutional investors, to influence corporate behavior through shareholder resolutions. In 1977, a group called Infact organized a consumer boycott of the Nestle Corporation to protest the firm's marketing of baby formula, and numerous boycotts of other corporations followed.

In 1994, a new approach emerged when Richard Grossman and Ward Morehouse formed the Program on Corporations, Law and Democracy (POCLAD), to study the legal entity called 'the corporation', pointing out that it derives all its power from state legislatures that grant 'corporate charters' – pieces of paper granting certain rights and privileges to groups of individuals while limiting their liability for their actions.

In the United States, in the early nineteenth century, corporations were chartered only for narrow purposes, such as construction of a canal or a toll road, and for a finite period of time (typically 20 years). Investors were held personally liable for corporate actions, and the corporation's total capitalization might be limited by legislative fiat. It is evident from the historical record that up until the Civil War, and even beyond, legislatures were reluctant to give broad authority to corporations. Many legislatures explicitly acknowledged that corporations are subordinate entities that exist only to serve public purposes and can be dissolved if they fail in that duty.

Initially, corporations could only fulfill the specific mandate in their charter. However, in 1886 a decision by the US Supreme Court seemed to give corporations the Constitutional rights enjoyed by individual humans (also known as 'corporate personhood'). From that time forward judges took the position that, like any other individual protected by the Bill of Rights and the Constitution, corporations have broad authority to do anything not specifically prohibited by law. In the following 100 years, corporations grew exceedingly large and powerful.

Now the anticorporate tributary of the environment movement is working through legislatures, courts, city and county councils, and other venues, trying to limit the rights that corporations enjoy. Their argument is that large publicly held corporations should not be protected by the Bill of Rights because such entities are nothing like individuals: corporations can grow without limit; they cannot die; they have limited liability for their actions; they have no built-in conscience analogous to that of an individual; they have a fiduciary duty to return a more-or-less steady profit to investors, a duty they are required by law to uphold before all else; and therefore they have a powerful incentive to externalize their costs, for example, to find ways to avoid paying for environmental damage to which their operations may contribute.

The phenomenon known as 'globalization' has focused a different kind of attention on corporations. In recent years major US corporations have worked hand in glove with the US government to create conditions conducive to 'free' trade – meaning, at base, the unrestricted flow of goods and capital (though not labor) across international borders. Many in the environmental and labor movements fear that globalization will undermine the authority of national governments and will thereby weaken or reverse hard-won environmental and labor standards in the United States and abroad. As a consequence US labor and environmental groups now work more closely together than at any previous time, and both are cooperating with counterparts abroad to an unprecedented degree. Within the larger environmental movement, the international antiglobalization tributary is one of the most significant developments of the past decade.

Animal Rights

The animal rights perspective can be traced to the founding of the American Society for the Prevention of Cruelty to Animals in 1866. The American Humane Associations was founded in 1877 as a network of local organizations aiming to prevent cruelty to children and animals. By the 1890s, several state Antivivisection Societies were founded to oppose the cruel treatment of animals, first in medical and scientific research, and later in education and product testing.

Slowly the animal welfare approach came to be supplemented by an animal rights approach. The Vegan Society was formed in 1944 on the principle that humans should avoid, to the extent possible, the use of animal products for food or for any other purpose. People for the Ethical Treatment of Animals (PETA), founded in 1980, defines animal rights this way:

Animal rights means that animals deserve certain kinds of consideration – consideration of what is in their own best interests regardless of whether they are cute, useful to humans, or an endangered species and regardless of whether any human cares about them at all (just as a mentally challenged human has rights even if he or she is not cute or useful or even if everyone dislikes him or her). It means recognizing that animals are not ours to use – for food, clothing, entertainment, or experimentation.

The animal welfare and animal rights movements can both be seen as part of a centuries-old movement toward pan-species democratization – toward the view that nonhuman species have a fundamental place in nature and a basic right to lead their own lives.

In a sense this approaches the view of nature held by the indigenous people of North America when Europeans first arrived (though indigenous people usually eschew a vegetarian diet because they believe that an omnivorous diet is fundamental to the sacred natural order).

At this writing the most militant branch of the environmental movement includes the Animal Liberation Front (ALF) and the Earth Liberation Front (ELF), both of which are committed to destroying property, though not life, as a way to stop the cruel exploitation of animals (ALF) and to expose and derail destructive development (ELF).

Appropriate Technology

Questions about the manageability of complex technologies have caused many people to ask whether there were inherently safer ways to provide food, shelter, energy, transportation, and consumer products. And since the United States had put Neil Armstrong on the moon in 1969, the question was phrased, "If we can put a man on the moon, shouldn't we be able to invent nondestructive technologies?"

The 'appropriate technology' branch of the environmental movement had been sparked by E.F. Schumacher, who published the book *Small is Beautiful* in 1973. From 1950 to 1970 Schumacher held an important government position as chief economic advisor to the British Coal Board. In 1955 Schumacher spent time in Burma, where he developed the principles of what he called Buddhist economics: the belief that good work was essential for proper human development and that 'production from local resources for local needs is the most rational way of economic life'. He subsequently became a pioneer of what is now called appropriate technology: earth- and user-friendly technologies matched to the scale of community life.

The appropriate technology branch of the environmental movement remained small and obscure until 1987 when the United Nations' World Commission on Environment and Development (dubbed the Brundtland Commission for its chairperson, Gro Harlem Brundtland, then the Prime Minister of Norway) published the book-length study, *Our Common Future*. In essence, the Brundtland report leveled a fundamental critique at the world industrial system: it was not sustainable because it was incompatible with nature. A sustainable system is one that survives or persists throughout its full expected life span.

The Brundtland Commission identified three steps needed to make the human economy sustainable: (1) improved efficiency (technical improvements, producing more with less, recycling); (2) reducing the human population explosion; and (3) redistributing wealth from overconsumers to the world's poor.

The Commission also said that, in order to achieve sustainability, the global human economy would need to grow by a factor of 5–10 in total size to eliminate poverty and give everyone the wherewithal to protect the environment. This prescription matches that of traditional economists, who have long asserted that the solution to poverty and environmental destruction lies with economic growth (meaning physical growth in the flow of energy and materials, from extraction, through use, to final disposal). In this view, growth in the total size of the global economic pie assures that even those holding a tiny slice will gain something even without intentional redistribution, and nations will then be able to afford to protect their natural environments.

At the historical rate of global economic growth $(3.5\% \text{ year}^{-1})$, a fivefold increase in the global economy would occur in 47 years and 10-fold in 67 years.

Shortly after publication of the Brundtland report, 'sustainability' was on everyone's lips and the appropriate technology movement suddenly gained new attention. This branch of the environmental movement is clearly on the ascendancy today, as more and more people – including thousands of scientists and engineers – acknowledge that trying to 'conquer' nature has led time after time to unintended consequences that damage ecosystems, harm human health and ultimately are self-defeating. Nature cannot be conquered, at least not in the way people assumed it could in 1950.

This new perspective still has many different names, indicating that it is not yet a fully coherent approach to industrial design. The National Academy of Sciences calls it 'industrial ecology'. Greenpeace calls it 'clean production'. Recycling professionals and activists sometimes call it 'zero waste', others call it 'toxics use reduction'. The United Nations Environment Program calls it 'cleaner production'. Many chemists call it 'green chemistry' and many engineers call it 'green engineering'. Many biologists call it 'biomimicry' after a book of the same name by Janine Benyus published in 1998. Biomimicry means 'a new way of viewing and valuing nature'. Biomimicry 'introduces an era based not on what we can extract from the natural world, but on what we can learn from it'.

To a very real degree these emerging disciplines are informed – consciously or not – by the worldview held by the indigenous people of North America when the Europeans first arrived: If we will observe carefully, and behave respectfully, nature will show us how to live in community with all living things.

The Faith-Based Environmental Movement

Some members of the US Judeo-Christian religious community have long been concerned with public health, sustainable agriculture, water and air pollution, urban land use, and other issues now labeled 'environmental'. However, the 'faith-based environmental movement' traces its roots to January 1, 1990, when Pope John Paul II said, "Men and women without any particular religious conviction, but with an acute sense of their responsibilities for the common good, recognize their obligation to contribute to the restoration of a healthy environment. All the more so should men and women who believe in God the Creator, and who are thus convinced that there is a well-defined unity and order in the world, feel called to address the problem..."

In a widely circulated 'Open Letter to the American Religious Community' in 1991, a group of 32 Nobel laureates expressed grave doubts about the sufficiency of humankind's response to the environmental crisis. In 1993, the National Religious Partnership for the Environment was formed "to further integrate the mission of care for God's creation throughout religious life, once and for all.... Humankind must better understand its role in the greater web of life. Destruction of habitat requires a change in the values of civilization. Changes of thought and behavior require transformation of heart and spirit. These are the perennial concerns of religion."

In the United States, where 80% of the population professes the Christian faith and 33% attends weekly worship services, this tributary of the environmental movement has the potential to reach large numbers of people with the compelling message that the Earth belongs to God and humans have a duty to care for it.

New Decision-Making Techniques

By 1990, risk assessment had been used for more than 20 years to establish allowable residues of pesticides on food; to assess the dangers of living near toxic waste sites; to determine acceptable levels of air and water pollution; to decide how to prioritize expenditures on environment-related government programs, and on and on.

Just when new information about the effects of chemical exposures was provoking new questions about the scientific bases of risk assessment, communitybased environmental groups were coming to realize that risk assessment was not a value-neutral scientific tool, but was in fact quite political, in the sense that it could be used to reach predetermined conclusions through the deliberate choice of assumptions, uncertainty factors, and judgments. Furthermore heavy reliance on risk assessment had the effect of placing decisions in the hands of experts instead of the hands of the people who would be affected by the decisions.

These concerns led to the development of new decision-making criteria, which are still evolving now. Some examples:

• The 'polluter pays' principle, which was adopted by the Organization for Economic Cooperation

and Development (OECD) in 1974 and reaffirmed in the US's Comprehensive Environmental Response, Compensation, and Liability Act (CER-CLA, or 'Superfund') in 1980.

- The 'substitution principle', adopted in Sweden's 1990 Act on Chemical Products, which reads, in part, 'Anyone handling or importing a chemical product must take such steps and otherwise observe such precautions as are needed to prevent or minimize harm to man or the environment. This includes avoiding chemical products for which less hazardous substitutes are available'.
- The principle of reverse onus, or reverse burden of proof, says that when there are scientifically based suspicions of harm about a chemical or product or process, the burden is on the producer or user to convince government authorities, beyond a reasonable doubt, that the product or process should not be restricted and that it is the least-damaging alternative available. This principle was recommended for adoption by the US and Canada in 1990 by the International Joint Commission (IJC, created by the Boundary Waters Treaty of 1909 to oversee international matters in the Great Lakes). To date, the United States and Canada have not acted on the IJC's recommendation, but Sweden adopted the principle of reverse onus for chemicals in 1990.
- In 1993, the World Bank suggested an ethical criterion for choosing among development projects: the least harmful alternative should be selected.
- Though not specifically intended for use in environmental decision-making, the political philosophy of John Rawls, with its focus on benefiting the least well-off and protecting the most vulnerable, has been gaining influence since it emerged in 1971.
- The public trust doctrine has evolved as a foundation stone for environmental protection. This ancient legal doctrine holds that our common heritage – the environment – is held in trust, with government the trustee and present and future generations the beneficiaries. Under this doctrine the trustee has an affirmative and inalienable duty to protect the trust property (nature, human health, the genetic integrity of living things, accumulated human knowledge, and, arguably, more) from the exploitive tendencies of the beneficiaries themselves.
- Biological criteria for environmental decisions:
 Synthetic chemicals should be eliminated if they are toxic or persistent in the environment. In their joint 1978 Water Quality Agreement, the United States and Canada defined a 'toxic

substance' as 'a substance which can cause death, disease, behavioral abnormalities, cancer, genetic mutations, physiological or reproductive malfunctions or physical deformities in any organism or its offspring, or which can become poisonous after concentration in the food chain or in combination with other substances'. The International Joint Commission defined 'persistent' chemicals as those having a half-life of 8 weeks or longer. The half-life is defined as the time it takes for half of any substance to degrade once it has been released into the environment.

- Synthetic chemicals that bioaccumulate should be eliminated. A substance bioaccumulates if its concentration increases as it moves through the food chain. For example, DDT may be found at 1 part per million (ppm) in fish and at 10 ppm in fish-eating birds. Thus DDT bioaccumulates and therefore would be a candidate for elimination.
- The Natural Step: It was invented by a Swedish pediatric oncologist Karl-Henrik Robert with assistance from physicist John Holmberg. Robert, a respected cancer researcher, concluded in the mid-1980s that humans are destroying the natural environment because they lack fundamental principles for making decisions about technologies. He said then, "Up to now, much of the debate over the environment has had the character of monkey chatter amongst the withering leaves of a dying tree."

The Natural Step defines four 'system conditions' that must prevail for a society to become sustainable.

System condition no. 1: In order for a society to be sustainable, nature's functions and diversity will not be systematically subject to increasing concentrations of substances extracted from the Earth's crust.

System condition no. 2: Nature's functions and diversity will not be systematically subject to increasing concentrations of substances produced by society.

System condition no. 3: Nature's functions and diversity must not be systematically impoverished by physical displacement, over-harvesting, or other forms of ecosystem manipulation.

System condition no. 4: Resources must be used fairly and efficiently in order to meet basic human needs globally.

• The precautionary principle, which originated in Germany in the 1970s in response to damage by acid rain in the beloved Black Forest. The original German concept, 'Vorsorgeprinzip', was developed to guide environmental planning. It translates best as 'the forecaring principle' but it also carries the connotation of foresight and preparation for the future, not merely precaution.

In 1992, the US government ratified the precautionary principle by signing the 'Rio Declaration' of the United Nations Conference on Environment and Development. Article 15 of the Rio Declaration says, "In order to protect the environment, the precautionary approach shall be widely applied by States [nations] according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation." Cost-effective means lowest cost.

In one form or another, the language of precaution has now been adopted in many international treaties and conventions, such as the North Sea Declaration (1987), The Ozone Layer Protocol (1987), the Ministerial Declaration of the Second World Climate Conference (1990), the Maastricht Treaty that created the European Union (1994), the United Nations Fisheries Convention (1995), The London Convention Protocol on ocean dumping (1996) and the Cartagena Protocol on Biosafety (2000), among others.

In the United States, the precautionary principle has evolved since 1992. A 1998 formulation, known as the Wingspread Statement, says,

When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically.

In this context the proponent of an activity, rather than the public, should bear the burden of proof.

The process of applying the Precautionary Principle must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives, including no action.

All formulations of the precautionary principle share three common precepts:

- 1. where there is reasonable suspicion of harm based on evidence,
- 2. and we have scientific uncertainty about cause and effect,
- 3. then we have a duty to take action to prevent harm.

The central feature of the precautionary approach is the way it alters society's traditional response to uncertainty. Since at least the publication on John Stuart Mill's On Liberty (1869), westerners have assumed that individuals enjoyed the fundamental freedom to behave as they saw fit until their behavior harmed another person (or harmed the larger society in some way), at which point society assumed the right to intervene. Traditionally, suspicion of harm accompanied by uncertainty has not provided a sufficient justification for society's intervention. Instead, convincing proof of harm, bordering on certainty as to cause and effect, has been required.

During the 1960s and 1970s, the study of environmental and human health problems revealed that by the time scientific consensus has been achieved regarding the causes of a particular harm, great damage can occur. Diethylstilbestrol (DES) was shown to cause cancer in laboratory animals in 1938 but it was nevertheless prescribed to millions of women until it was banned in 1971 after it was shown that children of DES-treated women were developing cancers and reproductive problems; lead in paint was known to poison children in the 1920s, or even earlier, yet it was not banned until 1976; studies in the 1930s indicated asbestos could cause lung cancer but its use in building materials was allowed to continue into the 1970s. This list could be readily extended with additional instances in which reasonable suspicion of harm was ignored while the search for scientific certainty unfolded – all at enormous cost to individuals and society.

Opponents of a precautionary approach say they fear it will stifle technical innovation, but proponents believe it will spur innovation through the systematic search for least-harmful ways of meeting society's needs.

In 2000, the European Environment Agency formally adopted the precautionary principle to guide all its environmental policies. In the United States the federal government has generally resisted adopting a precautionary approach, but precautionary thinking is beginning to be embraced at the local level. In 2003, the City and County of San Francisco, California adopted the precautionary principle to guide all policies. Many municipalities across the United States have adopted a precautionary approach to pest control, based on the assumption that chemical pesticides may be harmful to nonpest species and should be used only as a last resort.

The essence of the precautionary principle is perhaps best summed up in the time-worn adage, 'Better safe than sorry', and 'A stitch in time saves nine'. This approach seems to be slowly replacing the earlier philosophy of development, which was closer to 'Nothing ventured, nothing gained' and even in many cases, 'Damn the torpedoes, full speed ahead!'

New Ways of Seeing

In 1987, a new academic discipline emerged, called 'ecological economics', based on a new view of the world and the role of humans in it. The new view reveals a world that is no longer empty, but is now full – of humans and their artifacts. In this view, the human economy is now large enough to stress the recuperative powers of the ecosystems upon which the human economy depends. This branch of the environmental movement is thus offering a fundamental challenge to 300 years of economic thinking, which has always assumed that the human economy was in danger of remaining too small to meet all human wants, not that it might grow too large.

In support of their 'full world' hypothesis, ecological economists adduce various kinds of evidence:

- The human economy uses, directly or indirectly, ~40% of the net primary product of terrestrial photosynthesis. This means that, with one more doubling of human population (expected in 40–45 years), humans will be appropriating 80% of net terrestrial primary productivity – leaving little room for other than domesticated species.
- One result of human appropriation of the earth's terrestrial resources is soil degradation, which is widespread; worldwide, rates of soil loss exceed rates of soil formation by at least a factor of 10.
- Another result of human appropriation of terrestrial resources is the rapid loss of species, which is now proceeding at a rate somewhere between 100 and 1000 times the historical rate of extinction.
- The human contribution to atmospheric carbon dioxide (a 30% global increase in 200 years) and methane (which doubled in concentration during the same period) indicates that the human economy is now capable of disrupting ecosystems at a global scale. The US National Academy of Sciences has warned that climate change caused by the accumulation of 'greenhouse gases' (carbon dioxide, methane, nitrous oxide, and others) in the atmosphere may be the most pressing international issue of the present century.
- The buildup of greenhouse gases in the atmosphere, and the rupture of the earth's stratospheric ozone shield by chlorofluorocarbons (CFCs), indicate that the human economy has already exceeded the assimilative and regenerative capacities of the biosphere to absorb some human wastes. There is considerable evidence to support this general proposition. For example, persistent synthetic toxicants are now measurable from the peaks of the highest mountains to the floors of the deepest oceans and everywhere in between. It is

evident that nature is unable to degrade certain synthetic compounds as rapidly as humans are able to produce them.

Obviously if this 'full world' view is correct, then relying on global economic growth to solve problems of poverty and environmental degradation could be self-defeating. Indeed, ecological economists assert that the Brundtland Commission's proposed solution to poverty and environmental destruction – expanding the size of the global economy by a factor of 5 to 10 – is ecologically impossible. In this new view, poverty will have to be alleviated – and environmental degradation checked – by economic growth in poor regions matched by a reduction in the size of the economy in wealthy regions.

Unceasing growth is not observed in nature, giving support to the ecological economists' view that the human economy must eventually achieve a steady state. In this view, 'growth' must cease but 'development' can continue indefinitely. Growth is defined as increase in material throughput whereas development is qualitative improvement, such as gains in the efficiency of resource use. This distinction between 'growth' and 'development' is central to ecological economics. In the 'full world' view, there are definite ecological limits to growth, but not to development.

Ecological economists argue that the 'full world' hypothesis calls for new scientific approaches, new solutions for economic problems long ignored by traditional economists, new emphasis on conserving nature's bounty, and new 'incentives based' policies for managing and protecting the environment.

New Scientific Approaches

Ecological economists say the 'full world' hypothesis will require humans to re-organize their intellectual activities, shifting the fundamental scientific paradigm from Newtonian physics to ecology. Newtonian physics views the world as linear, separable, reducible to its component subsystems, which can be readily aggregated to model the behavior of the whole system. In contrast, an ecosystem perspective develops a worldview adapted to complex living systems – constantly interacting and evolving, nonlinear, and not scalable by simple aggregation.

In the ecological view, human knowledge of the evolving world is fraught with fundamental uncertainties that are large and likely to remain so, spawning a scientific approach that has less confidence in its predictions and prescriptions than was common in an earlier era. The ecological approach has produced a generation of scientists – among them, conservation biologists – who advocate greater humility and a more precautionary approach than was common in the past, with an orientation toward learning from nature for the purpose of working with it rather than subduing it.

Problems Traditionally Ignored by Economists

The full-world hypothesis, combined with the desire to design sustainable economic systems, confronts ecological economists with three economic problems – scale, distribution, and allocation. The problems of scale and distribution have traditionally been ignored by economists.

The scale problem requires economists to ask how large the human economy can become in relation to the biosphere from which it draws raw materials and to which it returns wastes. Neoclassical economists have never addressed this problem of scale, believing as they do that scarcities will always generate price signals that cause new technical solutions to emerge, making everyone better off - the very definition of progress. However, for reasons given above, ecological economists believe that the first priority must be to establish the ecological limits of sustainable scale and then establish policies to assure that the material throughput of the economy remains within those limits. Without limits on the scale of the human enterprise, damage to ecosystems may deprive us of essential ecosystem services (such as regulation of atmospheric temperature, or protection from ultraviolet radiation from the sun) making us all worse off. Traditional market mechanisms take no notice of scale, so limits must be determined by informed choices, then translated into explicit policies, ecological economists believe. Discovering ecological limits will require urgent scientific effort in coming decades, they say.

Distribution has to do with the fair and equitable apportionment of economic goods and opportunities. Solutions to sustainability that are seen as unfair may be rejected or dismissed out of hand by decisionmakers. As with scale, traditional market mechanisms take no notice of fair or equitable distribution, so a desirable distribution must be consciously chosen and implemented through explicit policies. Ecological economists say that the clearest implication of the full world hypothesis is that the current level of per capita resource use in wealthy countries cannot be generalized to poor countries, given the present global population, because present global resource use already appears to be unsustainably large. If the scale of the total global economy is bounded by ecological limits and resource scarcities, then poverty can be alleviated by distribution policies that promote growth in poor regions balanced by economic shrinkage in wealthy regions.

After questions of scale and distribution have been settled, then markets and prices can be relied upon to achieve their traditional purpose – an efficient allocation of resources: how many shoes will be produced at a particular price, versus how many bicycles, and so forth. Allocative efficiency by itself does not ensure sustainability, which is why questions of scale and distribution must be settled first, as ecological economists see it.

New Emphasis on Conserving Natural Capital

One consequence of a full world, ecological economists say, is that the human economy has evolved from one in which human capital was the limiting factor to one in which remaining natural capital is the limiting factor. Natural capital is the stock that produces a flow of natural resources – forests that yield lumber, petroleum reserves that yield petrochemicals, and so on. If the pattern of scarcity has fundamentally evolved to a new condition – as ecological economists believe it has – then economic policies should aim to preserve, and where possible increase, natural capital and enhance the efficiency of its use, rather than merely continuing to liquidate natural capital to accumulate human capital.

Traditional economists teach that human capital is a nearly perfect substitute for natural capital – as we exhaust one natural resource, human ingenuity will always find a substitute. Ecological economists emphasize that human capital is far more likely to complement natural capital than to substitute for it, a view that highlights the importance of preserving our remaining natural capital and using it more efficiently. Fishing boats complement, but do not substitute for, fish, and sawmills complement, but do not substitute for, trees.

Neoclassical economists take it on faith that technological advances, driven by higher prices generated by scarcities, will always be able to overcome resource limits, and that new technologies can substitute for ecosystem services that become degraded. Ecological economists are not so optimistic because they believe human activity is ultimately constrained by ecological limits.

Herman Daly offers three criteria for the maintenance of natural capital:

1. For renewable resources, the rate of harvest should not exceed the rate of regeneration.

- 2. The rates of waste generation should not exceed the assimilative capacity of the environment.
- 3. The depletion of a nonrenewable resource should require comparable development of renewable substitutes for that resource.

Ecological economists believe that traditional economists have, so far, not taken into account the fundamental shift that has occurred in the pattern of scarcity – from human capital as the limiting factor to natural capital as the limiting factor – perhaps partly because the changes wrought by exponential growth appear so quickly. With a constant rate of growth, in the same time it took the world to move from 1% full to 2% full, the world will move from half full to completely full – a change that ecological economists see occurring in the present era.

New Policies

The environmental movement developed in response to the perceived failure of government to protect our common heritage, the natural environment, from harmful human activities and technologies. Ecological economists trace this failure to government's reliance on a limited set of policy mechanisms – chiefly regulation.

State and local governments have proved to be ineffective regulators because they compete with each other to achieve economic growth, creating a 'race to the bottom'. In principle, this defect could be remedied by federal regulations to which all states must conform. However, even federal standards suffer lapses in compliance and enforcement so, for example, many major municipalities have failed to meet federally mandated air quality standards.

Perhaps the most fundamental criticism of the regulatory system is that it assumes that emissions are harmless until violation of a regulation can be proved, or harm can be shown beyond a reasonable doubt. Since the vast majority of emissions are not subject to any regulations, the public bears the burden of proving harm, a very difficult burden to meet, for reasons discussed above.

Although the regulatory system has unquestionably prevented much harm, relevant questions remain: Has regulation adequately protected the public trust? Can regulation provide adequate protection against potential harms of current and emerging technologies such as pesticides, nuclear proliferation, biotechnology and nanotechnology? Can regulation provide sufficient protection for public health and the environment at least cost?

Ecological economists would likely answer all three questions in the negative. They – and many

traditional economists – favor new 'incentives based' environmental management techniques to supplement or replace regulation, including some or all of these:

- taxing emissions;
- issuing a limited number of marketable permits to pollute;
- adding 'product charges' to the prices of products that pollute;
- in come cases, subsidizing polluters who abate;
- creating property rights in open-access resources;
- labeling products with their contents;
- educating consumers;
- imposing deposit-refund systems on manufacturers of products; and
- prohibiting the discounting of future benefits.

Emission fees, or taxes, are fairly straightforward. They require government to set a fee per unit of emission, monitor emissions, and collect the fees. Emission fees must be set high enough to provide a continuous incentive for emitters to abate, yet not so high as to be perceived as grossly unfair or unreasonable. This requires knowledge of the costs of abatement and of the harms caused by various emissions – a difficult problem inherent in the regulatory system as well. Exceptionally toxic materials – for example, highly radioactive elements, lead, mercury, and polychlorinated biphenyls (PCBs), to name a few – would not be candidates for control by emission fees alone, but would remain under strict regulatory limits.

Marketable permits to pollute offer an economically efficient way to reduce pollution from numerous sources in a region, at least total cost to the economy. This is so because the cost of pollution abatement varies from firm to firm. Firms that can abate cheaply can be expected to do so, and can then sell their unused pollution permits to firms having higher abatement costs. A total cap on allowable pollution in a region would be set by the total number of pollution permits issued. As time passed, the total amount of permitted pollution could be ratcheted down. Difficulties with this scheme arise from the obvious injustices that can occur when firms with obsolete plants in poor neighborhoods find it cheaper to buy pollution permits than to upgrade and abate, potentially creating dangerous 'hot spots' of pollution in communities of color or low income.

Ecological economists are quick to point out that the economic efficiencies to be gained by 'incentives based' approaches should never be allowed to trump other critical, deeply held values such as fairness, justice, scientific integrity, and political acceptability achieved through democratic participation. The ecological economists' vision summarized:

- 1. Earth is materially finite and not growing; therefore, there are limits to the size of the human economy that can be supported. The human economy is the product of human population and percapita resource use and it is the total product that must be limited, not merely population.
- 2. A sustainable future is possible, offering a high quality of life to all creatures (human and nonhuman), subject to the limits inherent in a materially closed system, but achieving the vision will require new ways of doing things, many of which remain to be discovered.
- 3. In ever-changing complex systems, such as human economies interacting with ecosystems, fundamental uncertainty is large and likely to remain so, and some processes are irreversible – which counsels humility and a precautionary approach.
- 4. Because human capital and natural capital complement each other, and hardly ever substitute for one another fully, new emphasis must be given to retaining and enhancing natural capital, finding ways to make it more productive without destroying it. In sum, we must learn to live off the interest derived from natural capital while preserving the capital stock itself.
- 5. Management should be proactive, experimental and adaptive, using incentives to change human behavior, relying less on regulation than in the past. In other words, more carrots, fewer sticks, and much experimentation to discover what works.

Ecological economists believe that good environmental management must be experimental and adaptive because ecosystems are ever-changing and therefore our knowledge of them is fraught with uncertainties. Rather than relying solely on scientists to determine the best approach, resource managers must experiment, observe, and adapt their management techniques to what they are learning. Ecological economists expect local communities and the interested public to play an important role in experimentation, monitoring, and learning. This is quite a different approach from older resource management techniques in which scientists were expected to determine the truth about a problem, managers to apply the scientific information, and the public to sit passively by.

Summary

The US environmental movement is large, diverse, and growing. However, it has not yet achieved the

coherence of previous social movements like the nineteenth-century crusade to end slavery, or the 100-year struggle that won women the right to vote. Toxics activists, cancer survivors, animal rights groups, traditional conservationists, and workers focused on job safety all know of each other's existence but do not often coordinate their efforts, and they do not yet share a few simple goals toward which they are all striving.

As environmentalism has developed over the past 50 years, major shifts in emphasis have become apparent:

- The overarching goal is slowly shifting from managing problems after they appear to avoiding problems before they occur. This incorporation of the first principle of public health – prevention – into the industrial enterprise has begun, but it has also met implacable opposition from some who are committed to business as usual. It remains to be seen to what extent humans can develop the capacity to foresee and forestall while retaining the corporate structure that presently provides so many of the benefits of industrial production.
- There is no 'away', so none of us can any longer claim to throw anything away. Everything must go somewhere, which has spawned attempts at comprehensive materials management – cradle to cradle thinking, not cradle to grave. This new approach has generated a host of innovative ideas including biomimicry, green chemistry, zero waste, clean production, and so forth. Presently these ideas reside at the fringe of industrial innovation, but how long will they remain there?
- An earlier focus on preserving wild lands and protecting endangered species has been expanded to include the need to protect all living things. Two basic arguments support this expanded view:
- In the modern era, humans have learned anew that their well-being is dependent upon the mosaic of biological systems that make up the biosphere. And because the biosphere is constantly evolving and therefore may never be fully understood in its totality of relationships, no one today can reliably discern which parts of it are expendable without sacrificing long-term stability. Therefore it seems only prudent to conserve all the parts.
- The reemergence of an ancient ethical perspective, that all living things have an inherent right to lead their own lives, beyond any instrumental value they may have for humans.
- Human health requires more than the control of infectious diseases. As the ancients knew, it first requires relatively clean and safe places in which to live, grow, play, learn, and work. And it

requires active community attention to the social determinants of health including income, education, living conditions, and the corrosive dynamics of social isolation, racism, inequality, and injustice.

• For 100 years or more the conservation and public health movements relied almost exclusively on experts to design and implement solutions to problems that were viewed through the twin lenses of engineering and the physical sciences. In the last quarter of the twentieth century, citizens began to insist that other valid ways of knowing must be brought to bear as well – perspectives of local knowledge, spiritual values, history, ethics, environmental justice, fairness, and democratic participation in decisions by those who are affected.

Still, despite heroic efforts by all concerned, a basic conflict persists between the needs of the natural environment and the needs of the human industrial enterprise. Although in principle nothing prevents humans – and all other creatures – from sharing the Earth and enjoying 'the good life' without undermining the biological integrity of the planet, in the modern era that goal has so far remained elusive and unmet. Nevertheless, rapidly growing recognition of the urgent nature of the problem – and new ways of conceptualizing, understanding, and responding to it – give reason for hope.

See also: Clean Air Act (CAA), (US); Clean Water Act (CWA) (US); Environmental Protection Agency (US); Food and Drug Administration (US); Genetically Engineered Foods; Lead; Mercury; Occupational Safety and Health Act, US; Organisation for Economic Cooperation and Development; Polychlorinated Biphenyls (PCBs); Risk Assessment, Ecological; Risk Assessment, Human Health; Toxic Substances Control Act.

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Environmental Change See Global Environmental Change.

Environmental Health

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Environmental health is a discipline that examines human health effects from exposures to harmful agents in the environment. 'Environment' may include the outdoors, home, workplace, or public buildings. This field incorporates aspects from many diverse fields, including: (1) environmental, occupational, and food toxicology; (2) environmental and occupational medicine; (3) food safety; (4) occupational health and safety; (5) industrial hygiene; (6) public health; (7) epidemiology; (8) environmental policy and law; and (9) psychology and sociology.

Potentially harmful agents in the environment include biological, physical, and chemical agents. Harmful biological agents, or pathogens, may include bacteria, viruses, and parasites. Pathogens spread by person-to-person contact are sometimes included in the field of environmental health, but more often they are limited to those spread by environmental contamination as a result of human activities, such as sewage disposal and livestock production. Environmental health often addresses diseases caused by noncontagious pathogens and biotoxins. Noncontagious diseases are contracted directly from the environment rather than spread person-to-person, such as Anthrax and Escherichia *coli* infections. Biotoxins are poisons produced by bacteria or fungi, and may be taken up by ingestion or inhalation. Examples include botulinum toxin, aflatoxins (produced by fungi), and toxins produced black mold growing in houses. Diseases spread by

arthropod vectors (mosquitoes, flies, ticks, mites, and fleas) and zoonoses may also be included under environmental health. Zoonoses are diseases that are contracted from animals, either by direct contact (Ebola, hanta virus, rabies, monkey pox, etc.) or via arthropod vectors (e.g., west Nile virus, St. Louis encephalitis). In this case, wild or domestic animals may provide a reservoir for the disease. Within the context of environmental health, interest in these diseases is often focused on anthropogenic (manmade) environmental disturbances, for example, global warming, agriculture, affect the spread of such diseases.

Physical agents in the environment that may cause illness include solar ultraviolet (UV) radiation, ionizing radiation (produced by radioactive materials and X-rays), extreme temperatures, noise, vibrations, and particulates. The most famous particulates inducing adverse health effects include asbestos and silica dust. Other physical agents, such as electric or magnetic fields and microwaves, may also cause adverse health effects, but there is of yet not enough solid evidence to support or refute this hypothesis.

Effects of environmental chemical agents on human health perhaps represent the bulk of environmental health research. People may be exposed to harmful chemicals in the outdoor air, surface water (lakes, rivers, oceans, etc.), soil, indoor air, at the workplace, in food, or from consumer products. Exposures to chemicals (or other agents) in the workplace are called 'occupational exposures'. Food exposure to chemical or biological agents may occur as a result of agricultural applications, environmental pollution, or are formed when foods are cooked. Effects of food additives, chemicals produced by plants, and organisms associated with spoilage usually come under the subject of food safety or food toxicology. Consumer products include objects that may give off gases or vapors or leach chemicals into the water (e.g., carpeting, upholstery, plastics), items that may contain hazardous materials (e.g., batteries), or chemicals used in the home, yard, garden, or garage. Also, exposure to biological or chemical agents as a result of warfare or terrorist attacks has recently become a prime concern of environmental health workers.

Exposure to environmental agents may be through the skin, lungs, or digestive system. Effects from environmental exposures to harmful agents depend upon duration, frequency, and severity of exposure, as well as susceptibility of the individual. Susceptibility may depend upon age, nutritional status, sex, or genetic makeup (heredity). The duration, frequency, and severity or exposure depend upon many factors including locality, occupation, behavior, socioeconomic status, and population density.

Effects of exposures may encompass environmentally induced diseases and syndromes such as cancer (including leukemia), birth defects, neurobehavioral disorders, autoimmune diseases, acquired allergies, multiple chemical hypersensitivity, infections, and 'chronic fatigue syndrome'. Other effects of particular concern include effects on the immune, reproductive (including sterility and fertility), and endocrine (hormone) systems, nerve toxins, and brain development. Disorders of various organs that are routes or exposure or function in detoxification of chemicals - including lungs, skin, digestive tract, liver, and kidney - are also common. Organs with high rates of cell division, such as the skin, bone marrow, gonads, and developing embryo/fetus, are often highly susceptible to environmental agents. Also, some organs tend to accumulate ('bioaccumulate') toxic chemicals. For example, fat-soluble chemicals may bioaccumulate in fatty organs such as liver, brain, and breasts, while certain metals and radioactive materials may accumulate in bone.

Monitoring

In cases where people may exposed to hazardous agents on a regular basis, environmental surveillance may be carried out. This may consist of regular medical check-ups, environmental monitoring, biomonitoring, and dosimetry. Environmental monitoring includes collection of environmental media (air, water, soil) for chemical analysis, or may include real-time monitoring using devices that detect exposures to hazardous agents immediately or nearly so. Biomonitoring includes collection of biological samples, usually fluids or expired air, for determination of chemical concentrations or biomarker analysis. Finally, dosimetry is often determined using small devices worn on the person that give an indication of the dose of hazardous agents to which people were exposed.

Protecting the public from hazardous agents depends upon knowledge of the health effects of such exposures. One method of gathering information on the health effects of environmental chemicals is by using toxicity tests on animals' (usually rodents) in vitro systems (cultured cells or cellular components in flasks or test tubes). A second method of determining health effects of hazardous agents is by clinical challenge studies, clinical observations, and case studies. Challenge studies are when volunteers are exposed to low levels of chemicals or other agents under carefully controlled clinical settings. Clinical observations involve identifying clusters of disease associated with toxic exposures. Case studies are indices where individuals or a small group of people are exposed to high doses of a contaminant as a result of accidental poisonings or industrial accidents.

Another tool used by environmental health professionals to determine health effects of environmental agents is the field of epidemiology, which studies the incidence and progression of diseases in populations. Epidemiological studies may be prospective or retrospective. Prospective studies predict the scope and magnitude of diseases that have not yet been manifested, while retrospective studies assess the cause and/or magnitude of disease that already occur. Epidemiological studies may also be descriptive or analytical. Descriptive epidemiology uses vital statistics (birth and death rates), patterns of disease, or incidence of disease in exposed and unexposed populations at a single point in time ('cross-sectional' studies). Analytic epidemiology calculates risk factors for hazardous agents by either identifying exposed and unexposed segments of the population and comparing their disease frequencies ('cohort' or 'longitudinal' studies), or by identifying diseased and healthy individuals and determining their exposure histories ('case-control' studies). Another branch of epidemiology is molecular epidemiology, which incorporates biomarkers into descriptive or analytical studies in order to determine individual susceptibility to environmental hazards.

Unfortunately, however, assigning a cause to environmentally induced diseases is often difficult for the following reasons: (1) there is often a latency period of months, years, or decades between exposure and onset of disease; (2) there are often multiple causes of each disease; (3) there are few diseases that are specific to any one agent; and (4) there are usually a host of confounding factors (e.g., age, gender, socioeconomic status, and behavioral factors such as smoking and consumption of caffeine or alcohol) that may influence incidence of disease. For these reasons, epidemiological studies often employ an established set of criteria for establishing causality for any environmental disease.

Determination of exposure and toxic effects of chemicals also requires knowledge of toxicokinetics. Toxicokinetics is the study of changes in the levels of toxic chemicals and their metabolites over time in various fluids, tissues, and excreta of the body, and determines mathematical relationships to explain these processes. These processes depend upon uptake rates and doses, metabolism, excretion, internal transport, and tissue distribution. Methods for determining these processes include studies with laboratory animals, volunteer human subjects, persons accidentally exposed to high doses of chemicals, and experiments with tissue or organs cultured in the laboratory. Computer simulations of such processes are often formulated using complex mathematical equations.

Protection of the public from exposures or effects of chemicals involves constructing safety standards, regulations, and exposure limits. This process usually relies upon human health risk assessment, which is a process of quantifying the likelihood, magnitude, and duration of human health effects from hazardous environmental agents. Human health risk assessment of hazardous chemicals consists of the following steps: (1) hazard definition and identification: establishing cause and effect relationships using animal or in vitro toxicity studies, clinical studies, epidemiology, and quantitative structure activity relationships (QSARs: prediction of a chemical's toxicity from its molecular structure); (2) establishing dose-effect relationships (i.e., what is the magnitude and duration of an effect for a given degree of exposure); (3) exposure assessment: includes environmental chemistry and surveillance, mathematical and computer simulation modes of environmental behavior of chemicals, toxicokinetics, and identification of all likely exposure pathways (intentional ingestion of contaminated food or water, accidental ingestion of soil, inhalation of gases, vapors, and particles, and absorption through the skin); and (4) risk characterization, which involves integration of the above three steps. Like epidemiology, risk assessments may be prospective (or predictive) or retrospective. Prospective studies assess possible occurrence and severity of health risks in which environmental exposure is hypothetical, but likely to occur. In retrospective studies, humans are environmentally or occupationally

exposed to hazards and the potential for health risks (and how to mitigate them) is assessed.

Environmental health professionals, policy makers, and government officials use the outcome of risk assessments for risk communication (informing the public of possible risks and how to avoid them) and risk management (weighing policy alternatives and selecting appropriate regulatory action). Regulatory actions may include establishing exposure limits for certain chemicals, setting emissions standards for environmental pollutants, or taking protective actions. Protective actions may include: (1) isolation: prohibiting the public from, or advising against, entering certain areas; (2) shielding: using physical shields or protective clothing to prevent exposures; (3) time: limiting the time people may spend in hazardous areas; (4) treatment: treating environmental media to reduce or eliminate toxic or infectious agents; and (5) prevention strategies, such as vaccination against infectious agents or eating antioxidants to prevent toxic effects of certain chemicals.

To an increasing degree, environmental management programs are integrating protection of the environment and protection of human health. This may include integrating human health and ecological risk assessments. This also includes integration of effects of pollutants on ecosystem and human health. For example, an algal bloom caused by fertilizer pollution may deplete dissolved oxygen in the water and affect the natural ecosystem, and produce chemicals that are toxic to humans. Such integrated assessments are a practical means of reducing effort and money used in environmental management and protection.

See also: Asbestos; Biomarkers, Human Health; Biomonitoring; Botulinum Toxin; Chemicals of Environmental Concern; Ecotoxicology; Epidemiology; Pharmacokinetics/Toxicokinetics.

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Environmental Hormone Disruptors

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The idea that exposure to small amounts of certain chemicals in the environment might disrupt normal endocrine function in wildlife and humans garnered widespread attention in the mid-1990s, and debate over the importance of this phenomenon continues today. Effects that have been potentially linked to these chemicals include feminization of males in various aquatic and avian species; declines in sperm quality; increased incidence of abnormal or incomplete genital development in human males; and increases in certain endocrine-mediated cancers such as breast, testicular, and prostate cancer. Concern for compounds that could cause such adverse effects initially focused on chemicals with estrogenic activity, and thus they were commonly referred to as environmental estrogens, or xenoestrogens. The initial focus on xenoestrogens has since expanded to include compounds with androgenic activity, as well as thyroid-active chemicals. Thus, today these compounds are commonly referred to as endocrine disrupting chemicals (EDCs). Compounds that have been implicated as EDCs include certain chlorinated organic compounds, principally some pesticides such as DDT and kepone, as well as polychlorinated biphenyls; some plasticizers and breakdown products of polycarbonate plastic, such as pthalates, and bisphenol A (BPA); and some pharmaceutical agents such as diethylstilbestrol (DES).

The endocrine system comprises glands located throughout the body, hormones synthesized by these glands, and specialized receptor molecules in target tissues that recognize and bind with these hormones. Naturally occurring hormones in the human body, such as the sex hormones estrogen and testosterone, thyroid hormone, and insulin (among others) are involved in a variety of processes including growth and development, sexual differentiation and behavior, metabolism, and reproductive function. Appropriately timed changes in the circulating concentrations of hormones exert control over these

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processes by binding with specific receptor molecules in target tissue, and thus eliciting the appropriate biological effects. At least in the laboratory, some EDCs, in sufficient concentrations, act as agonists, by binding with hormone receptors to trigger the receptor's biological actions. Other EDCs act as antagonists, by binding with hormone receptors in a way that prevents access of the naturally occurring hormones to their receptor, without triggering the receptor's biological actions. EDCs can also alter either the production or metabolism of certain hormones. The concern with EDCs is that they could potentially produce hormonal stimuli that are of inappropriate timing, magnitude, or biological context, which could thus result in unwanted biological effects such as feminization of males or increases in endocrine-mediated cancers. Because only very small amounts of naturally occurring hormones in the body are required to elicit their effects, some scientists believe that likewise only very small quantities of environmental EDCs would be required to elicit inappropriate endocrine effects. There is particular concern for exposures occurring during development, when timing and magnitude of hormone signals are critically important for many developmental processes.

Several lines of evidence helped to focus attention on the phenomenon of endocrine disruption. One was the observation, beginning in the 1950s, of reproductive problems in various wildlife populations, including reptiles, birds, and mammals. Some decades later, it was proposed that a common factor underlying these effects might be that environmental chemicals were affecting sex hormone-controlled processes. Then, in the late 1980s, there was the discovery that very low concentrations of the chemical *p*-nonylphenol, which is used in the manufacture of plastics, was leaching out of plastic test tubes and causing extensive cell proliferation in laboratory cultures of estrogen-sensitive breast cancer cells, even in the estrogen-free control cultures. At about the same time, in vivo studies in mice indicated that traits such as aggressiveness in females and prostate gland size in males could be influenced by intrauterine position.

In these studies, female mice that as fetuses were situated *in utero* between two males were more likely to be aggressive, while male mice that had been situated *in utero* between two female fetuses were more likely to have enlarged prostate glands. These studies demonstrated that even small variations in concentrations of hormones in the uterus could affect subsequent development.

Observations of modestly enlarged prostate glands, similar to those observed in male mice situated in utero between two female mice, were subsequently observed in male mice exposed in utero to low doses of DES, BPA (which is widely used in food and beverage containers), and the pesticide methoxychlor. These effects, which were observed at environmentally relevant doses, also included increased weight of preputial glands, decreased daily sperm production, and decreased epididymal weight. Not all studies, however, have shown effects with in utero exposure to low doses of EDCs. For example, additional studies with DES and BPA, several of which used the same study design and strain of mice as the initial studies, did not show any effect on the prostate gland, sperm production, or other organs. Based on a review of both positive and negative studies regarding low-dose in utero effects of BPA, an National Institute of Environmental Health Sciences (NIEHS)/Environmental Protection Agency (EPA) Endocrine Disruptors Low Dose Peer Review Panel concluded that these low dose effects have not yet been established as a 'general and reproducible finding'. Possible reasons for the discrepancies among studies include differences in intrauterine position, diet, living conditions (e.g., type of bedding and type of housing, and animals per cage), within strain differences, seasonal variation, and differences in body weight and prostate weight for control animals.

In addition to the question of whether in utero exposure to low doses of environmental EDCs is actually associated with specific adverse effects in laboratory animals, there is also the question of whether effects observed in laboratory animals, such as rats and mice, are relevant for humans. Although there are definite similarities between laboratory animals and humans in terms of the key hormones that are involved during gestation (e.g., estrogens and progestins), there are several differences that could play a role in relative susceptibility to environmental EDCs. One key difference relates to both the types and levels of estrogens observed in the fetus. Whereas pregnant rats and mice produce estradiol and estrone, pregnant humans also produce estriol, in addition to estradiol and estrone. Moreover, estrogen concentrations are greater in the human fetus as compared to the mouse and rat, and the proportion

of unbound (i.e., biologically active) estrogen is also greater in the human fetus. As a consequence of the greater concentration of biologically active estrogens, the human fetus may be less sensitive to low concentrations of EDCs than rats or mice.

Although many chemicals are suspected of being potential EDCs, based, for example, on their ability to bind to hormone receptors, very few chemicals have been clearly established as endocrine disruptors in vivo. This is because currently there is insufficient data to establish causal relationships between exposure to many suspected EDCs, particularly at the low concentrations typically found in the environment, and effects potentially associated with endocrine disruption. In humans, the only chemical that has been clearly established as causing endocrine-mediated adverse health effects is the synthetic estrogen DES. DES was given to pregnant women in the 1940s-1960s to prevent miscarriages, thus resulting in relatively high exposure to the developing fetus. The primary effect of in utero DES exposure was abnormal development of the uterus and vagina as well as vaginal cancer in daughters whose mothers had taken DES. These effects, which were clearly associated with DES exposure, are not indicative of effects for most environmental EDCs, as the magnitude of exposure to DES far exceeded levels of concern for environmental EDCs. In addition, most other estrogenic substances, including the naturally occurring estradiol, are much less potent than DES. In fact, most estrogenic EDCs are far less potent than naturally occurring estradiol, thus requiring much greater concentrations of EDCs as compared with estradiol to elicit an effect.

Outside of the experience with DES, there is no direct link between exposure to environmental EDCs and specific endocrine-mediated effects in humans, although some observers have attributed several temporal trends to exposure to EDCs. These include reports of declines in sperm quality (generally referring to sperm quantity, but also to morphology and motility in some cases), as well as increases in hypospadias (abnormal location of the urethral opening in males), cryptorchidism (undescended testes), and testicular cancer. Many of these studies lack information on actual exposure to environmental EDCs, however, and associations between chemical exposure and effect are only speculative. For many of these end points, there are alternative and equally plausible factors that could explain the observed trends, including changes in diet, a more sedentary lifestyle, changes in diagnosis, or changes in collection methods (for declines in sperm quality). In some cases, the observed trend is not temporally consistent with exposure to EDCs. For example, incidence of testicular cancer began to increase after World War I, while exposure to environmental EDCs increased most dramatically after World War II. Thus, exposure to environmental EDCs cannot necessarily be implicated as the primary cause for the observed increase in rates of testicular cancer. In other cases, such as for breast cancer, most studies indicate that there is no correlation with actual tissue concentrations of EDCs.

When likely exposure concentrations are taken into account, the most important EDCs to consider are not industrial chemicals but rather phytoestrogens, such as the isoflavones that are abundant in dietary soy products. Exposure to EDCs in the environment (e.g., through exposure to contaminated soil, water, or air) is typically dwarfed by our intake of these naturally occurring phytoestrogens. Soy products, which have long been consumed as part of the typical Asian diet, are increasingly consumed in Western diets. Few data exist for humans regarding potential effects of phytoestrogens, particularly for in utero exposures, although data in animals indicate that exposure to phytoestrogens at high enough levels may be associated with developmental abnormalities. Most notably, infertility has been observed in sheep grazing on clover with high concentrations of isoflavone precursors and in captive cheetahs following addition of soy meal to their diet. On the other hand, isoflavones may offer protection against breast cancer, particularly for postmenopausal women. Studies in animals also suggest that early exposure to soy products protects against formation of breast tumors later in life.

Evidence linking exposure to EDCs with specific effects is perhaps more convincing in wildlife than in humans, particularly for aquatic wildlife. For example, reduced genitalia in male alligators in Florida's Lake Apopka has been attributed to a massive spill of DDT in the late 1980s. There is also evidence that natural and synthetic estrogens in effluent from wastewater treatment plants may be causing male fish to produce elevated levels of the reproductive protein vitellogenin, which is ordinarily produced at elevated levels only in females. While such observations are certainly important in terms of their significance for aquatic populations, their significance for humans and other mammals is less certain, due to differences in physiology and metabolism. For example, although there is a high degree of similarity of the hormones and their receptors between aquatic species and mammals, there are also significant differences in hormone function and regulation. In addition, most effects that have been well established in wildlife are generally associated with localized exposure to relatively high concentrations of chemicals,

such as DDT in Lake Apopka, or effluent from sewage treatment plants.

In response to heightened concern for exposure to potential endocrine disruptors in the environment, the US EPA chartered the Endocrine Disruptors Science and Technical Advisory Committee (EDSTAC), with the charge of recommending a screening and testing strategy for potential endocrine disrupting chemicals. EDSTAC proposed an Endocrine Disruptors Screening Program (EDSP) focusing on disruption of endocrine effects related to estrogen. androgen, and thyroid hormones for both humans and wildlife. The EDSP consists of a tiered approach involving sorting and prioritization of chemical substances and mixtures for further screening and testing, based on existing data. 'Screening' of prioritized chemicals identifies chemical substances and mixtures that are potentially capable of affecting endocrine control, while subsequent and more sophisticated 'testing' studies confirm, characterize, and quantify the nature of endocrine disrupting properties. US EPA is currently in the process of establishing screening priorities, and ensuring that Tier 1 Screening and Tier 2 Testing assays are scientifically validated. The overall aim of the EDSP is to facilitate identification of EDCs, so that ultimately the US EPA can take appropriate action, if necessary. At present there are 87000 chemicals or chemical mixtures that are slated at least for screening in the EDSP.

Apart from the number of chemicals that need to be evaluated, evaluating potential EDCs for their ability to cause endocrine disruption presents several unique challenges to toxicologists. As illustrated by conflicting results from studies with BPA, the ability to detect effects at very low doses may depend on a variety of factors seemingly unrelated to exposure, such as diet, living conditions, and specific animal strain. In addition, it will be important to consider cross-species physiological differences in order to determine the relevance of findings in laboratory animals for humans or wildlife. Thus, one challenge will be to define the precise conditions under which laboratory responses can predict the existence and magnitude of true risks for humans or wildlife exposed to low levels of environmental EDCs. Another challenge is the possibility that EDCs may display either a U-type or inverted U-type dose-response curve (i.e., a dose-response curve for which low doses may be more potent than high doses). This means that the dose range for studies evaluating EDCs may need to be extended below doses at which no adverse effects are observed. Considering these challenges, it will likely be some time before there is a general consensus among scientists as to whether the endocrine disruption phenomenon is real, particularly for low-dose exposures.

See also: Developmental Toxicology; Endocrine System; Estrogens I: Estrogens and Their Conjugates; Estrogens IV: Estrogen-Like Pharmaceuticals; Reproductive System, Female; Reproductive System, Male; Toxicity Testing, Developmental; Toxicity Testing, Reproductive.

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Environmental Processes

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Environmental processes include fate and transport of pollutants in the environment, which are components of the field of environmental chemistry. The fate of pollutants in the environment depends upon the 'compartment' in which they occur: air, surface water, ground water, soil, sediment (the 'mud' at the bottom of water bodies), or living organisms. The water compartment may include ground water, surface water (water bodies such as rivers, streams, lakes, ponds, oceans, etc.), or pore water (water in between soil or sediment particles). Within each compartment, the chemical may remain unaltered, but more often is altered by biotic (living) or abiotic (nonliving) components of the environment.

Alterations may include transformation, degradation, and changes in speciation or ionization. Transformation occurs when a contaminant is chemically altered by the addition of oxygen, hydrogen, or nitrogen, or is combined with or bound to another chemical. Abiotic transformations may include chemical oxidations or reductions in aerobic or anaerobic environments, respectively. Biotic transformations may be carried out by bacteria and fungi in the environment, or may take place within the bodies of plants and animals. Transformations may either make the chemical more or less toxic, depending on the reaction involved. If the chemical is broken down into smaller molecules, this process is called degradation. Abiotic degradation may occur through reactions of the chemical with oxygen, acids, alkalis, other chemicals, or by exposure to sunlight ('photolysis' or 'photodegradation'). Biotic degradation ('biodegradation') may be carried out by plants or animals, but bacteria and fungi accomplish the bulk of the biodegradation in natural systems. If a chemical resists biotic and abiotic degradations, it is termed a 'persistent' chemical. Some especially persistent chemicals, DDT, for example, may remain in soils and sediments for decades.

Many chemicals can also exist as various species or states of ionization. For example, nitrogen can exist as nitrate, nitrite, or ammonia, arsenic can exist as arsenate or arsenite, and lead can exist as lead nitrate or lead chloride. The species or ionization state may depend upon abiotic variables such as soil or water pH, amount of dissolved oxygen in the water, and presence of other chemicals. Alternatively, bacteria and fungi may change the species or ionization state of a chemical. For example, bacteria can convert arsenite to arsenate, and add methyl groups to ionic mercury to produce methylmercury.

Chemicals may also be taken up by plants and animals from the environment. Plants can take up pollutants through roots or leaves, while animals can take in pollutants by ingestion of contaminated food or water, absorption through skin or gills, or inhalation into the lungs. Chemicals can be absorbed from the lungs by inhaled vapors or gases, particulate matter (e.g., dust), or aerosols (tiny droplets suspended in the air). Animals may also ingest from soil or sediment, and absorb chemicals through the skin from air, water, soil, or sediment. Chemicals may also be passed along in the food chain ('trophic transfer'). Certain chemicals that are lipophilic (i.e., accumulate in fat) may reach greater and greater concentrations in animals higher and higher in the food chain. Such a process is called 'biomagnification'.

The degree to which a pollutant is taken up, which also determines its potential toxicity, is determined by its bioavailability. Bioavailability refers to the ability of a chemical to move from the environment into a living organism. Bioavailability depends upon the ionization state and speciation of a chemical. Because certain organic compounds and clays can strongly bind various hydrocarbon chemicals and metals, the amount of organic carbon and clay in the soil, sediment, and water determines the bioavailability of these compounds. Bioavailability of metals is also dependent on the amount of sulfur precipitates of other metals in soils and sediments.

The fate of chemicals in the environment depends not only on processes taking place within compartments, but also by chemical partitioning between compartments. For example, there may be exchange of chemicals between air and water or soil. Movement from the water or soil into the air is accomplished by volatilization and evaporation of volatile or semivolatile compounds. Movement of chemicals from the air to water or soil is accomplished by deposition or diffusion into the water. Chemicals can also move from water to soil or sediment and vice versa. If a solid chemical in the soil or sediment dissolves into the water, this is called 'dissolution', while the opposite is called 'precipitation'. If a chemical dissolved in water attaches to a soil or sediment particle, this is called 'adsorption', while the opposite is called 'desorption'. The fugacity of a chemical, that is, its tendency to remain within a compartment, is affected by the properties of that chemical, as well as the chemical and physical properties of the environments such as temperature, pH, and amount of oxygen in water and soil. Wind or water currents, wave action, water turbulence, or disturbance of soil or sediment (through the action of air or water currents, animals, or human activities) may also affect partitioning of chemicals.

Chemicals can also be transported within and between compartments. Transport may be via convection, diffusion, or bulk transport. Convection occurs when environmental contaminants dissolved or dispersed in air or water are carried along by air or water currents. Diffusion through air or water occurs relatively slowly, and is of importance only over small distances. However, diffusion (as well as evaporation and volatilization) may be enhanced by convection, and diffusion, convection, and volatilization may act in concert to promote transport of contaminants between compartments. The third type of transport, bulk transport, occurs when pollutants are adsorbed to soil or sediment particles and are carried along by wind or water currents. Bulk transport of contaminated dust by the wind may transport the contaminants from the soil to the air compartments. Bulk transport from air to water or soil compartments may also occur when rain forms around contaminated dust particles or suspended particulate pollutants, or when gaseous pollutants dissolve in rain droplets and fall onto the soil or water surfaces. Chemicals may also be transported from water to air or soil compartments when strong waves or waterfalls produce aerosols that are carried away by the wind. Flooding may also transport contaminants from the water to the soil, either due to contaminants dissolved or dispersed in the water or from bulk transport of contaminated sediment particles. In addition, when rainwater falls on contaminated soil, it may flow overland and carry dissolved or dispersed contaminants or contaminated soil particles to surface waters, or percolate through the soil to carry pollutants to ground or surface waters. Finally, plant growth and activities of animals and humans may facilitate transport or contaminants within and between compartments.

Transport and fate of pollutants also depends upon the source of pollutants. Contaminants that originate from a definable source, for example, a smokestack or effluent (wastewater discharge) pipe, are known as 'point sources'. Sometimes, however, the source of the pollutant is more diffuse; for example, when pesticides are carried by runoff from a large area into a river. These sources are called 'nonpoint sources'. Often, point and nonpoint sources may be combined or interconverted; for example, if nonpoint runoff from a city is channeled into a discharge pipe via storm sewers.

There are also several methods to determine patterns of fate and transport of pollutants in the environment. In some cases, microcosms and mesocosms are used to study fate, biodegradability, bioavailability, and transport within compartments. Field surveys may also be used to study fate and transport of pollutants in contaminated environments. Such studies involve collection and analysis of biota, water, air, soil, or sediment. In some cases, radioactively labeled contaminants ('tracers') may be introduced in mesocosms or noncontaminated environments in order to determine their fate and patterns of transport. Finally, mathematical models are often used to produce computer simulations to study fate and transport on a large scale. Often, these models rely on the 'mass balance' concept, which asserts that the total mass of a contaminant introduced into an ecosystem must be accounted for by summing the masses present in each compartment.

See also: Ecotoxicology.

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Environmental Toxicology

Chris Theodorakis

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Environmental toxicology can be broadly defined as the effects of environmental contaminants ('pollution') on living organisms. Such contaminants are also known as anthropogenic (man-made) or 'xenobiotic' (foreign to living organisms) chemicals. As a discipline, environmental toxicology is more of an applied science rather than a basic science, but environmental toxicological investigations may be slanted more toward basic research or applied research. Studies that have more of a basic research slant may include, for example, investigations of the mechanism of action of a particular chemical on a particular organism. Research in the more applied direction may include biomonitoring, toxicity tests, or ecological risk assessments. The diverse field of environmental toxicology may include effects on individual organisms, effects on humans, or effects at higher levels of biological organization (population, community, or ecosystem). The study of the latter effects is a subdiscipline known as 'ecotoxicology'. Toxic effects of xenobiotics on humans are also covered elsewhere in this encyclopedia. Therefore, the discussion below will focus on individual-level effects on nonhuman organisms. Such effects may include overt toxic responses (e.g., acute and chronic toxicity) or sublethal effects.

Acute and Chronic Toxicity

Toxicity tests examine the effects of xenobiotics on living organisms under controlled laboratory

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conditions. Acute toxicity tests are typically shortterm tests (most commonly, 96 h in duration) and measure such endpoints as growth and mortality. In species with short generation times, reproductive endpoints may also be used. For terrestrial and aquatic plant species, the rate of photosynthesis or chlorophyll content may also be measured. Toxic effects are typically reported as LD₅₀ for terrestrial species and LC₅₀ for aquatic species (lethal dose or lethal concentration 50, the dose or concentration that is lethal to 50% of the test organisms). Nonlethal endpoints, such as growth or reproduction, may be expressed as ED_{50} or EC_{50} (effective dose or concentration, e.g., the concentration required to inhibit growth or reproduction by 50%). Toxicity tests are commonly determined using fish, crustaceans, insects, birds, algae, aquatic and terrestrial plants, and rodents. Alternative tests may use microorganisms such as bacteria. The duration of exposure is usually included in reporting acute toxicity, for example, 96 h LC₅₀.

Chronic toxicity tests may span the entire life-cycle of the organism (i.e., from zygote to age of first reproduction). However, chronic tests are often difficult to perform because of their long duration (9–30 months for fish tests) and are very expensive, which makes their routine use prohibitive. For this reason, three alternatives to full life-cycle tests include partial life-cycle tests, early life-stage tests, and short-term chronic tests. Partial life-cycle tests were developed for organisms that require >12 months to reach reproductive maturity. They are typically long enough to span a period from gonadal maturation until the first reproduction. Early life-stage tests expose organisms from the embryonic through juvenile stages, because these life stages are thought to be most sensitive to effects of environmental toxicants. Short-term chronic assays last from 4 to 7 days. Results of the chronic tests are usually expressed as lowest-observed-effects level or concentration (LOEL and LOEC, respectively) and the no-observedeffects level or concentration (NOEL and NOEC). The LOEL and LOEC are defined as the lowest toxicant level or concentration causing an effect that is statistically significantly different from the control (no toxicant present), while NOELs and NOECs are the highest toxicant levels concentrations for which the effect is not significantly different from control.

There are a variety of factors that can affect the toxicity of chemicals to living organisms. These include intrinsic factors of the organism such as species, genetic constitution, age, sex, nutritional status, and overall health. This can also include extrinsic factors such as temperature, photoperiod, and, for aquatic or soil organisms, pH, water hardness, and oxygen availability. The presence of other chemicals can also affect the toxicity of a particular chemical. These toxic interactions can include additivity (toxicity of chemical A + B = toxicity of chemical A +toxicity of chemical B), synergism (toxicity of chemical A+B > toxicity of chemical A + toxicity of chemical B), or antagonism (toxicity of chemical A + B < toxicity of chemical A + toxicity of chemicalB). In fact, humans and other organisms are usually exposed to complex mixtures of chemicals in the environment, and the effects of such mixtures on living systems has yet to be completely understood.

Sublethal Effects

Except in extreme cases (oil or chemical spills, overapplication of pesticides, etc.), environmental contamination does not result in acute toxicity in field situations. Thus, in the natural environment, pollution generally induces sublethal responses. Sublethal responses may include growth and reproductive indices, embryo/fetal development, larval metamorphosis, hormone and endocrine function, immune function, bioenergetics and metabolic rate, and overall health of the organism. Such sublethal responses can be determined during chronic or subchronic toxicity tests in the laboratory or in field surveys of indigenous organisms. Reproductive and developmental toxicity tests have also been developed using sea urchins, frogs, algae, chickens, rodents, and the aquatic organisms known as Hydra.

Although they may not directly affect survival, such responses may affect the fitness of organisms and eventually growth and sustainability of populations. Another type of sublethal effect includes so-called 'biomarkers of environmental contamination', which can be defined as alterations of physiological, cellular, biochemical, or molecular structures or processes that are indicative of contaminant exposure and effects.

Sublethal effects may also include behavioral traits of exposed organisms. Such altered behaviors may include simple behaviors or more complex behaviors. Simple behaviors include general activity level, abnormal or disoriented behavior, and avoidance of contaminated media (air, water, soil, etc.). These may occur at earlier or at lower exposure levels than overt mortality, and may be used as an early warning of toxic effects. More complex behaviors may include alterations or feeding, foraging, or predator-prey relationships, schooling in fish, migration, and homing, or reproductive behaviors. In general, simple behaviors are easier to standardize in laboratory tests, but may be less relevant to effects on fitness components (survival, growth, and reproduction) or ecological endpoints (effects on populations, communities, and ecosystems) that are more complex behaviors.

Toxicokinetics

Toxicokinetics refers to the uptake, excretion, metabolism, and distribution of environmental contaminants within the body of organisms. This discipline was derived from pharmacokinetics, which focuses on pharmacologic drugs rather than toxicants. Processes involved in toxicokinetics include uptake, elimination, and biotransformation. Uptake of contaminants can occur from ingestion of contaminated food, water, soil, or sediment, by absorption from air and water through respiratory surfaces such as lungs or gills, or absorption through skin when organisms come into contact with contaminated water, soil, or sediment. Elimination from various organs can occur through excretion of the toxicants, metabolic conversion into other chemicals, or total metabolic breakdown of the contaminants ('biodegradation'). Elimination from all possible organs and tissues is known as 'depuration'. Metabolic conversion of contaminants results in a less toxic product and the process is known as 'detoxification', but sometimes metabolic conversion results in a more toxic product, a process known as 'bioactivation'. Toxicokinetics can be determined with living organisms, but can also be simulated using computer simulation models (mathematical equations describing movements of chemicals). Such models rely on real data, however, as input and for determination of accuracy.

The rates of uptake, excretion, and metabolism and the equilibrium (or steady state) concentration of chemicals in the body ('body burden') rely on characteristics of the chemical, the environment, and the animal. (*Note*: equilibrium or steady state refers to a condition when body burden does not change over time.) Characteristics of the chemical include its water or fat solubility, its volatility, stability, and how rapidly it can be metabolized. Characteristics of the environment include temperature, soil or water pH, and presence of other chemicals or contaminants. Characteristics of the animal include age, sex, breeding condition, species, and health condition. Because a chemical must be taken up to be toxic, and because body burden affects toxicity, characteristics of the chemical, organism, and environment also affect the toxicity of chemicals.

These characteristics also determine whether or not a chemical bioconcentrates (body burden concentration > environmental concentration due to absorption from skin or respiratory organs) or bioaccumulates (body burden concentration > environmental concentration due to all routes of uptake). Similar to toxicity tests, bioaccumulation tests are laboratory exposures designed to assess the potential for bioaccumulation or bioconcentration for a chemical.

See also: Biomonitoring; Ecotoxicology; Environmental Health.

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Eosinophilia-Myalgia Syndrome

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Introduction

During the summer and fall of 1989, an epidemic of an apparently new disease occurred throughout the United States. The illness was characterized by blood eosinophilia (greatly increased numbers of the type of white blood cell usually associated with allergic reactions), myalgias (severe muscle pain), fever, joint pain, rash, itching, and generalized swelling and was termed the eosinophilia–myalgia syndrome (EMS). It was initially recognized in October 1989 when physicians in New Mexico identified three women with similar clinical findings; all three had consumed L-tryptophan prior to onset of illness. Soon after, additional cases were recognized throughout the United States and in several other countries. Some cases ultimately resulted in death or severe disability.

Epidemiological studies initiated in early November 1989 by the health departments of New Mexico and Minnesota demonstrated a strong association between antecedent tryptophan consumption and EMS. A national surveillance program to investigate the new disease was initiated by the US Centers for Disease Control (CDC). On November 11, 1989, the US Food and Drug Administration (FDA) issued a nationwide warning that advised consumers to discontinue use of tryptophan food supplements. Six days later, the agency requested a nationwide recall of all dietary supplements that would provide a daily dose of more than 100 mg of tryptophan. The recall was expanded on March 22, 1990, to include all products containing tryptophan at any dose (with the exception of protein supplements, infant formulae, and intravenous solutions that incorporated small amounts of tryptophan for nutritional requirements).

With the removal of tryptophan from the consumer markets, the number of new EMS cases diminished rapidly. Nevertheless, over 1500 persons were affected by the illness in the United States, with 37 known deaths. While the epidemiological and chemical investigations indicate that the epidemic of EMS was caused by contaminated L-tryptophan, the precise contaminant(s) or metabolites causing the disease is still uncertain.

Prevalence and Reasons for L-Tryptophan Usage

L-Tryptophan usage was widespread in the United States in 1989. In Oregon and Minnesota, $\sim 2\%$ of the household members surveyed had used tryptophan at some time between 1980 and 1989. The most common reasons for tryptophan use were insomnia, premenstrual syndrome, and depression; other reasons included anxiety, headaches, behavior disorders, obesity, and smoking cessation. Although most consumers purchased tryptophan for therapeutic use, it was marketed as a food supplement and widely available in the United States without a prescription. This product was not approved or regulated by the FDA.

L-Tryptophan is an essential amino acid; however, sufficient quantities are present in the diet of most US citizens without the need for supplements. The typical daily US diet contains 1-3 g of tryptophan, which satisfies the recommended daily dose of 3 mg kg^{-1} body weight (or $210 \text{ mg} (70 \text{ kg})^{-1}$ individual). It is metabolized to serotonin and therefore theoretically might have sedative and antidepressant properties.

Eosinophilia–Myalgia Syndrome National Surveillance Data

As of June 1993, 1511 EMS cases had been reported to the US CDC, including 37 deaths. The case definition developed by the CDC for epidemiological surveillance included (1) blood eosinophil count greater than 1000 ml^{-1} , (2) generalized debilitating myalgia, and (3) no evidence of infection or neoplasm that would explain the clinical findings. National surveillance data of July 1990 revealed that 84% of patients were female, 97% were non-Hispanic white, and 86% were over 34 years old (median age, 49 years). One-third of the patients required hospitalization. Ninety-seven percent of the patients with EMS had reported tryptophan use before onset of the disease, in doses ranging from 10 to $15\,000\,\text{mg}\,\text{day}^{-1}$ (median, $1500\,\text{mg}\,\text{day}^{-1}$). The prevalence of EMS was higher in the western United States than in other parts of the country, apparently paralleling the higher rate of tryptophan consumption in those states. The true prevalence of EMS was most likely underestimated by surveillance reports because persons with mild disease were excluded by the surveillance case definition. In addition, some cases may not have been reported to state or federal health agencies because the syndrome was not recognized by the patient or their physician.

Cases of EMS were also reported in Canada and Europe. In Germany, more than 100 persons became ill with EMS (as delineated by the CDC case definition). Unlike in the United States, tryptophan was available only by prescription in Germany, and thus case histories on German patients were well documented. As in the epidemiological investigations in the United States (see below), all of the tryptophan associated with EMS in Germany was traced back to a single manufacturer in Japan.

Epidemiologic Studies

After initial studies implicated the consumption of tryptophan as a major risk factor for EMS, US state and federal health agencies began investigations to further examine this association. Consumers of tryptophan were classified as either case (EMS patients) or control (non-EMS tryptophan users), and the lots of tryptophan consumed by each group were traced back to determine the tryptophan source. Before the epidemic, L-tryptophan had been manufactured by six companies, all in Japan. Analysis of the tryptophan sources for case patients and controls demonstrated a strong association between EMS and consumption of tryptophan manufactured by a single company, Showa Denko K. K. (Tokyo), a large petrochemical company.

In the Oregon study, 98% of case patients had consumed tryptophan manufactured by Showa Denko compared to 44% of controls. In the Minnesota study, 29 (97%) of the 30 case patients consumed tryptophan that was traced back to Showa Denko compared to 21 (60%) of the 35 controls (odds ratio (the ratio of the odds of the disease occurring in exposed individuals relative to the odds of the disease occurring in unexposed individuals), 19.3; 95% confidence interval, 2.5-844.9). Highperformance liquid chromatography (HPLC) analysis (see below) of the tryptophan ingested by the one case of EMS in Minnesota that was not traced back to Showa Denko showed a chromatogram that was characteristic of the company's product, revealing that the tryptophan was, in fact, produced by Showa Denko. All later trace-back studies support the association between tryptophan manufactured by Showa Denko K. K. and the occurrence of EMS.

Data from a cohort of tryptophan users in a South Carolina psychiatric practice provide an estimate of the rate of occurrence of EMS (attack rate) in persons exposed to the etiologic agent. Of 157 people who consumed a single brand of tryptophan (comprising only three lots of tryptophan manufactured by Showa Denko), 29% were diagnosed as definite cases of EMS, and an additional 23% were classified as 'possible cases' because they had some clinical findings of EMS (such as eosinophilia without myalgia) but did not meet the strict CDC surveillance case definition. Thus, the pooled attack rate was 52% among persons exposed to the etiologic agent. Among those taking more than 4g of this brand of tryptophan per day, the definite EMS attack rate was 59% and the pooled (definite and possible EMS) attack rate was 84%. Therefore a dose-response relationship appears to have been established. These data also suggest that most if not all individuals are susceptible to EMS if exposed to sufficient quantities of the etiologic agent.

Risk Factors

Two risk factors for EMS, other than consumption of implicated tryptophan lots, have been identified: the amount of tryptophan consumed and the age of the individual. The risk of developing EMS increased with larger dosages of tryptophan and with increasing age. The tryptophan dosage most likely reflects the degree of exposure to the etiologic agent: Persons who consumed larger doses of tryptophan probably had higher amounts of toxic metabolites formed, or had a greater probability of encountering tryptophan tablets that were contaminated with the causative chemical(s). The reason for the increased risk of EMS with age is unclear; it may be due to age-dependent physiologic changes in renal or hepatic function that delay the metabolism or clearance of a toxic substance, or to age-dependent changes in the immune system. No other host factors were found to alter significantly the risk of developing EMS.

Clinical Features

EMS is a syndrome with multiple clinical presentations and variable severity. The clinical course of EMS consists of an early (acute) phase and a late, long-lasting (chronic) phase. During the early phase, most patients developed severe myalgias, and perhaps in conjunction with weakness, joint pain, rash, shortness of breath, cough, headache, swelling, or paraesthesias (numbness and tingling) these symptoms prompted a visit to their physician. A complete blood count (CBC) would then reveal profound eosinophilia (sometimes up to $30\,000\,\text{cells}\,\text{ml}^{-1}$; normal <5 cells ml⁻¹). In different groups of EMS patients, the median eosinophil count has been reported to be 4000–6000 cells ml⁻¹.

The majority of patients also had an elevated leukocyte count with modestly elevated levels of aldolase, a marker of muscle injury; however, creatine phosphokinase, another indicator of muscle injury, was normal in most patients. This inconsistency between the levels of these two muscle-associated enzymes, previously described in some patients with systemic sclerosis and the toxic oil syndrome (TOS) (see below), is helpful in differentiating EMS from other myopathies (muscle diseases) and from eosinophilic fasciitis (EF) (see below). Approximately one-half of patients had abnormal liver function tests, although the changes were mild. The erythrocyte sedimentation rate, rheumatoid factor, and levels of IgE, complement, and cryoglobulin (all markers of immune dysfunction) were normal in most patients tested.

For some patients, cessation of tryptophan ingestion led to resolution of the symptoms; in other patients the use of high dose corticosteroids appeared to be helpful. However, for some patients, the disease evolved into a chronic phase, with cutaneous, neuromuscular, pulmonary, cardiac, and cognitive involvement. The most common features of chronic EMS are fatigue, muscle cramping, myalgia, paraesthesias with objectively demonstrated hypesthesias (lessened sensitivity to touch), chronic joint pain, scleroderma-like skin changes, and proximal muscle weakness. In one study, 88% of EMS patients continued to manifest more than three of these clinical symptoms after 3 years.

Pathologic studies have demonstrated a perivascular, lymphocytic infiltrate with eosinophils in the dermis, fascia, and skeletal muscle, with variable numbers of eosinophils. The perivascular infiltrate was accompanied by thickening of the capillary and arteriolar endothelium in dermal, fascial, and muscle vessels. The frequent occurrence of microangiopathy (disease of the small blood vessels) in biopsy specimens suggests that ischemia (deficiency of blood supply) may contribute to tissue injury. Deposition of major basic protein (an eosinophil-specific protein) in affected tissue of some patients suggests that cytotoxic eosinophil degranulation products may also play a role in the pathogenesis of EMS.

The histopathologic examination of affected skin showed thickening of the fascia, deep dermal fibrosis, and accumulation of mononuclear cells and eosinophils. *In situ* hybridization and immunohistochemical studies have demonstrated increased production of type I and type VI collagen in the extracellular matrix of the affected fascia. Thus, the dermal and fascial fibrosis of patients with EMS is likely due to stimulation of collagen synthesis by fibroblasts.

Most patients with EMS reported paraesthesias, and in some patients severe peripheral neuropathy was the most prominent clinical feature. In a few, persistent paraesthesias have been accompanied by axonal and demyelinating abnormalities on electrophysiologic testing. Muscle biopsies showed a characteristic histopathologic picture, with extensive inflammation (fasciitis) and fibrosis in the connective tissue surrounding the muscle, but little evidence of muscle fiber damage. Perineural inflammation and type II muscle fiber atrophy with denervation features have been observed, but muscle fiber necrosis was uncommon. The severe myalgias may be related to inflammation of nerves in the fascia or muscle, peripheral nerve injury caused by granule proteins, possibly eosinophil-derived neurotoxin, or ischemia of nerves caused by occlusive microangiopathy.

Lung biopsies performed in a small number of patients revealed a vasculitis and perivasculitis with a chronic interstitial pneumonitis. Disturbances of cardiac rhythm and conduction have also been documented. Examination of cardiac autopsy specimens has demonstrated neural lesions throughout the conduction system, similar to the neuropathology seen in skeletal muscle. Inflammatory lesions of the small coronary arteries were also present. The prevalence of cardiac abnormalities among all patients with EMS is unknown, although life-threatening rhythm disturbances appear to be uncommon.

The most commonly observed disease process leading to death of patients with EMS was progressive polyneuropathy (disease involving the peripheral nerves) and myopathy (disease of muscles) that produced complications of pneumonia and sepsis or respiratory failure due to weakness. Two-thirds of EMS patients died of these complications. Other causes of mortality were cardiomyopathy (disorder affecting the muscles of the heart), primary pulmonary disease, arrhythmia (deviation from the normal rhythm of the heart), and stroke.

The response to therapy has been disappointing. Multiple therapeutic interventions have been suggested, but no clearly effective treatment has been identified. In the early phase, glucocorticoid treatment (usually prednisone) was generally helpful in treating pneumonitis, myalgias, and edema and in reducing the eosinophil count. However, some patients have not responded to high doses of prednisone, and others have had an exacerbation of symptoms when the dose was tapered. There is no evidence that prednisone therapy alters the natural history of the disease or the risk of neuropathy. Other treatments that have been used include nonsteroidal anti-inflammatory drugs, cyclophosphamide, hydroxychloroquine, D-penicillamine, methotrexate, octreotide (a somatostatin analog), and plasmapheresis. Many of these therapies have been tried in patients with severe illness, but insufficient information is available to assess efficacy.

The clinical and histopathologic findings of EMS overlap those of EF, a scleroderma-like syndrome characterized by tender swelling and induration (hardening) of the subcutaneous tissue, primarily in the arms and legs. Some cases of EF, in retrospect, were associated with tryptophan ingestion. However, EF is probably triggered by additional factors because few, if any, cases of EF occurring before 1986 can be attributed to the ingestion of tryptophan. Several clinical and laboratory features distinguish EMS from EF. Patients with EMS had greater frequency and severity of myalgias, fever, peripheral neuropathy, and other visceral organ involvement than patients with EF. Moreover, positive antinuclear antibody and a dichotomy between elevated serum aldolase and nonelevated creatine phosphokinase were features of EMS, but not of EF. EMS appears to be a more severe disease than EF in terms of hospitalization rate, duration of symptoms, and mortality.

Taken together, the epidemiological and clinical findings in patients with EMS could be explained by changes in the manufacturing process of L-tryptophan from 1985 to 1988 which resulted in contamination of the product, with a likely increase in the quantities of contaminants in 1989.

Manufacture of L-Tryptophan

The L-tryptophan produced by Showa Denko K. K. was manufactured by fermentation using the bacterium *Bacillus amyloliquefaciens*. The biosynthetic pathway of L-tryptophan is shown in Figure 1. Several new strains (I–V), each modified slightly to increase the biosynthesis of tryptophan, were introduced sequentially during the years preceding the outbreak of EMS (Table 1).

In December 1988, the company introduced a new strain of *B. amyloliquefaciens* (strain V), which had been genetically modified to increase the synthesis of 5-phosphoribosyl-1-pyrophosphate, an intermediate in the biosynthesis of tryptophan (see Figure 1). After fermentation, tryptophan was extracted from the broth and purified using a series of filtration, crystallization, and separation processes. The purification procedures included contact with powdered activated carbon and then granulated activated carbon. The amount of powdered activated carbon in each batch was usually

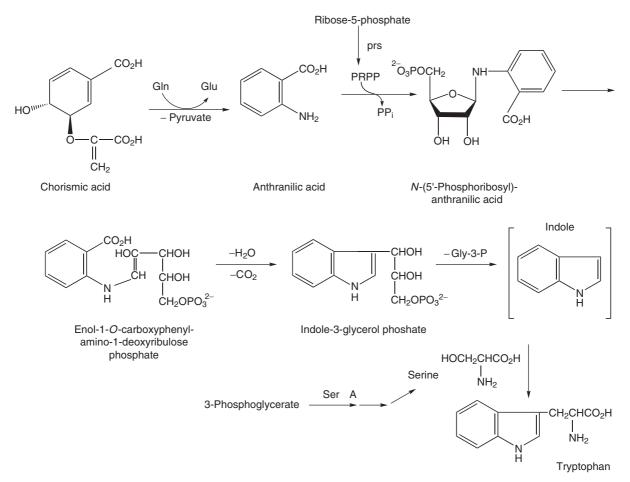


Figure 1 Biosynthesis of ∟-tryptophan. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia–myalgia syndrome and tryptophan production: A cautionary tale. *Trends in Biotechnology* 12: 346–352, with permission from Elsevier.)

Strain ^a	Modification
I	Original strain of <i>B. amyloliquefaciens</i> IAM 1521
II	The tryptophan operon (coding for all enzymes catalyzing reactions from chorismate to L-tryptophan, as well as for those involved in the biosynthesis of serine and 5-phosphoribosyl-1-pyrophosphate (PRPP)) of strain I was duplicated through chromosomal integration
III	The isolated tryptophan operon was attached to a more efficient promoter prior to integration into chromosomal DNA of strain II
IV	The ser A gene (coding for phosphoglycerate dehydrogenase ^b) was amplified using a plasmid vector with strain III
V	The <i>prs</i> gene (coding for ribose phosphate pyrophosphokinase ^c) was isolated and integrated into the chromosome of strain IV

^aStrains II-V were derived by successive modifications of strain I.

^b Phosphoglycerate dehydrogenase catalyzes the conversion of 3-phosphoglycerate to 3-phosphohydroxypyruvate, an intermediate in the biosynthesis of serine.

^cRibose phosphate pyrophosphokinase catalyzes the phosphorylation of ribose-5-phosphate to give PRPP.

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20 kg through 1988. In 1989, the amount of powdered activated carbon used to purify some batches of tryptophan was reduced to 10 kg. From October 1988 to June 1989, a portion of some fermentation batches also bypassed a filtration step that employed a

reverse-osmosis membrane (ROM) filter to remove chemicals with a molecular weight of more than 1000 Da. According to the company, these changes did not significantly alter the purity of the tryptophan powder, which was maintained at 99.6% or greater.

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Univariate analysis of retail lots of tryptophan consumed by case patients and controls demonstrated an association between development of EMS and the ingestion of tryptophan processed with 10 kg of powdered carbon per batch (odds ratio, 9.0; 95%) confidence interval, 1.1-84.6; p = 0.014) and the use of B. amyloliquefaciens strain V (odds ratio, 6.0; 95% confidence interval, 0.8-51.8; p = 0.04). Thus, both a reduction in the amount of powdered activated carbon and use of B. amyloliquefaciens (strain V) were significant manufacturing changes, but the independent contribution of each manufacturing change could not be assessed because of the high correlation between them. Bypass of the ROM filter was not significantly associated with the case lots. Studies carried out by Showa Denko suggested that the 'biochemical and physiological characteristics' of B. amyloliquefaciens (strain V) did not differ from those of earlier strains.

Contaminants Associated with EMS

Once the link between EMS and manufactured L-tryptophan had been established, chemical analyses of bulk tryptophan lots were performed by researchers at the Mayo Clinic (Rochester, MN), FDA (Washington, DC), CDC (Atlanta, GA), and the Japanese National Institute of Hygienic Sciences (Tokyo) to determine if any contaminants were associated with EMS. HPLC was used to separate the contaminants in tryptophan and revealed that each manufacturer's tryptophan produced a unique chromatographic pattern, or 'fingerprint', that was distinctive for the product from each company, as shown in Figure 2. The chromatographic pattern consisted of multiple peaks, each of which represented a trace chemical constituent other than tryptophan, which eluted as a large, broad peak between 11 and 15 min. L-Tryptophan manufactured by each of the six companies contained impurities. The chromatogram for Showa Denko tryptophan included five 'signature' peaks that were present in all tryptophan manufactured by this company (see Figures 2 and 3). Initial comparison of individual peaks in case and control lots of Showa Denko tryptophan revealed a single peak (called 'peak E' or 'peak 97') that was significantly associated with case lots (Figure 3). The chemical structure of peak E was subsequently determined to be 1,1'-ethylidenebis [L-tryptophan], or EBT (Figure 4). Two other contaminants were subsequently reported to be associated with case lots of tryptophan manufactured by Showa Denko. One of the peaks, labeled UV-5, eluted before tryptophan (Figure 3) and was determined to be 3-(phenylamino)-L-alanine (PAA)

(Figure 4). The other peak (UV-28) eluted much later than EBT and is as yet uncharacterized. Recent HPLC studies revealed more than 60 trace contaminants in Showa Denko tryptophan, six of which are associated with EMS. The structures of three are known (EBT, PAA, and 'peak 200' (2[3-indolylmethyl]-L-tryptophan)), but the other three have not yet been characterized. One of the uncharacterized contaminants, called 'peak AAA', was the contaminant most significantly associated with EMS and was recommended for characterization.

The amount of EBT present in Showa Denko tryptophan varied markedly in the period 1987-89 (Figure 5), presumably reflecting alterations in the manufacturing conditions. It is likely that levels of all of the contaminants varied with time. These data are consistent with the hypothesis that a contaminant(s) in tryptophan is responsible for EMS and for the sporadic cases of EF between 1986 and 1988. Recent statistical analyses of EBT, adjusted for serial autocorrelation (to take into account that sequential lots of tryptophan may be related), revealed that higher levels of EBT are still associated with EMS, but the association (p = 0.120) did not achieve statistical significance. Nonetheless, the results do not vindicate EBT as a cause of EMS because misclassification of lots as case or control could weaken the association and the methods used to account for the lack of independence of observations over time probably reduce the power of the statistical analysis.

Investigation into the origin of contaminant PAA reveals that it can be formed from aniline and serine by heating at 80°C for 6 h under alkaline conditions (pH 11). Although aniline was not used in the biosynthesis of tryptophan, small amounts of aniline are formed from anthranilic acid, a biosynthetic precursor of tryptophan (see Figure 1), after heating at 80°C for 6 h under acidic conditions (pH 2). The industrial process used by Showa Denko to purify tryptophan from the fermentation broth consisted of several steps, including anion exchange at pH 10.5, cation exchange at pH 11, and heat treatment at 80–90°C. Thus, the fermentation and purification processes used to produce tryptophan may have led to the formation of PAA as a by-product.

Connection with Toxic Oil Syndrome

The clinical and pathologic findings of EMS bear a striking resemblance to those of the TOS, which occurred as an epidemic in Spain during the spring and summer of 1981. Over 20 000 persons were affected, and several hundred deaths have been attributed to TOS. The similarities between EMS and

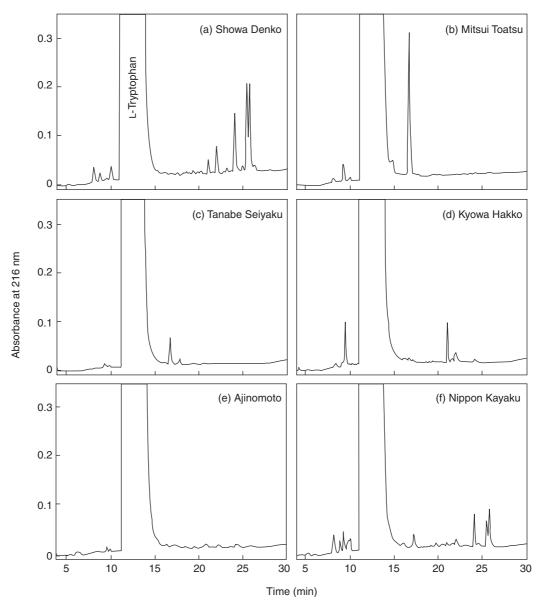


Figure 2 Typical HPLC chromatograms of L-tryptophan manufactured by the six different companies. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia – myalgia syndrome and tryptophan production. A cautionary tale. Trends in Biotechnology 12: 346–352, with permission from Elsevier.)

TOS are summarized in Tables 2 and 3. Unlike EMS, respiratory symptoms (cough or dyspnea) were prominent and severe in TOS during the first week of illness (acute phase). Other early symptoms included fever, malaise, headache, nausea, and pruritic (itchy) rash. In some patients, the disease progressed to an intermediate and chronic phase that resembled EMS more closely. The intermediate phase (2–8 weeks after onset) was characterized by eosinophilia and leukocytosis (raised numbers of leukocytes). Patients whose illness progressed to the late phase developed muscle cramps and severe myalgias, peripheral edema, scleroderma-like skin changes, and polyneuropathy. The histopathological changes

of skin, nerve, and skeletal muscle are remarkably similar between EMS and TOS.

The pathophysiology of both TOS and EMS involves an immunological component. Generally, early skin biopsies in both TOS and EMS showed edema and inflammatory infiltrates. Inflammatory lesions of arteries and cardiac neural structures in both EMS and TOS patients were primarily composed of lymphocytes. Persistent elevated levels in the serum level of the soluble fraction of IL-2 receptor were noted in both EMS and TOS patients, suggesting chronic immune activation.

Epidemiologic investigations implicated ingestion of adulterated rapeseed oil that had been imported

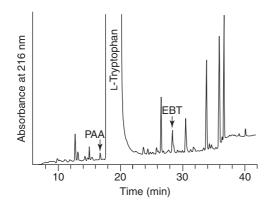
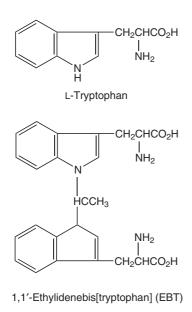
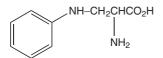


Figure 3 HPLC chromatogram of EMS-associated L-tryptophan. HPLC conditions differ from those used in **Figure 2**. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia–myalgia syndrome and tryptophan production: A cautionary tale. *Trends in Biotechnology* 12: 346–352, with permission from Elsevier.)





3-(Phenylamino)alanine (PAA)

Figure 4 Chemical structures of tryptophan and of contaminants EBT and PAA associated with EMS.

from France. At the time, rapeseed oil could not be legally imported into Spain as a food substance, only as an industrial lubricant after denaturation with aniline, a toxic chemical. The oil had been denatured with aniline (to give a concentration of 2% aniline by weight) as required by law. However, the oil was then illegally de-denatured in Spain by a refining process that removed almost all of the aniline and was

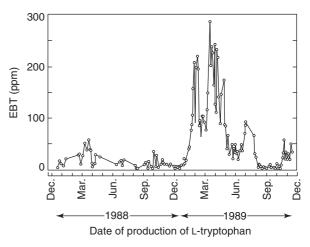


Figure 5 Levels of 1,1'-ethylidenebis[L-tryptophan] (EBT) in lots of L-tryptophan produced by Showa Denko K. K. during 1988 and 1989. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia–myalgia syndrome and tryptophan production: A cautionary tale. *Trends in Biotechnology* 12: 346–352, with permission of Elsevier.)

Table 2	Comparis	on of the	e clinio	cal	features	of	eosinoph	nilia–
myalgia	syndrome	(EMS),	toxic	oil	syndror	ne	(TOS),	and
eosinoph	ilic fasciitis	(EF)						

	EMS	TOS	EF
Female (%)	80	90 (late)	50
Myalgia	+++	++	\pm
Dyspnea/cough	+	+ + +	_
		(early)	
Pruritus	++	+	_
Rash	+	+	-
Swelling, edema	++	++	++
Muscle weakness	+	+	_
Scleroderma-like lesions/	++	++	+ + +
fasciitis			
Heart involvement	±	+	-
Axonal polyneuropathy	++	++	-
Arthritis	+	+	+

+, Occasional; ++, common; +++, very common.

Reproduced from Varga J (1993) L-Tryptophan-associated eosinophilia–myalgia syndrome: Clinical and pathological features of an evolving new disease and current concepts of etiology. *Journal of Intensive Care Medicine* 8: 229–242.

subsequently mixed with 10–30% of other seed oils, about 30% of animal fats, and up to 5% of a poor quality olive oil or, alternatively, chlorophyll to produce the desired color. The resulting adulterated oil was sold as pure olive oil, typically in unlabeled 51 containers by street vendors and itinerant salesmen.

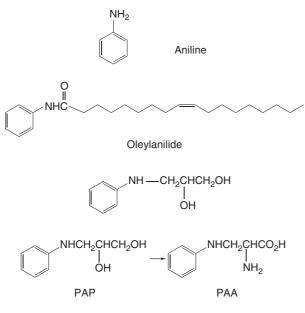
Chemical analyses of implicated oil samples and 'control' oil samples demonstrated that free aniline and aniline derivatives were significantly associated with case-related samples. Fatty acid anilides, in particular oleylanilide (Figure 6), have been reported to be markers of TOS-causing oil. Another

 Table 3
 Comparison of the laboratory features of EMS, TOS, and EF

	EMS	TOS	EF
Eosinophilia	+++	++	+
Elevated IgE	-	\pm	_
Elevated aldolase	++	+	_
Antinuclear antibody	++	+	_
Lymphocytes in lesion	+	+	_
Eosinophils in lesion	±	±	\pm
Vasculitis	+	+	\pm

+, Occasional; ++, common; +++, very common.

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3-Phenylamino-1, 2-propanediol (PAP)

Figure 6 Chemical structures of oleylanide and 3-phenylamino-1,2-propanediol (PAP) associated with TOS.

contaminant, 3-phenylamino-1,2-propanediol (PAP) (Figure 6), has been isolated from implicated oil and is chemically similar to the tryptophan contaminant PAA. Efforts to evaluate the biologic activity of the aniline contaminants have been limited by the absence of an animal model for TOS.

The striking similarities between EMS and TOS suggest that they may share the same final pathway that leads to neuromuscular damage. The recent discovery of a chemically related aniline derivative in tryptophan preparations implicated in causing EMS suggests of a related etiology. Recently, PAP has been demonstrated to undergo biotransformation to PAA by both rat hepatocytes and human liver tissue *in vitro*, linking the two diseases to a common chemical, namely PAA (see below). This finding is the first reported chemical link between TOS and EMS.

EMS Not Apparently Associated with L-Tryptophan

Some patients with EMS reported no history of tryptophan ingestion. An EMS-like syndrome has been associated with use of L-5-hydroxytryptophan (5-HTP). HPLC analysis of the 5-HTP that might have caused the symptoms revealed the presence of an impurity not present in 5-HTP preparations that did not cause symptoms. The structure of the impurity has not been reported. In addition, a recent pharmacoepidemiological study in Canada identified several EMS patients with no history of tryptophan ingestion. These reports suggest that factors other than tryptophan ingestion can lead to the induction of EMS or EMS-like diseases.

Investigations of the Etiology and Pathogenesis

Animal Models

Several studies using animals have been performed; however, the results of studies have been inconclusive, with no animal model tested replicating all of the clinical features of the disease. Initially, the Lewis rat showed promise as a model for EMS. Muscle biopsies of Lewis rats given either implicated tryptophan (containing EBT) or US Pharmacopoeia (USP) grade tryptophan (without EBT) demonstrated perimysial inflammation in seven of nine animals receiving implicated tryptophan compared to 0 of 10 receiving USP grade tryptophan. A significant increase in fascial thickening was also observed in rats receiving implicated tryptophan. However, leukocyte counts and eosinophil counts remained normal in both groups. Gastrointestinal changes were also noted with an increased number of degranulating inflammatory cells in the lamina propria of the rats that received case-implicated tryptophan. Recently, however, control L-tryptophan alone was observed to cause mild myofascial thickening, alterations in peripheral blood mononuclear cell (PBMC) phenotypes, and pancreatic pathology in Lewis rats, suggesting that tryptophan itself may play a role in EMS and other fibrosing diseases. Another recent study using C57BL/6 mice found that EBT caused inflammation and fibrosis in the dermis and subcutis, including fascia and perimysial tissues, mimicking some of the clinical features of EMS. Eosinophilia was not observed.

These findings, however, may not be reproducible. F-344 and Lewis rats, as well as BALB/c mice, were treated with one of the following substances: feed or food-grade L-tryptophan, tablets containing L-tryptophan, isopropanol extracts from L-tryptophan, synthetic EBT or PAA, and 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (a breakdown product of EBT). No EMS-like symptoms were observed in any of the animals tested. The tryptophan preparations were manufactured by Showa Denko; however, neither the specific lots numbers nor their association with EMS were indicated. Thus, it is difficult to interpret the results of this study in light of the fact that only certain lots of L-tryptophan manufactured by Showa Denko were linked to EMS. A recent toxicologic study using PAA supports the negative findings; PAA (1, 10, and 100 mg $kg^{-1} day^{-1}$) was administered by gavage to Sprague-Dawley rats for up to 13 consecutive weeks. No EMS-like symptoms were observed. Overall, the animal studies suggest that Lewis rats and C57BL/ 6 mice may be useful in replicating only certain aspects of EMS.

In Vitro Models

In vitro investigations have attempted to clarify the mechanism of immune activation but so far have provided limited data. Studies testing the hypothesis that implicated tryptophan or EBT can trigger PBMCs to release cytokines have been equivocal, although one study found that EBT activates eosinophils and induces IL-5 production from T cells. Another recent study found that certain lots of L-tryptophan could stimulate PBMCs to release granulocyte-macrophage colony-stimulating factor (GM-CSF); this response, however, was caused by endotoxin contamination and not associated with case lots of tryptophan. The mechanism of immune activation is clearly complex and may be difficult to reproduce with an in vitro assay. Similar difficulties have been encountered in the study of immune system activation in TOS.

Despite the negative findings of *in vitro* studies, there is evidence that cytokines may play a role in the pathogenesis of EMS. It is known that IL-3, IL-5, and GM-CSF can each induce eosinophil production and enhance *in vitro* survival. In one study, EMS patients had significantly elevated serum levels of IL-5 and a higher proportion of hypodense eosinophils compared to normal controls. Elevated levels of IL-3 and GM-CSF were not observed. Their results suggest that IL-5 is the cytokine that triggers eosinophilia and converts peripheral blood eosinophils to the hypodense phenotype. The mechanism responsible for the elevation of IL-5 levels in the blood is undefined.

Possible Pathogenetic Mechanisms

Although the sequence of events leading to the pathologic changes of EMS is undoubtedly complicated, a framework for possible mechanisms can be advanced. One hypothesis involves a direct effect of the etiologic agent on mononuclear cells, leading to production of cytokines, including IL-5. This cytokine could then activate tissue eosinophils and convert them to a hypodense phenotype. Effector functions would be augmented with release of cytotoxic molecules from eosinophils. Once activated, eosinophils can release additional cytokines such as IL-3, GM-CSF, IL-5, and transforming growth factor- α . A cascade of interacting cytokines could then lead to recruitment of additional inflammatory cells and increased collagen synthesis by fibroblasts.

The predominance of inflammatory changes in the fascia suggests that mediators produced by mesenchymal cells (fibroblasts and endothelial cells) may also play a role in the pathogenesis. For example, fibroblasts augment IL-5-dependent eosinophil survival and stimulate conversion to the hypodense phenotype. Fibroblasts can also produce IL-8, which recruits neutrophils and lymphocytes when injected *in vivo*. Thus, one can speculate that the etiologic agent interacts with these cells to stimulate release of inflammatory mediators and increase collagen synthesis.

Another general hypothesis involves incorporation of the etiologic agent into metabolic or biosynthetic pathways that utilize chemically related compounds. EBT and PAA are amino acids with structural similarities to tryptophan and phenylalanine, respectively, and might function as an analog with adverse immunologic effects. If EBT, PAA, or one of their metabolites is recognized by an analogous transfer RNA, it might be incorporated into a nascent protein molecule, stimulating an autoimmune response.

The biotransformation of the toxic oil contaminant PAP to the L-tryptophan contaminant PAA may link TOS and EMS to a common chemical agent, namely PAA. Both PAP and PAA are metabolized further to the *p*-hydroxylated forms, HPAP and HPAA (Figure 7). Such compounds readily autoxidize to the benzoquinoneimine, which is reactive toward nucleophiles such as the sulfhydryl and amino moieties present on many biological molecules. Thus, upon oxidization, HPAA and HPAP may react with macromolecules as a hapten to form immunogenic targets. HPAA possesses some chemical properties

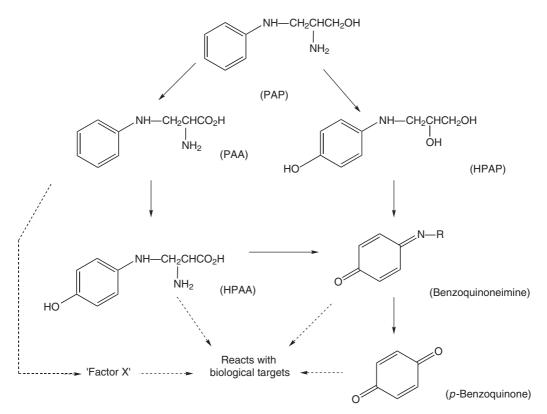


Figure 7 Hypothetical scheme for the bioactivation of PAP and PAA.

similar to that of homogentisic acid (HGA), a hydroquinone derivative implicated in the causation of alkaptonuria, a connective tissue disorder resulting from an inherited abnormality in phenylalanine and tyrosine metabolism. HGA interacts with connective tissue reversibly or is oxidized enzymatically by an enzyme (polyphenol oxidase) present in connective tissue to benzoquinoneacetic acid, which covalently bonds to macromolecules.

One may hypothesize that HPAA reacts similarly, as shown in Figure 7. PAP is initially metabolized to PAA or HPAP. PAA is then converted to HPAA. Both HPAP and HPAA can undergo oxidation to the benzoquinoneimine. Benzoquinoneimines can hydrolyze to *p*-benzoquinone, and both benzoquinoneimines and benzoquinone readily react with nucleophilic molecules. It is also possible that PAA is metabolized to another undetermined molecule, shown as factor X (Figure 7), that reacts with biological targets. Thus, HPAA may haptenize proteins, and T-cell activation could result from hapten recognition.

The oxidation of HPAA and HPAP to benzoquinones may require an enzyme with properties similar to polyphenol oxidase. The inflammatory pattern and a lack of an animal model for EMS may be explained if the enzyme is localized to the connective tissue (fascia) and is specific to humans. In addition,

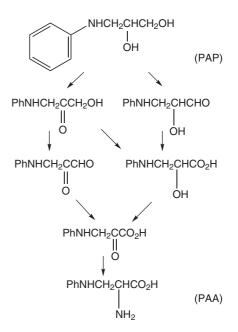


Figure 8 Hypothetical metabolic pathway for the biotransformation of PAP to PAA.

the observation of greater respiratory symptoms during the early phase of TOS in comparison with EMS may result from accumulation and metabolism of PAP or HPAP in the lung.

The biotransformation of PAP to PAA must proceed through various intermediates (Figure 8). The first steps most likely involve stepwise oxidation of the diol to an α -keto acid or some other keto intermediate, which then undergoes transamination to give PAA. It is likely that any one of these intermediates, in the presence of hepatocytes, can be metabolized to PAA. Thus, many molecules related to PAP (e.g., phenylamino compounds) may be channeled down this pathway to give PAA. This model suggests that numerous molecules with similar chemical structures to PAP will give rise to PAA and, if PAA is indeed responsible for EMS and TOS, the model predicts that an entire class of molecules, as shown in Figure 8, can cause EMS/TOS-like diseases, consistent with the reports of EMS cases not associated with tryptophan ingestion.

Summary

EMS occurred as an epidemic in the United States during 1989–90, with isolated cases occurring before and after the major epidemic. EMS is a multisystemic disease that resulted from the ingestion of L-tryptophan manufactured by one company. The illness is clinically and pathologically similar to EF and the TOS. The syndrome is likely triggered by one or more contaminants in tryptophan. Contaminants currently studied include EBT and PAA, although other uncharacterized contaminants have recently been discovered and may likewise be responsible. One or more of these chemicals may cause EMS by an undefined mechanism, or they may be surrogate markers for another unidentified substance that triggers the syndrome. Consumption of high tryptophan doses and increased age have been identified as risk factors. Patients who ingested tryptophan and were diagnosed with EF during 1986-88 had most likely EMS. The recent demonstration of the biotransformation of PAP to PAA suggests that both EMS and TOS share a common etiology, namely PAA. Ongoing research is focused on identification of contaminants in implicated tryptophan and on establishing an animal model of the diseases. Success in these endeavors would greatly increase our understanding of eosinophilic diseases in general and hopefully prevent the outbreak of future epidemics.

See also: Blood; Epidemiology; Immune System; Neuro-toxicity.

Further Reading

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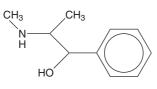
Ephedra

Vishal S Vaidya and Harihara M Mehendale

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- CHEMICAL NAME: Ephedra
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 299-42-3 (Ephedrine)
- COMMON NAMES: Ephedra sinica family (ephedraceae); Chinese ephedra, Ma Huang
- RELATED SPECIES: *Ephedra distacha* (European ephedra); *E. trifurca* or *E. viriditis* (desert tea); *E. nevadensis*; *E. americana* (American ephedra); *E. gerardiana* (Pakistani ephedra)

- SYNONYMS (Ephedrine): Benzenemethanol, α-(1-(Methylamino)ethyl)-, $(R-(R^*,S^*))$; Biophedrin; Eciphin; Efedrin; Ephedral; Ephedrate; Ephedremal; Ephedrin; Ephedrine; (-)-Ephedrine; (L)-Ephedrine; Ephedrital; Ephedrol; Ephedrosan; Ephedrotal; Ephedsol; Ephendronal; Ephoxamin; Fedrin; α -Hydroxy- β -methyl amine propylben-1-Hydroxy-2-methylamino-1-phenylprozene; α -Hydroxy- β -methylaminopropylbenzene; pane; Isofedrol; Kratedyn; Ma Huang; Manadrin; Mandrin: α -(1-(Methylamino)ethyl)benzenemethanol; α -(1-(Methylamino)ethyl)benzyl alcohol; $1-\alpha-(1-Methylaminoethyl)$ benzyl alcohol; 1-2-Methylamino-1-phenylpropanol; 2-Methylamino-1-phenyl-1-propanol; Nasol; Nci-C55652; N-Methyl-1-phenyl-1-hydroxy-Norephedrine, 2-methylaminopropane; 1-Phenyl-2-methylaminopropanol; Sanedrine; 1-Sedrin; Vencipon; Zephrol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Herbal supplements; Ephedra alkaloids; Adrenergic agents; Natural products
- CHEMICAL FORMULA: C₁₀H₁₅NO
- CHEMICAL STRUCTURE:



Uses

The medicinal use of *Ephedra sinica* in China dates from ~2800 BC. Ma Huang (the stem and branch) was used primarily in the treatment of common cold, asthma, hay fever, bronchitis, edema, arthritis, fever, hypotension, and urticaria. Ephedra has been used to treat bronchoconstriction for centuries, because of its activity at β_2 -adrenergic receptors. It contains pseudoephedrine, ephedrine, and other similar alkaloids. These are sympathomimetics that either directly or indirectly stimulate α - and β -adrenergic receptors. It has become less extensively used with the advent of more selective agonists.

Background Information

Ephedra plants are erect, branching shrubs found in desert or arid regions throughout the world. The 1.5–4 ft shrubs typically grow on dry, rocky, or sandy slopes. The many slender, yellow-green branches of ephedra have two very small leaf scales at each node. The mature, double-seeded cones are visible in the fall.

Ephedra is one of the plants that are a source of ephedrine alkaloids, including ephedrine and pseudoephedrine. Chemically synthesized, ephedrine, and pseudoephedrine are regulated under the Federal Food, Drug, and Cosmetic Act as drugs. In contrast, the Dietary Supplement Health Education Act (DSHEA)-regulated dietary supplements that contain ephedrine alkaloids, the safety and effectiveness of drug products containing ephedrine alkaloids in drug products have to be proven by the manufacturer.

Within the last 10 years, the use of dietary supplements containing ephedrine alkaloids was extensively promoted in the United States for aiding weight control and boosting sports performance and energy (Table 1). Drinks like Ripped Fuel claimed to give athletes a quick jolt of energy and gained substantial popularity not only in just athletes but also in those wanting to get a better workout in health clubs. Ephedra contains a natural alkaloid ephedrine, similar to the hormone epinephrine (adrenaline), a stimulant that acts on the central nervous system (CNS), dilates the bronchial tubes in the lungs, elevates blood pressure, and increases heart rate thereby giving a feeling of jolt of energy. The use of ephedra can also increase feelings of alertness and reduce or suppress appetite.

Since 1994, the Food and Drug Administration (FDA) and Centers for Disease Control collected reports of over 100 deaths and 500 reports of adverse events associated with ephedrine-containing dietary supplements over a 2 year period. The NCAA banned the use of ephedra-containing products since 1997, and the Olympics banned the use of ephedra for over a decade.

Scientists have conducted several studies and the totality of the available data showed little evidence of the effectiveness of ephedra except for modest, short-term weight loss without any clear health benefit, while confirming that the substance raises blood pressure and otherwise stresses the circulatory system. These effects are linked to significant adverse health outcomes, including heart attack and stroke. On February 6, 2004, the FDA issued a final rule prohibiting the sale of dietary supplements containing ephedrine alkaloids (ephedra) because such supplements present an unreasonable risk of illness or injury (Table 2).

Exposure Routes and Pathways

Herbal medicines are widely perceived by the public as being healthful and innocuous. An estimated three billion servings of ephedra are reportedly consumed

Proposed indications	Popular products containing ephedra extracts	Label ingredient indicating ephedra compounds	Adverse effects
Weight loss	Metabolife	Ephedra	Nervousness
Asthma	Ripped Fuel	Ma huang	Dizziness
Common cold	Diet Fuel	Ephedrine	Tremor
Hay fever/allergies	Stacker 3	Ephedra sinica	Alternations in blood pressure or heart rate
Increasing energy	Natural TRIM	Sida cordifolia	Headache
Congestion	Hydroxycut	Epitonin	Gastrointestinal distress
Weight lifting formula	Xenadrine RFA-1	Pseudoephedrine	Chest pain
0 0	Metab-O-Lite	Methyl ephedrine	Myocardial infarction
	Metabolift	2	Hepatitis
	Up Your Gas		Stroke
	Truckers Luv It		Seizures
	Yellow Jackets		Psychosis
			Death

Table 1 Facts about ephedra

Table 2 Federal and State regulatory actions against ephedra and ephedrine-containing alkaloids

Date	Action	Authority
November 1989	Ephedrine and pseudoephedrine placed on a list of controlled substances used in the manufacture of illegal drugs on a list of controlled substances used	Drug Enforcement Agency (DEA)
1991–94	State regulations controlling sale of ephedrine and/ or ephedra	Arizona, Arkansas, California, Florida, Hawaii, Idaho, Missouri, Nevada, New Mexico, Ohio, Oklahoma, Oregon, Texas, Virginia, Washington
1993	Exemptions for Ma Huang products with less than 25 mg of total ephedrine alkaloids	Arizona, Nevada, and Washington
August 1994	Ephedrine placed in Schedule V of Ohio's Controlled Substance Act. Sale of ephedra banned in Ohio	Ohio's Drug Laws Board
June 1996	Proposed warning and dose limitations on dietary supplements containing ephedra	Food and Drug Administration (FDA)
January 1997	Texas withdraws proposed regulations to ban ephedra	Texas Board of Health
March 1997	Ban on ephedra sales amended in Ohio. Bill permits natural products stores to sell the herb containing limited alkaloid levels	Ohio's Drug Laws Board
February 2000	Withdrawal of proposed ephedra rules	FDA
February 2004	Final rule prohibiting the sale of dietary supplements containing ephedrine alkaloids (ephedra) because such supplements present an unreasonable risk of illness or injury	FDA

yearly, making it an extremely popular stimulant contained in diet pills and sports drinks. The quantity of ephedrine in dietary supplements, as reported on package labels, is typically about 20 mg per serving, and the usual dose frequency is two to three times per day. However, these products may contain larger or smaller amounts of ephedra alkaloids that are listed on the product label. For example, 11 of 20 supplements tested either failed to list the alkaloid content on the label or had greater than a 20% difference between the amount listed on the label and the actual amount. Therefore, even in the absence of simultaneous ingestion of other known or unknown stimulants, consumers of ephedra are often overdosed.

Toxicokinetics

Ephedrine is rapidly absorbed after oral, intramuscular, or subcutaneous administration. It is strongly bound to human saliva, and binding is independent of pH. L-Ephedrine-(14)c injected intraperitoneally in rats was metabolized to l-norephedrine and 4-hydroxy-l-ephedrine plus minor products. The average half-life is 6 h, although acidifying the urine will decrease the half-life considerably and alkalinization will increase the half-life. The major route of elimination of ephedrine is as the unchanged drug in the urine.

Mechanism of Toxicity

The basic pharmacological action of Ephedrine is that of a sympathomimetic. It does not contain a catechol moiety and is effective after oral administration. The drug stimulates heart rate and cardiac output and variably increases peripheral resistance; as a result, ephedrine usually increases blood pressure. Stimulation of the α -adrenergic receptors of smooth muscle cells in the bladder base may increase resistance to the outflow of urine. Activation of β -adrenergic receptors in the lungs promotes bronchodilation. Ephedrine stimulates the cerebral cortex and subcortical centers to produce its effects in narcolepsy and depressive states.

Ephedra can produce the same side effects as ephedrine, that is, increased blood pressure and heart rate, insomnia, and anxiety. A highly potent CNS stimulant, ephedrine may even induce toxic psychosis at high dosages. The FDA advisory review panel on nonprescription drugs recommended that ephedrine not be taken by patients with heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to the enlargement of the prostate gland. Nor should ephedrine be used in patients on antihypertensive or antidepressant drugs. Ephedrine administered to chick embryos has resulted in cardiovascular teratogenicity and embryotoxicity at doses as low as 1 mmol per egg. The teratogenic effect of ephedrine is potentiated by caffeine.

Acute and Short-Term Toxicity (or Exposure)

Animal

There are very few animal studies conducted investigating the toxic effects of ephedra. Studies using ephedrine in animals suggest that if it is given in excessive amounts, it produces symptoms of sympathetic stimulation, manifested by anxiety and restlessness. If the dosage is large, muscular tremors and even convulsions may occur.

Human

Probable lethal dose in man is 50 mg kg^{-1} .

Chronic Toxicity (or Exposure)

Animal

The only chronic carcinogenecity data available is for ephedrine sulfate which states that there was no evidence of carcinogenicity for F344/N rats or B6C3F1 mice of either sex receiving 125 or 250 ppm ephedrine sulfate in the diet for 2 years. Ephedrine sulfate was not mutagenic in four strains of *Salmonella typhimurium* (TA100, TA1535, TA97, or TA98), with or without Aroclor 1254 induced male Sprague–Dawley or Syrian hamster liver S9 activation. Ephedrine sulfate did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells.

Human

Many cases of serious adverse effects and even fatalities have been reported that were linked with ephedra or ephedrine administration over the last 10 years. Haller and Benowitz published a review of 140 reports of adverse events related to the use of ephedra alkaloids that were submitted to the FDA between June 1997 and March 1999. Using standardized rating system for assessing causation, 31% of the cases were considered to be definitely or probably related to the use of ephedra alkaloid-containing supplements, and another 31% were deemed to be possibly related. Among these adverse events, 47% involved in cardiovascular symptoms and 18% involved the CNS. Hypertension was the most frequent adverse effect, followed by palpitations, tachycardia, or both stroke and seizures. Ten events led to death and 13 cases produced permanent disability.

The central nervous-simulating effects of ephedrine may result in nervousness, anxiety, apprehension, fear, tension, agitation, excitation, restlessness, weakness, irritability, talkativeness, or insomnia. Dizziness, lightheadedness, and vertigo may occur, especially with large doses. Tremor or tremulousness, and hyperactive reflexes have also been reported. Large parenteral doses of ephedrine may cause confusion, delirium, hallucinations, or euphoria. Ephedrine may deplete norepinephrine stores in sympathetic nerve endings and tachyphylaxis to the cardiac and pressor effects of the drug may develop. In addition, after several doses of ephedrine are administered, hypotension more severe than that originally being treated may result from direct cardiac depression and vasodilation.

The overall conclusions drawn from all these studies are that the adverse effects of ephedra may be amplified, sometimes culminating in death, due to the following reasons:

- 1. *Individual susceptibility*: Individuals undergoing very stressful situations such as athletes/football players who practice in very hot temperatures, extensive workout for muscle building or people who fast to achieve weight loss are particularly susceptible. Ephedra puts an undue stress in these individuals by increasing the blood pressure and causing additional stress on the cardiovascular system, blood supply in the brain, which may result in heart attack or stroke.
- 2. Additive stimulant effects of caffeine: Caffeine is present in many products that contain ephedra alkaloids, and those who take these products might also be consuming considerable quantities of caffeine in coffee, tea, and soft drinks. Caffeine can enhance the undesirable effects of ephedrine on the heart, blood supply system, and brain function.
- 3. Variability in contents: The quantity of ephedrine in dietary supplements, as reported on package labels, is typically $\sim 20 \text{ mg}$ per serving, and the usual dose frequency is two to three times per day. However, these products may contain larger or smaller amounts of ephedra alkaloids that are listed on the product label. For example, 11 of 20 supplements tested by Bill Gurley, PhD, an associate professor in the College of Pharmacy at the University of Arkansas for Medical Sciences, either failed to list the alkaloid content on the label or had greater than a 20% difference between the amount listed on the label and the actual amount. Therefore, even in the absence of simultaneous ingestion of other known or unknown stimulants, consumers of ephedra are often overdosed.
- 4. *Preexisting medical conditions*: The likelihood of adverse effects of ephedrine is heightened in individuals with a history of high blood pressure, heart or thyroid disease, diabetes, kidney disease or difficulty urinating, glaucoma, a seizure disorder, depression, prostate enlargement, history of stress, or are involved in stressful activities.
- 5. Taking ephedra along with other drugs: If taken with other drugs simultaneously, ephedra may cause serious complications. Antidepressants, allergy, asthma, or cold medications containing ephedrine, pseudoephedrine or phenylpropanola-

mine, caffeine-containing drugs or soft drinks are known examples of substances that exaggerate the adverse effects of ephedra.

Clinical Management

Vital Signs

The clinical effects following overdose depend on the receptor selectivity (alpha and/or beta effect). Most patients require only observation for a period of 4–8 h. Pharmacologic intervention is required only in severely symptomatic patients (cardiac arrhythmias, hypertensive crisis, seizures, hyperthermia). Severe overdose effects may most commonly result in hypertension, tachycardias, followed by bradycardia, and arrhythmias, seizures, cerebral hemorrhages and ischemia or vasoconstriction, psychosis, and hyperthermia.

Antidote and Emergency Treatment

This includes protecting the patient's airway and supporting ventilation and perfusion, monitoring and maintaining, within acceptable limits, the patient's vital signs, blood gases, and serum electrolytes, besides monitoring electrocardiogram continuously. In alert patients, removing ephedrine from the stomach by inducing emesis with ipecac, followed by activated charcoal (as long as ileus is not present); in depressed or hyperactive patients, removing ephedrine by airway-protected gastric lavage. For supraventricular or ventricular tachycardia, administering a β -adrenergic blocker, such as propranolol by slow intravenous administration, is necessary to control cardiac arrhythmias; however, in asthmatic patients, a cardioselective β -adrenergic blocker (e.g., acebutolol, atenolol, metoprolol) may be more appropriate. The β -blocker should be used with caution in asthmatic patients because it could induce severe bronchospasm or an asthmatic attack. Nitroprusside or phentolamine infusion may be used for marked hypertension, if necessary. For 'true' hypotension, administration of intravenous fluids, elevation of legs, or administration of an inotropic vasopressor, such as norepinephrine, should be considered. To control convulsion, administer diazepam. For refractory seizures, general anesthesia with thiopental or halothane and paralysis with a neuromuscular blocking agent may be necessary. Pyrexia can be controlled by cool applications and by slow intravenous administration of 1 mg dexamethasone per kilogram of body weight.

Further Reading

- Food and Drug Administration, HHS (2004) Final rule declaring dietary supplements containing ephedrine alkaloids adulterated because they present an unreasonable risk. Final rule. *Federal Registry* 69: 6787–6854.
- Haller CA and Benowitz NL (2000) Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *New England Journal of Medicine* 343: 1833– 1838.
- Rados C (2004) Ephedra ban: No shortage of reasons. FDA Consumption 38: 6–7.

Ephedrine See Speed.

Epichlorohydrin

Brad Hirakawa

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 106-89-8
- SYNONYMS: 1-Chloro-2,3-epoxypropane; 3-Chloro-1,2-epoxypropane; Epi-chlorohydrin; Chloromethyloxirane; Chloropropylene oxide; Glycerol epichlorohydrin; Glycidyl chloride; NCI-C07001; Propane, 1-chloro-2,3-epoxy-; SKEKhG; Gammachloropropylene oxide; 3-Chloro-1,2-propylene oxide; (DL)-α-Epichlorohydrin; Glycerol epichlorohydrin; ECH; RCRA waste number U041
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Solvent
- CHEMICAL FORMULA: C₃H₅ClO
- CHEMICAL STRUCTURE:



Uses

Epichlorohydrin is usually prepared from propene and is mainly used in the manufacture of glycerol and epoxy resins. It is also used: in the manufacture of elastomers, glycidyl ethers, cross-linked food starch, surfactants, plasticizers, dyestuffs, pharmaceutical products, oil emulsifiers, lubricants, and adhesives; as a solvent for resins, gums, cellulose, esters, paints, and lacquers; and as a stabilizer in chlorine-containing substances such as rubber, pesticide formulations, and solvents.

Exposure Routes and Pathways

Inhalation and dermal exposure are the most common routes of exposure.

Toxicokinetics

Epichlorohydrin is absorbed rapidly into the body through the skin, after ingestion or inhalation. Epichlorohydrin is itself a reactive epoxide and is metabolized by binding to glutathione and by hydration via epoxide hydrolase. The same hemoglobin adduct has been detected in humans and rats. Epichlorohydrin is distributed widely throughout the body. The highest tissue concentrations in rodents were found in the nose after inhalation and in the stomach after ingestion. In rats, regardless of the route of exposure, most absorbed epichlorohydrin is metabolized rapidly, part being excreted as carbon dioxide via the lungs and part as water-soluble compounds via the urine.

Mechanism of Toxicity

Epichlorohydrin is an alkylating agent (mutagenic, affects DNA). Epichlorohydrin is also an irritant, sensitizer, and corrosive.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} is 90 mg kg^{-1} in rats and 236 mg kg⁻¹ in mice. The inhalation LC_{50} is 250 ppm per 8 h in rats. Epichlorohydrin caused reproductive toxicity (testicular damage) in male rats exposed by inhalation to concentrations as low as 50 ppm per 6 h day⁻¹ over 50 days.

Human

The lowest published single toxic dose is 20 ppm via inhalation. The kidneys and respiratory tract are the target organs after acute epichlorohydrin exposure. Symptoms include nausea, vomiting, and abdominal distress. It can also cause facial swelling, eye and nasal mucosal irritation, respiratory tract irritation, bronchitis, dyspnea, central nervous system depression, hepatomegaly, and kidney lesions.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence in experimental animals for the carcinogenicity and teratogenicity of epichlorohydrin exposure via oral and inhalation routes. Epichlorohydrin was tumorgenic in rats exposed (inhalation) to concentrations as low as 100 ppm per 6 h day^{-1} over 30 days.

Human

Epichlorohydrin has been shown to cause chromosomal aberrations in humans, and is classified as a probable human carcinogen (group 2A).

Clinical Management

Emesis is not recommended. Activated charcoal slurry with or without saline cathartic and sorbitol can be used after oral ingestion. In case of inhalation exposure, good ventilation should be maintained. Skin decontamination should be performed with repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min. Liver and kidney function should be monitored. Consult a physician as soon as possible.

Environmental Fate

Environmental contamination by epichlorohydrin mainly occurs via air ducts and waste disposal of heavy ends in industries that produce or use epichlorohydrin. Epichlorohydrin can also be lost to the environment via industrial water, during transport and storage, by volatilization during use, and by inadvertent industrial production. Epichlorohydrin is relatively volatile and would therefore readily evaporate from near-surface soils and other solid surfaces. If released into water it will be lost primarily by evaporation (half-life is 29 h in a typical river) and hydrolysis (half-life is 8.2 days). It will not adsorb appreciably to sediment. If spilled on land, it will evaporate and leach into the groundwater where it will hydrolyze. In the atmosphere, epichlorohydrin will degrade by reaction with photochemically produced hydroxyl radicals (estimated half-life is 4 days). It will not bioconcentrate appreciably in aquatic organisms.

Exposure Standards and Guidelines

Threshold limit value, 2 ppm; 7.6 mg m^{-3} (skin) (American Conference of Governmental Industrial Hygienists, 1992–1993).

See also: Pollution, Water; Respiratory Tract.

Further Reading

- Giri AK (1997) Genetic toxicology of epichlorohydrin: A review. *Mutation Research* 386(1): 25–38.
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- Kolman A, Chovanec M, and Osterman-Golkar S (2002) Genotoxic effects of ethylene oxide, propylene oxide and epichlorohydrin in humans: Update review (1999–2001). *Mutation Research* 512(2–3): 173–194.

Relevant Websites

- http://ntp-server.niehs.nih.gov National Toxicology Program.
- http://www.inchem.org INCHEM website.
- http://www.epa.gov US Environmental Protection Agency website.

Epidemiology

Shayne C Gad

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Epidemiology looks at the association between adverse effects seen in humans and a selected potential 'cause' of interest, such as the use of or exposure to a chemical, a disease agent, radiation, a drug, or a medical device. Epidemiology is sometimes simply defined as the study of patterns of health in groups of people. Behind this deceptively simple definition lies a surprisingly diverse science, rich in concepts and methodology. For instance, the group of people might consist of only two people, such as the case of a father suffering from rheumatoid arthritis and his daughter with vertigo. In both father and daughter, the pattern of affected areas was remarkably similar, which might suggest that the distribution of joint lesions in rheumatoid arthritis is genetically determined. At the opposite extreme, studies of the geographic distribution of diseases using national mortality and cancer incidence rates have provided clues about the etiology of several diseases such as cardiovascular disease and stomach cancer. The patterns of health studied are also wide-ranging and may include the distribution, course, and spread of disease. The term 'disease' also has a loose definition in the context of epidemiology and might include ill-defined conditions, such as organic solvent syndrome and sick-building syndrome, or consist of an indirect measure of impairment such as biochemical and hematological parameters or lung function measurements.

Epidemiology and toxicology differ in many other ways, but principally in that epidemiology is essentially an observational science, in contrast to the experimental nature of toxicology. The epidemiologist often has to make do with historical data that have been collected for reasons that have nothing to do with epidemiology. Nevertheless, the availability of personnel records such as lists of new employees and former employees, payrolls and work rosters, and exposure monitoring data collected for compliance purposes has enabled many epidemiological studies to be conducted in the occupational setting. Thus, the epidemiologist has no control over who is exposed to an agent, the levels at which they are exposed to the agent of interest, or the other agents to which they may be exposed. The epidemiologist has great difficulty in ascertaining what exposure has taken place and certainly has no control over lifestyle variables such as diet and smoking.

Despite the lack of precise data, the epidemiologist has one major advantage over the toxicologist.

An epidemiological study documents the actual health experiences of human beings subjected to real-life exposures in an occupational or environmental setting. The view has been expressed that uncertainty in epidemiology studies resulting from exposure estimation may be equal to or less than the uncertainty associated with extrapolation from animals to humans. Regulatory bodies such as the US Environmental Protection Agency (EPA) are starting to change their attitudes toward epidemiology and recognize that it has a role to play in the process of risk assessment. However, there is also a complementary need for epidemiologists to introduce more rigor into the conduct of their studies and to introduce standards akin to the Good Laboratory Practices standards under which animal experiments are performed.

Measurement of Exposure

Epidemiologists have placed much greater emphasis on the measure of response than on the measure of exposure. They claim that this is because most epidemiologists have been trained as physicians and are consequently more oriented toward measuring health outcomes. It is certainly true that a modern textbook of epidemiology says very little about what the epidemiologist should do with exposure assessments. However, this is probably as 'much a reflection of the historical paucity of quantitative exposure information as a reflection on the background of epidemiologists. Nevertheless, it is surprising how many epidemiological studies do not contain even a basic qualitative assessment of exposure. The contrast between epidemiology and toxicology is never more marked than in the area of estimation of dose response. The toxicologist can carefully control the conditions of exposure to the agent of interest; moreover, the toxicologist can be sure that the test animals have not come into contact with any other toxic agents. An industrial epidemiologist conducting a study of workers exposed to a hepatotoxin will certainly have to control for alcohol intake and possibly for exposure to other hepatotoxins in the work and home environments. Nevertheless, it can be argued that epidemiological studies more accurately measure the effect on human health of 'real-life' exposures.

If an exposure matrix has been constructed with quantitative estimates of the exposure in each job and time period, then it is a simple matter to estimate cumulative exposure. It is a more difficult process when, as is common, only a qualitative measure of exposure is available (e.g., high, medium, and low). Even when exposure measurements are available, it may not be sensible to make an assumption that an exposure that occurred 20 years ago is equivalent to the same exposure yesterday. The use of average exposures may also be questionable, and peak exposures may be more relevant in the case of outcomes such as asthma and chronic bronchitis. Noise is a good example of an exposure that must be carefully characterized and where the simple calculation of a cumulative exposure may be misleading.

Study Designs

This section provides a brief introduction to the most important types of studies conducted by epidemiologists. It is an attempt to briefly describe the principles of the major types of epidemiological studies in order to provide insight into the reporting of epidemiological studies and the assumptions made by epidemiologists. The next section will discuss the similarities and differences between the methodologies of toxicology and epidemiology.

Cohort Studies

Historical Cohort Study When the need arises to study the health status of a group of individuals, there is often a large body of historical data that can be utilized. If sufficient information exists on individuals exposed in the past to a potential workplace hazard, then it may be possible to undertake a retrospective cohort study. The historical data will have been collected for reasons that have nothing to do with epidemiology. Nevertheless, the availability of personnel records, such as registers of new and former employees, payrolls, work rosters, and individuals' career records, has enabled many epidemiological studies to be conducted, in particular, mortality studies.

The principles of a historical cohort study can also be applied to follow a cohort of workers prospectively. This approach will be discussed further in the next section, although it should be emphasized that many historical data studies have a prospective element in so far as they are updated after a further period of follow-up. The discussion of historical cohort studies in this section will concentrate on mortality and cancer incidence studies. However, there is no reason why hearing loss, lung function, or almost any measure of the health status of an individual should not be studied retrospectively if sufficient information is available.

Mortality and cancer incidence studies are unique among retrospective cohort studies in that they can be conducted using national cancer and mortality registers even if there has been no medical surveillance of the work force. A historical cohort study also has the advantages of being cheaper and providing estimates of the potential hazard much earlier than a prospective study. However, historical cohort studies are beset by a variety of problems. Principal among these is the problem of determining which workers have been exposed and, if so, to what degree? In addition, it may be difficult to decide what an appropriate comparison group is. It should also be borne in mind that in epidemiology, unlike animal experimentation, random allocation is not possible and there is no control over the factors that may distort the effects of the exposure of interest, such as smoking and the standard of living.

The principles of historical cohort studies are described in the following subsections.

Cohort Definition and Follow-Up Period A variety of sources of information are used to identify workers exposed to a particular workplace hazard, to construct an occupational history, and to complete the collection of information necessary for tracing (see below). It is essential that the cohort be well defined and that criteria for eligibility are strictly followed. This requires that a clear statement be made about membership of the cohort so that it is easy to decide whether an employee is a member or not. It is also important that the follow-up period be carefully defined. For instance, it is readily apparent that the follow-up period should not start before exposure has occurred. Furthermore, it is uncommon for the health effect of interest to manifest itself immediately after exposure, and allowance for an appropriate biological induction (or latency) period may need to be made when interpreting the data.

Comparison Subjects The usual comparison group for many studies is the national population. However, it is known that there are marked regional differences in the mortality rates for many causes of death. Regional mortality rates exist in most industrialized countries but have to be used with caution because they are based on small numbers of deaths and estimated population sizes. In some situations the local rates for certain causes may be highly influenced by the mortality of the patients being studied. Furthermore, it is not always easy to decide what the most appropriate regional rate for comparison purposes is, as many employees may reside in a different region from that in which the plant is situated.

An alternative or additional approach is to establish a cohort of unexposed workers for comparison purposes. However, workers with very low exposures to the workplace hazard will often provide similar information. Analysis and Interpretation In a cohort study the first stage in the analysis consists of calculating the number of deaths expected during the follow-up period. In order to calculate the expected number of deaths for the cohort, the survival experience of the cohort is broken down into individual years of survival, known as 'person years'. Each person year is characterized by the age and sex of the cohort member and the time period when survival occurred. The person years are then multiplied by age-, sex-, and time period-specific mortality rates to obtain the expected number of deaths. The ratio between observed and expected deaths is expressed as a standardized mortality ratio (SMR) as follows:

$$SMR = \frac{observed \ deaths}{100 \times expected \ deaths}$$

Thus, an SMR of 1.25 represents an excess mortality of 25%. An SMR can be calculated for different causes of death and for subdivision of the person years by factors such as the level of exposure and time since the first exposure.

Interpretation of cohort studies is not always straightforward; there are a number of selection effects and biases that must be considered. Cohort studies routinely report that the mortality of active workers is less than that of the population as a whole. It is not an unexpected finding since workers usually have to undergo some sort of selection process to become or remain workers. Nevertheless, this selection effect, known as the 'healthy worker' effect, can lead to considerable arguments over the interpretation of study results, particularly if the cancer mortality is as expected but the all-cause mortality is much lower than expected. However, even an experimental science such as toxicology is not without a similar problem of interpretation, namely, the problem of distinguishing between the effects of age and treatment on tumor incidence.

Proportional Mortality Study

There are often situations in which one has no accurate data on the composition of a cohort but does possess a set of death records (or cancer registrations). In these circumstances a proportional mortality study may sometimes be substituted for a cohort study. In such a mortality study the proportions of deaths from a specific cause among the study deaths is compared with the proportion of deaths from that cause in a comparison population. The results of a proportional mortality study are expressed in an analogous way to those of the cohort study with follow-up corresponding to the observed deaths from a particular cause; it is possible to calculate an expected number of deaths based on mortality rates for that cause and all causes of death in a comparison group and the total number of deaths in the study. The ratio between observed and expected deaths from a certain cause is expressed as a proportional mortality ratio (PMR) as follows:

$$PMR = \frac{observed \ deaths}{100 \times expected \ deaths}$$

Thus, a PMR of 125 for a particular cause of death represents a 25% increase in the proportion of deaths due to that cause. A proportional mortality study has the advantage of avoiding the expensive and timeconsuming establishment and tracing of a cohort but the disadvantage of little or no exposure information.

Prospective Cohort Study Prospective cohort studies are no different in principle from historical cohort studies in terms of scientific logic, the major differences being timing and methodology. The study starts with a group of apparently healthy individuals whose health and exposure are studied over a period of time. As it is possible to define in advance the information that is to be collected, prospective studies are theoretically more reliable than retrospective studies. However, long periods of observation may be required to obtain results.

Prospective cohort studies or longitudinal studies of continually changing health parameters, such as lung function, hearing loss, blood biochemistry and hematological measurements, pose different problems from those encountered in mortality and cancer incidence studies. The relationships between changes in the parameters of interest and exposure measurements have to be estimated and, if necessary, a comparison made of changes in the parameters between groups. These relationships may be extremely complicated, compounded by factors such as aging, and difficult to estimate because there may be relatively few measurement points. Furthermore, large errors of measurement in the variables may be pre1sent because of factors such as within-laboratory variation and temporal variation within individuals. Missing observations and withdrawals may also cause problems, particularly if they are dependent on the level and change of the parameter of interest. These problems may make it difficult to interpret and judge the validity of analytical conclusions. Nevertheless, prospective cohort studies provide the best means of measuring changes in health parameters and relating them to exposure.

Case–Control Study

In a case-control study (also known as a casereferent study) two groups of individuals are selected for study, of which one has the disease whose causation is to be studied (the cases) and the other does not (the controls). In the context of the chemical industry, the aim of a case–control study is to evaluate the relevance of past exposure to the development of a disease. This is done by obtaining an indirect estimate of the rate of occurrence of the disease in an exposed and an unexposed group by comparing the frequency of exposure among cases and controls.

Principal Features Case-control and cohort studies complement each other as types of epidemiological study. In a case-control study the groups are defined on the basis of the presence or absence of a given disease and, hence, only one disease can be studied at a time. The case-control study compensates for this by providing information on a wide range of exposures that may play a role in the development of the disease. In contrast, a cohort study generally focuses on a single exposure but can be analyzed for multiple disease outcomes. A case-control study is a better way of studying rare diseases because a very large cohort would be required to demonstrate an excess of a rare disease. In contrast, a case-control study is an inefficient way of assessing the effect of an uncommon exposure, when it might be possible to conduct a cohort study of all those exposed.

The complementary strengths and weaknesses of case-control and cohort studies can be used to advantage. Increasingly, mortality studies are being reported which utilize 'nested' case-control studies to investigate the association between the exposures of interest and a cause of death for which an excess has been discovered. However, case-control studies have traditionally been held in low regard, largely because they are often poorly conducted and interpreted. There is also a tendency to overinterpret the data and misuse statistical procedures. In addition, there is still considerable debate among leading epidemiologists themselves as to how controls should be selected.

Analysis and Interpretation In a case–control study it is possible to compare the frequencies of exposures in the cases and controls. However, what one is really interested in is a comparison of the frequencies of the disease in the exposed and the unexposed. The latter comparison is usually expressed as a relative risk (RR), which is defined as

$$RR = \frac{\text{rate of disease (exposed group)}}{\text{rate of disease (unexposed group)}}$$

It is clearly not possible to calculate the RR directly in a case-control study since exposed and unexposed groups have not been followed in order to determine the rates of occurrence of the disease in the two groups. Nevertheless, it is possible to calculate another statistic, the odds ratio (OR), which, if certain assumptions hold, is a good estimate of the RR. For cases and controls the exposure odds are simply the odds of being exposed, and the OR is defined as

 $V = \frac{\text{cases with exposure/controls with exposure}}{\text{cases without exposure/controls without exposure}}$

An OR of 1 indicates that the rate of disease is unaffected by exposure of workers to the agent of interest. An OR greater than 1 indicates an increase in the rate of disease in exposed workers.

Matching Matching is the selection of a comparison group that is, within stated limits, identical with the study group with respect to one or more factors (e.g., age, years of service, and smoking history), which may distort the effect of the exposure of interest. The matching may be done on an individual or group basis. Although matching may be used in all types of study, including follow-up and cross-sectional studies, it is more widely used in case–control studies. It is common to see case–control studies in which each case is matched to as many as three or four controls.

Nested Case–Control Study In a cohort study, the assessment of exposure for all cohort members may be extremely time-consuming and demanding of resources. If an excess of incidence of death has been discovered for a small number of conditions, it may be much more efficient to conduct a case–control study to investigate the effect of exposure. Thus, instead of all members being studied, only the cases and a sample of noncases would be compared with regard to exposure history. Thus, there is no need to investigate the exposure histories of all those who are neither cases nor controls. However, the nesting is only effective if there are a reasonable number of cases and sufficient variation in the exposure of the cohort members.

Other Study Designs

Descriptive Studies

There are large numbers of records in existence that document the health of various groups of people. Mortality statistics are available for many countries and even for certain companies. Similarly, there is a wide range of routine morbidity statistics, in particular, those based on cancer registrations. These health statistics can be used to study differences between geographic regions (e.g., maps of cancer mortality and incidence presented at a recent symposium), occupational groups, and time periods. Investigations based on existing records of the distribution of the disease and of possible causes are known as descriptive studies. It is sometimes possible to identify hazards associated with the development of rare conditions from observation of clustering in occupational or geographical areas.

Cross-Sectional Study

Cross-sectional studies measure the cause (exposure) and the effect (disease) at the same point in time. They compare the rates of diseases or symptoms of an exposed group with an unexposed group. Strictly speaking, the exposure information is ascertained simultaneously with the disease information. In practice, such studies are usually more meaningful from an etiological or causal point of view if the exposure assessment reflects past exposures. Current information is often all that is available but may still be meaningful because of the correlation between current exposure and relevant past exposure.

Cross-sectional studies are widely used to study the health of groups of workers who are exposed to possible hazards but do not undergo regular surveillance. They are particularly suited to the study of subclinical parameters such as blood biochemistry and hematological values. Cross-sectional studies are also relatively straightforward to conduct in comparison with prospective cohort studies and are generally simpler to interpret.

Intervention Study

Not all epidemiology is observational, and experimental studies have a role to play in evaluating the efficiency of an intervention program to prevent disease (e.g., fluoridation of water). An intervention study at one extreme may closely resemble a clinical trial with individuals randomly selected to receive some form of intervention (e.g., advice on reducing cholesterol levels). However, in some instances it may be a whole community that is selected to form the intervention group. The selection may or may not be random.

Veterinary Epidemiology

Humans are in close association with their pets and other animals (e.g., local wildlife and animals on a farm). Veterinary epidemiology, like human epidemiology, looks at the association between adverse effects and a selected potential 'cause' of interest, such as exposure to a chemical or a disease agent. For example, veterinary epidemiology can play a key role in emerging and global disease outbreaks, helping in the understanding and prevention of infections and other emerging diseases, including those transmitted from an animal to other animals, and those possibly transmitted from animals to humans. An example of a veterinary epidemiological study was one investigating the transmission of Salmonella typhimurium from cattle which had received no growth-promoting antibiotics to humans who had direct contact with the sick animals. Another example is severe acute respiratory syndrome (SARS). In the investigation of the origins of the SARS outbreak in China, viruses associated with SARS were isolated from Himalayan palm civets found in a live-animal market in Guangdong, China, and evidence of virus infection was also detected in other animals and in humans working at the same market. The detection of these viruses in small, live wild mammals in a retail market helped identify at least one means of the interspecies transmission, that is, infected animals sold in that market to human customers.

Conclusion

Epidemiological studies can be the most powerful and persuasive tools for establishing the hazards associated with chemical exposures or personal actions (such as cigarette smoking). However, due to all the factors discussed previously, such studies also tend to be somewhat insensitive. Unless one can clearly establish the symptoms and signs of a disease for which there is a causal connection, such studies lose the desired specificity.

See also: Analytical Toxicology; Carcinogen Classification Schemes; Carcinogenesis; Exposure; International Agency for Research on Cancer; Medical Surveillance; National Institute for Occupational Safety and Health.

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Relevant Websites

- http://www.cvm.uiuc.edu The Association for Veterinary Epidemiology and Preventive Medicine (AVEPM).
- http://www.pitt.edu Toxicology and Epidemiology (Online Supercourse). (More than 9000 faculty from 118

countries have contributed to an online library of more than 700 lectures with quality control and adherence to accepted pedagogic principles. The goal is to improve teaching and research in epidemiology and public health worldwide.)

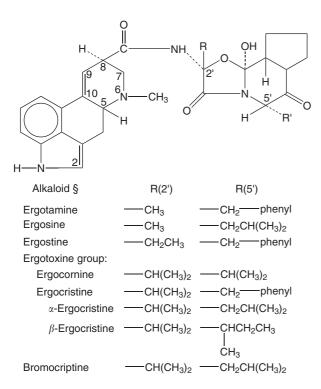
Epinephrine See Catecholamines.

Ergot

Christopher P Holstege

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- REPRESENTATIVE CHEMICAL: Ergotamine
- SYNONYMS: Bromocriptine; Dihydroergocornine; Dihydroergocristine; Dihydroergosine; Dihydroergotamine; Dihydroergotaxime; Ergobasine; Ergocornine; Ergocristine; Ergocryptine; Ergometrine; Ergonovine; Ergosine; Ergotamine; Ergotamine tartrate; Ergotaxime; Lergotrile; Lisuride; Lysergol; Metergoline; Methylergonovine; Methysergide
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alkaloid
- CHEMICAL STRUCTURE:



Uses

Ergot was used as early as the sixteenth century to strengthen uterine contractions. Currently, ergotamine tartrate is combined with caffeine and administered to relieve migraine headaches. Ergonovine has been used to treat postpartum hemorrhage. Derivatives of ergots are used to manage amenorrhea and as an adjunct in the treatment of Parkinson's disease. Hydrogenated ergot alkaloids have been used for symptoms of idiopathic mental decline in elderly patients.

Background Information

The ergot alkaloids are found within the sclerotium of the fungus *Claviceps purpurea*. The sclerotium is the hard tuber-like resting stage of this fungus and is a dark gray, purple, or black cylindrical structure measuring 1.5 cm in length and 0.5 cm in width. *C. purpurea* may be found on a number of different grains, with rye contamination most often reported. A cold winter followed by wet spring favors germination. If the sclerotia are not removed from contaminated grain by beating or sieving, humans or animals may accidentally ingest them.

Exposure Routes and Pathways

Historically, exposure occurred by consumption of contaminated grain, especially rye flour. Acute poisonings in humans are rare and are generally associated with overdosage with ergotamine tartrate medication. Poisoning by ergot-containing mixtures has been associated with attempts to induce abortion. Animal poisonings result from consumption of contaminated pasture grasses and grains. The last diagnosed human fatalities associated with consumption of ergotcontaining grains occurred in a French village in 1951.

Toxicokinetics

The degree of oral absorption of ergots varies depending on the specific agent. For example, ergotamine is poorly absorbed orally and a considerable amount is eliminated by first-pass metabolism in the liver. On the other hand, ergonovine is rapidly and more completely absorbed following ingestion. Suppositories increase ergotamine bioavailability ~ 20 times that of ingested doses. Peak plasma levels are reached within 2 h. Symptoms typically appear within 4 h after intake. The volume of distribution is estimated to be 21kg⁻¹. Ergots are primarily metabolized by the liver and 90% of metabolites are eliminated in the bile. The elimination half-life varies depending on which ergot is ingested: ergotamine's half-life is 3 h whereas dihydroergotamine's half-life is 13 h. However, these elimination half-life values were determined at therapeutic doses. In overdose, the half-life of these agents would be expected to prolong.

Mechanism of Toxicity

The pharmacological mechanisms associated with ergot toxicity are complex and have not been fully delineated. Ergotamine interacts with serotonergic, dopaminergic, and α -adrenergic receptors. In the central nervous system, ergots have a sympatholytic effect. They also stimulate serotonergic receptors that contribute to its hallucinogenic activity. Peripherally, ergots act as α -adrenergic agonists resulting in peripheral vasospasm. The vasoconstrictive action of ergots can produce widespread arterial spasm. Endothelial injury associated with arterial spasm may cause local thrombosis and subsequent gangrene.

Acute and Short-Term Toxicity (or Exposure)

Animal

Peripheral vasoconstriction, particularly of the hind limbs and forelimbs, can produce hemorrhagic vesiculations that may progress to gangrene. Ergot induced embryotoxicity has been reported with associated fetal malformations and growth retardation. Ergotism may also result in miscarriages.

Human

Acute early symptoms of toxicity include nausea, vomiting, diarrhea, skin paresthesias, and chest pain. Headache, fixed miosis, hallucinations, delirium, hemiplegia, and convulsions may occur. Historically, 'ergotism' was divided into gangrenous ergotism and convulsive ergotism. St. Anthony's Fire was one descriptive name given to ergotism in Europe during the Middle Ages due to extremity burning pain associated with ergot toxicity. Peripheral ischemia most commonly manifests in the lower extremities, although ischemia of cerebral, mesenteric, coronary, and renal vascular beds may also occur. Legs may become pulseless, pale, and cyanotic. Hemorrhagic vesiculations, pruritus, and gangrene can occur.

Chronic Toxicity (or Exposure)

Animal

Pigs have demonstrated chronic ergot toxicity as blockade of lactation only. No other effects were noted.

Human

Studies of dihydroergotamine nasal spray have described increased incidence of allergic rhinitis and some gastrointestinal complaints. Patients taking ergot derivatives chronically for the management of headache may develop rebound headache (headache that develops from lack of drug, which can lead to continued drug use and worsening of headache symptoms).

In Vitro Toxicity Data

Studies in rat brains have shown that ergot alkaloids do not bind to brain benzodiazepine binding sites.

Clinical Management

Acute poisoning may be treated with decontamination using oral activated charcoal. Arterial spasms may be relieved by administration of vasodilators, such as nitroprusside, nitroglycerin, and phentolamine. Benzodiazepines should be administered to halt seizures and may be used to alleviate agitation associated with hallucinations. Anticoagulants such as heparin may be administered in cases of extreme vasoconstriction. Reaction to the ergot alkaloids is highly variable and clinical progress should be monitored carefully.

See also: Mold.

Further Reading

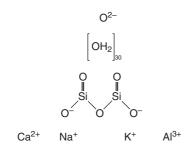
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Erionite

A Umran Dogan, Meral Dogan, and Salih Emri

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- CHEMICAL NAME: Erionite refers to a group of minerals: erionite-Ca, erionite-K, and erionite-Na
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 66733-21-9; CAS 12510-42-8
- SYNONYMS: Erionite-Ca; Erionite-Na; Erionite-K
- Classifications:
 - Erionite-Ca International Mineralogy Association (IMA) status: Approved Strunz ID: 8/J.26-94
 - Erionite-Na IMA status: Approved Strunz ID: 8/J.26-90
 - Erionite-K IMA status: Approved Strunz ID: 8/J.26-92 where 8=Silicates, J=Tectosilicates, 26= Zeolite Group, Wilhendersonite-Chabazite-Perlialite series
- CHEMICAL PROPERTIES: Si/Al ratio (used to be Si/ Al>2.4; Si/Al>3.0; Si/(Al + Fe)>2.9 for erionite) is no longer a criteria for discrimination between erionite and offretite, because of the extensive compositional overlap that exists between the two species. However, the Si–Al content in the tetrahedra framework is the major control on the unit cell volume dimensions in erionite. In addition, Mg cation is a major factor in controlling the crystallization of the mineral species.
- CHEMICAL FORMULAS: Erionite-Ca Composition of the type specimen is from Maze, Niigita Prefecture, Japan: (Ca_{2.28}K_{1.54}Na_{0.95}Mg_{0.86}) Al_{8.83}Si_{26.90}O₇₂·31.35H₂O (Mg>0.80). Erionite-Na Composition of the type specimen is from Cady Mountains, CA, USA: (Na_{5.59}K_{2.00}-Ca_{0.11}Mg_{0.18}Fe_{0.02})Al_{7.57}Si_{28.27}O₇₂·24.60H₂O. Erionite-K Composition of the specimen from Ortenberg, Germany: (K_{3.32}Na_{2.31}Ca_{0.99}Mg_{0.06}-Ba_{0.02})Al_{8.05} Si_{28.01}O₇₂·31.99H₂O
- CHEMICAL STRUCTURE:



Relationship to Other Species

Belbergite, Chabazite-Ca, Chabazite-K, Chabazite-Na, Chabazite-Sr, Gmelinite-Ca, Gmelinite-K, Gmelinite-Na, Levyne-Ca, Levyne-Na, Mazzite, Offretite, Perlialite, Tschernichite, Wilhendersonite, and other Erionite species.

Associated Minerals

Analcime, Chabazite, Clinoptilolite, Heulandite, Mordenite, Phillipsite, Offretite, Levyne, Smectite, Cristobalite, Quartz, Opal, Pyrite, Thenardite, Celandonite, Herschelite, Calcite, Dolomite, Halite, Gypsum.

Erionite Characterization Rules

The toxicity of the mineral is such that quantitative characterization of erionite is extremely important. Samples should be characterized by using one or more of the following techniques: (1) powder X-ray diffraction, (2) electron probe microanalysis or inductively coupled plasma-mass spectroscopy, (3) scanning electron microscopy equipped with wavelength dispersive spectroscopy (WDS) and/or energy dispersive spectroscopy (EDS), (4) transmission electron microscopy equipped with WDS and/or EDS and selected area electron diffraction, and (5) similar or better analytical techniques.

Crystal chemistry of erionites should be computed based upon the guidelines of the IMA Zeolite Report of 1997. The reliabilities of the crystal chemistries of these erionites should be evaluated using the balance error formula of $E = ((Al + Fe^{3+}) - (Na + K) + 2(Ca + Mg + Sr + Ba))/(Na + K) + 2(Ca + Mg + Sr + Ba) \times 100$. The results of chemical analyses of erionite are only considered to be reliable if the balance error (*E*%) is equal to or less than 10%.

In the crystal structure of erionite, Mg cations can be present up to 0.8 atom per cell. $Si + Al (+Fe^{3+})$ should be approximately equal to 36 atoms based upon 72 oxygen atoms in the erionite formula, although the Si/Al ratio alone cannot be used for identification.

Often the erionite specimens were incompletely or incorrectly characterized throwing doubt on the results of the work. Such experiments should only be performed with erionite minerals that have passed the quantitative characterization tests (both E% and Mg-content) and the type of erionite (-Ca, -Na, -K) must be identified properly. Failure to do so makes the results of the experiments problematic. Because of this correct identification of the mineral is imperative and the characterization guidelines described above must be followed.

Erionite Group Minerals

Erionite occurs in different types of rocks, rarely in pure form. It occurs in two major morphotypes, a short fiber form. Erionite's name came from 'erion', the Greek work for wool, because of its white, fibrous, wool-like appearance.

The basic structure of erionite is aluminosilicate tetrahedra. The oxygen is shared between two tetrahedra. The structure of erionite is chainlike, with six tetrahedra on each edge of the unit forming part of a chain of indefinite length. Erionite is not known to occur in other than fibrous form, in single needles or in clusters. In 1997, erionite was elevated to group status and individual members of erionite-Ca, erionite-Na, and erionite-K have been redefined.

Erionite-Ca

Ca is the most abundant extra framework cation. $T_{\rm Si}$ in the range of 0.68–0.79. Erionite-Ca from Shourdo, Georgia; Durkee, OR, USA; Beach Creek, OR, USA; Montresta, Nuoro, Italy; British Columbia, Canada; Montecchio Maggiore, Italy; Jindivick, Australia; Phillip Island, Australia; and Faedo, Vicenza, Italy passed the balance error and Mg-content test and reclassified as erionite-Ca.

Erionite-Na

Na is the most abundant extra framework cation. $T_{\rm Si}$ in the range of 0.74–0.79. Erionite-Na from Durkee, OR, USA; Cady Mountains, CA, USA; Lake Natron, Tanzania; Crooked Creek, OR, USA; Phillip Island, Australia; Campbell Glacier, Antarctica; Mt. Adamson, Antarctica; Dunseverik, Northern Ireland; Montecchio, Maggiore, Italy; and selected samples from Cappadocian region of Turkey passed the balance error and Mg-content test and re-classified as erionite-Na.

Erionite-K

K makes up 58% of extra framework cation; significant Na, Ca, and Mg are also present. T_{Si} in the range of 0.74–0.79. Erionite-K from Durkee, OR, USA; Rome, OR, USA; Yaquina Head, OR, USA; Reese River, NV, USA; Ortenberg Quarry, Germany; Rome, OR, USA; and selected samples from Cappadocian region of Turkey passed the balance error and Mg-content test and re-classified as erionite-K.

Undifferentiated Erionites

Some erionite data from the literature did not pass the balance error test or Mg-content test or both. Some data could not be re-classified as single erionite mineral. Italian samples reported from Montecchio were re-calculated as erionite-Ca and erionite-Na, respectively. Two sets of data from Phillip Island, Australia were re-calculated as both erionite-Ca and erionite-Na. Data from Durkee, OR, USA by three different authors were re-calculated and found as erionite-Ca, erionite-Na, erionite-K, respectively.

Localities

Type Localities

- Erionite-Ca: Maze, Niigita Prefecture, Chubu Region, Honshu Island, Japan.
- Erionite-Na: Cady Mountains, San Bernandino County, CA, USA.
- Erionite-K: Rome, Marion County, OR, USA.

Other Localities

Large number of erionite deposits have been reported from many countries including Antarctica (North Victoria Land), Australia (New South Wales), Bulgaria, Canada (British Columbia), China, France (Pays de la Loire), Germany (Baden-Wurttemberg, Bavaria, Hesse), Hungary, Iceland (Breidhdalur-Brufjordur, Hofsa, Hvalfjordur), Italy (Latium, Sardinia), Japan, Korea, Mexico, New Zealand (South Island), Romania, Russia (Eastern Siberia), South Africa, Turkey (Cappadocia), UK (Northern Ireland, Scotland), USA (Arizona, California, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming).

Uses of Erionite and Zeolite

Erionite is not known to be currently mined or marketed for commercial purposes. Natural erionite has been replaced by synthetic nonfibrous zeolites. However, erionite was used as a noble metal impregnated catalyst in a hydrocarbon-cracking process, and erionite-rich blocks was also used for house building materials. Its use to increase soil fertility and to control odors in livestock production has been studied.

Since 1978, the use of zeolites to solve environmental pollution and energy conservation problems showed promise and was expected to increase. Natural zeolites have many commercial uses in coal gasification and natural gas purification, selectively adsorb molecules from water or air, purify sludge effluents to potable standards, extract trace amounts of heavy metals so that existing water supplies may be reused, extract radioactive species from nuclear plant wastes, retain their ion-exchange capacities, and are resistant to nuclear degradation. In addition, they are used in agriculture to decrease ammonia released from animal wastes and retain the nitrogen in the solid wastes, which increases the fertilizer value of the solid material, reduce noxious fumes of ammonia and hydrogen sulfide when spreading in chicken houses, increase egg production when used in the chicken houses, absorb liquid waste and reduce odors when spreading on the floors of pig farms, feed supplements to increase the feed conversion value, control aquaculture environments and fish culture recirculating water systems, appear to exhibit antibiotic properties that reduce illness and death rates among farm animals. Further, zeolites are used in air/water/soil pollution to remove sulfur dioxide from coal and oil burning power plant emissions, are especially suited to low pH and high temperature exhaust systems, absorb oil spills, neutralize low pH soils; in oxygen production for enclosed and poorly ventilated spaces, river and pond aeration, reoxygenate downstream waters of paper and pulp plants, secondary sewage treatment. Zeolites also have miscellaneous uses in paper products, construction products, fluoride toothpaste, recycle-dialysis systems, solar energy collection, dehydration and rehydration resulting in the exchange of several hundred BTUs per pound.

Production of Erionite

Current commercial production and marketing of erionite is not known. Erionite was first described in 1898, but reports of occurrences were not published until 1959. Commercial mining of zeolites, including ores containing erionite, began in the 1960s. In the 1970s, two US companies mined erionite at two of six mineable deposits in the United States. Erionite was one of the four commercially important zeolites in the 1970s.

Background Information

Erionite was first described in 1898. The carcinogenity of erionite was called to the attention of the rest of the world from experiences in the Cappadocia region of Turkey where the cancer rate is about 1000 times greater than that observed elsewhere. Most cases of mesothelioma were found to occur in the villages of Tuzkoy, Sarihidir, Karain, and in neighboring villages. Erionite was first listed for its carcinogenicity in the Seventh Annual (1987) Report on Carcinogens from the International Agency for

Exposure Routes and Pathways

Inhalation is the exposure route for humans. Animal studies have used inhalation exposures and intrapleural or intraperitoneal injections. Current potential occupational exposure to erionite appears to be as the result of mining and producing other natural zeolites, some of which may contain erionite fibers. Environmental and residential exposure can occur via dusts containing erionite.

Toxicokinetics

Erionite has been shown to be an agent responsible for malignant mesothelioma. Mesothelioma is an aggressive tumor of the mesothelial cells lining the body cavities for which there is no cure and for which current therapies to reduce the effects of the disease are unsatisfactory. The source of exposure may be occupational or environmental and 2000– 3000 cases are diagnosed per year in the United States, many of which are associated with (amphibole) asbestos and erionite.

Erionite induced peritoneal mesotheliomas in male rats when administered by intraperitoneal injection, induced pleural mesotheliomas in rats of both sexes when administered by intrapleural injection or inhalation.

Erionite is also listed as a group-I known human carcinogen because the IARC Working Group reported that there is sufficient evidence for carcinogenicity based upon descriptive studies of three villages from Turkey. Erionite fibers were found in lung tissues of pleural mesothelioma cases of Karain, Tuzkoy, and Sarihidir villages of Turkey, where there was very high mortality from malignant mesothelioma. Therefore, erionite in the Cappadocian region of Turkey is believed to be responsible for the majority of the mesothelioma cases observed in that country.

Mechanism of Toxicity

The tumorigenesis of mineral fibers is governed by fiber dimensions and inherent differences in the physicochemical properties of the fibers (intrinsic fiber potencies), for example, studies of carcinogenic fibers have found that fiber geometry and length seem to be the most important factors, with long thin fibers having a greater proliferative and tumorigenesis capability than short fibers. Although the biological mechanisms are complex and there is little understanding of how durable mineral fibers cause mesothelioma, the association of erionite with mesothelioma is well established. Recently, a more complex relationship of erionite plus a genetic component to cause mesothelioma has been postulated. Work in progress may yet give a greater understanding of the biological mechanisms involved. What is clear is that erionite is one of the most carcinogenic minerals in the world and it requires the utmost care in handling.

Acute and Short-Term Toxicity (or Exposure)

Animal

There is sufficient evidence for the carcinogenecity of erionite to experimental animals.

A number of experiments have been conducted on the intrapleural and intraperitoneal administration of erionite in mice and rats. These experiments have all been positive, showing a very high mesothelioma yield (90% or above) for amounts of erionite above 0.5 or 1 mg. For higher doses, the time of appearance of tumors was decreased. Other tumors at the site of inoculation as well as lymphomas have been occasionally reported.

Natural erionite, synthetic nonfibrous zeolite with the composition of erionite, and crocidolite type asbestos were tested at a concentration of 10 mg m^{-3} inhalation in rats. Pleural mesotheliomas were found in 27 of 28 rats exposed to erionite; one pulmonary and one pleural tumor were found in 28 rats exposed to synthetic zeolite, and one lung carcinoma was reported in rats exposed to crocidolite.

The relative carcinogenic potency of erionite and asbestos have been compared. In experiments based upon intrapleural inoculation, erionite was 300–800 times more active than chrysotile, and 100–500 times more active than crocidolite. In intraperitoneal experiments, erionite was 20–40 times more active than chrysotile and 7–20 times more active than crocidolite.

Human

No human studies of acute and short-term exposures are available.

Chronic Toxicity (or Exposure)

Animal

No animal studies of chronic exposures are available; the long-term effects of short-term exposures have been studied as part of the evaluation of the carcinogenic potential of erionite.

Human

There is sufficient evidence for the carcinogenecity of erionite to humans. Most of the data on the carcinogenicity of erionite in humans come from the experience of the inhabitants of the erionite-contaminated villages in Cappadocia region of Turkey. It is reported that 25 malignant pleural mesothelioma (MPM) in population of 575 inhabitants of Karain village between 1970 and 1974; 28 MPM in Karain village between 1975 and 1979; 15 MPM, 12 malignant peritoneal mesothelioma (MPEM), and eight lung cancer in Tuzkoy village between 1978 and 1980. The incidence or mortality from mesothelioma was above 1% year⁻¹, a rate which is over 10 000 times higher than those seen among populations of nonoccupationally exposed to asbestos from Western Europe or North America.

Several mesothelioma cases were reported for persons who immigrated to Sweden but used to live in one of the Cappadocian villages (Karain). In this group of ~100 men who presently live in Sweden, seven cases of mesothelioma were observed. The incidence was approximately to 1% year⁻¹. In another study, 14 deaths due to MPM among 162 Turkish emigrants from Karain. In addition, there were five patients with mesothelioma (four MPM and one MPEM) who were still alive, at the time of the study performed. Thus it is calculated that risk of mesothelioma is for men 135 times and for women 1336 times greater than for the same sex and age groups in Sweden.

In Vitro Toxicity Data

Various in vitro studies have examined erionite in noncellular systems and the effects of erionite on isolated cells, mainly to attempt to understand how erionite induces chromosomal aberrations and carcinogenicity via cytotoxic effects and other possible mechanisms. One example is a study of the ability of erionite to initiate hydroxyl radical formation from hydrogen peroxide in a noncellular system. Other examples are the study of the possible mechanisms of reactive oxygen intermediate (ROM) production by human blood cells stimulated with erionite, and the release of superoxide as a biologic response of alveolar macrophages, hamster tracheal epithelial cells, and rat lung fibroblasts exposed to erionite. In *in vitro* mutagenicity studies, erionite induced unscheduled DNA synthesis and morphological transformation in cultured mammalian cells. No data

were available to evaluate the reproductive or prenatal toxicity of erionite in experimental animals.

Clinical Management

It takes 20–30 years for erionite to induce mesothelioma. However, there is no cure for the malignant mesothelioma. Surgery may extend the patient's life 9–12 months.

Environmental Fate

Most of the nonoccupational data on exposure to erionite refer to an agricultural area of Central Anatolia, Cappadocia, Turkey. Limited data are available from the USA, suggesting little exposure to fibers. People and animals must be prevented from contacting erionite; responsible person/group should take necessary precaution and must not permit people work or live in an area contaminated with erionite.

Ecotoxicology

Since erionite has been used in laboratory animals for carcinogenecity experiments and tests positive, it could be harmful to other animals if it is inhaled.

Other Hazards

Erionite is not known as flammable or explosive.

Exposure to Erionite

Airborne erionite fibers are generally respirable. Erionite is reported to be a minor component in some commercial zeolites.

Production and use of the erionite-contaminated zeolites may result in potential exposure for the workers and the general population who use zeolites in a variety of processes and products. All workers involved in the production or use of these zeolite-containing products are potentially exposed to erionite.

Fibrous and nonfibrous zeolites are common minerals in the western United States; there are 10 trillion tons of reserves and 120 million tons near the surface of the ground. Erionite fibers have been detected in samples of road dust in Nevada. United States residents of the Intermountain West may be potentially exposed to fibrous erionite in ambient air. Total dust exposures in an open pit zeolite (containing erionite) mine in Arizona for miners ranged from 0.1 to $13.7 \,\mathrm{mg \, m^{-3}}$; respirable dust in the mining area was $0.01-1.4 \,\mathrm{mg \, m^{-3}}$.

Exposure Standards and Guidelines

Erionite is listed as a group-I known human carcinogen by the International Agency for Research on Cancer (IARC) because there is sufficient evidence for the carcinogenicity in experimental animals. The US Occupational Safety and Health Administration (OSHA) regulates erionite under the Hazard Communication Standard, and as a chemical hazard in laboratories. The US Environmental Protection Agency (EPA) regulates erionite under the Toxic Substances Control Act (TSCA) as a chemical substance for which there are significant new uses, and specifies procedures for manufacturers, importers, or processors to report on those significant new uses.

See also: Carcinogen Classification Schemes; Carcinogenesis; Respiratory Tract; Toxicity Testing, Carcinogenesis.

Further Reading

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Relevant Websites

http://un2sg4.unige.ch - Athena Mineralogy.

- http://www.mindat.org Mindat.org: The Mineral Database.
- http://webmineral.com Webmineral.

Erythromycin

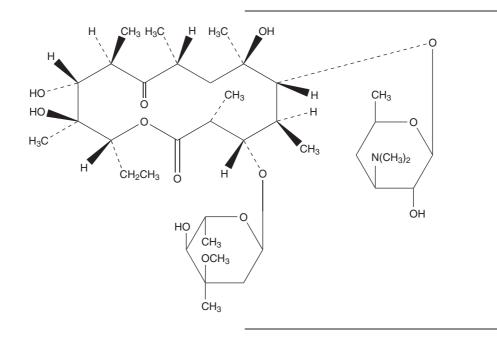
Michael D Reed

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- SYNONYMS: Numerous salts and brand names available. E-Mycin; EES; Ilosone; and many others
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Antibiotic; Macrolide
- CHEMICAL FORMULA:

Toxicokinetics

The ester and ester salts of erythromycin are variably absorbed (18–45%) from the small intestine into the systemic circulation. Overall, erythromycin bioavailability depends upon the formulation administered with greatest bioavailability observed with the estolate salt (Ilosone brand) and the least is observed with the base formulation. The bioavailability of the ethylsuccinate salt is highly variable. The majority of erythromycin formulations are more completely absorbed when administered in the fasting state whereas the ethylsuccinate salt is better absorbed



Uses

Erythromycin is indicated for the treatment of infections caused by erythromycin susceptible bacteria. The drug binds to the 50 S ribosomal subunit inhibiting bacterial RNA-dependent protein synthesis. Susceptible bacteria include most Gram-positive bacteria and the "atypical" pathogens.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to erythromycin. Numerous erythromycin salt preparations are available in tablet, capsule, or liquid preparations for oral administration. Other available forms include intravenous, topical (acne), and ophthalmic preparations. when given with food. As would be expected, peak serum erythromycin concentrations are variable and occur $\sim 2-4$ h after administration and are dependent upon which formulation ingested. Peak plasma concentrations after intravenous administration of the lactobionate or gluceptate salts occurs within 1 h of administration.

Once in systemic circulation, erythromycin is widely distributed in the body with an estimated $V_d \sim 0.6-0.71 \text{kg}^{-1}$. The drug is $\sim 70-90\%$ bound to plasma proteins. The vast majority of the drug is found in the intravascular space, reflecting its widespread clinical use for the treatment of intracellular pathogens. The drug undergoes extensive metabolism with only 5–10% excreted unchanged in the urine; the majority of the drug is excreted via the bile. Erythromycin's half-life is $\sim 2-3$ h. Erythromycin is a substrate for the cytochrome P450 (CYP 450) 3A4 isoenzyme. Once bound to CYP 3A, erythromycin stimulates its own metabolism via demethylation and oxidation to nitroalkane metabolites. These nitroalkane metabolites appear to form stable complexes with the iron of the CYP 3A4 isoenzyme, decreasing its functional capacity. Depending upon the erythromycin dose and duration of therapy, CYP 3A4 activity could be inhibited to undetectable activity and serves as the basis of erythromycin-associated metabolic-based drug-drug interactions.

Mechanism of Toxicity

The gastrointestinal hormone, motilin, is responsible for maintaining the normal, rhythmic peristaltic activity of the intestines. Erythromycin possesses high affinity for the motilin receptor which accounts for the drug's poor tolerability with routine clinical dosing (abdominal cramping, diarrhea) and accounts for the primary toxicity observed after oral overdose. In contrast, very high plasma erythromycin concentrations, which may be obtained after rapid administration of the intravenous formulation or after aggressive erythromycin dosing in any patient who cannot metabolize/excrete the drug (i.e., patient with poor hepatic and renal function) leading to the accumulation of high plasma concentrations, can experience life-threatening cardiac arrhythmias. In a concentration dependent manner, erythromycin will interfere with the potassium rectifier channels within the myocytes of the heart leading to QT prolongation on ECG and arrhythmia. The delayed potassium rectifier (Ik) channels are the potassium channels involved in repolarization of cardiac cells. Blockade of the cardiac Ikr (delayed potassium rectifier-rapid) channel produces a depressed peak in the voltage and a decrease in potassium cellular outflow predisposing the myocardium to early after repolarization, which when sizeable, results in dysrhythmia and most notably, Torsades de Pointes. (Similar to what is observed with astemizole, cisapride, and terfenidine.)

Acute and Short-Term Toxicity (or Exposure)

Animal

Erythromycin is regularly used in veterinary practice and seems to be tolerated well by many animal species. Like humans, animals can develop hypersensitive reactions to erythromycin. Also like humans, acute gastrointestinal effects are the most commonly seen adverse effects.

Human

Patients presenting with acute erythromycin overdose are usually asymptomatic or experiencing minor to moderate gastrointestinal side effects/ discomfort. Serious cardiac effects, including prolongation of the QT interval, arrhythmias (i.e., ventricular tachycardia, Torsades de Pointes, ventricular fibrillation, and heart block), may be observed after rapid intravenous administration and coincident with high, peak erythromycin plasma concentrations. The occurrences of these QT prolongation-associated arrhythmias are rare.

Chronic Toxicity (or Exposure)

Animal

Animals have been reported to develop similar symptoms to those seen in humans when larger doses are used. Horses are particularly sensitive to erythromycin induced gastrointestinal effects. At doses of $>5 \text{ mg kg}^{-1} \text{ day}^{-1}$, dogs have been reported to develop ventricular arrhythmias.

Human

Patients undergoing routine antibiotic use with erythromycins orally, generally tolerate the drug well. Gastrointestinal complaints are common. However, more serious effects have been noted, including profound cardiovascular toxicity (e.g., arrhythmias such as Torsades do Pointes), hepatic damage, neurologic effects (e.g., ototoxicity) associated with high serum levels and intravenous use, although one case describes toxic effects without elevated serum erythromycin concentrations or evidence of impaired renal or hepatic drug clearance.

In Vitro Toxicity Data

Mutagenicity assays of bacterial DNA repair in *Escherichia coli* have been negative with erythromycin.

Clinical Management

Though the clinical need for such measures would be expected to be rare, basic and advanced life-support measures as well as aggressive decontamination should be instituted as clinically necessary. Gastric decontamination may be performed dependent on the symptomatology of the patient and history of the exposure. Activated charcoal will effectively adsorb erythromycin. Gastrointestinal discomfort may be treated symptomatically or by reducing the dosage, if appropriate. Specific attention to maintaining normal fluid and electrolyte homeostasis should be addressed in significant overdoses involving young infants. Liver function tests should be monitored if hepatotoxicity is suspected. Erythromycin blood levels are not clinically useful.

See also: Cardiovascular System; Gastrointestinal System.

Further Reading

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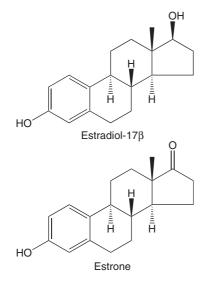
Estrogens I: Estrogens and Their Conjugates

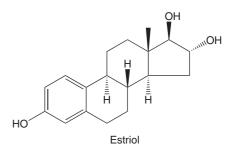
James L Wittliff and Sarah A Andres

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Estrogens

- Representative Chemicals: Estradiol-17β; Estrone; Estriol
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Estradiol-17 β (CAS 50-28-2); Estrone (CAS 53-16-7); Estriol (CAS 50-27-1)
- SYNONYMS:
 - Estradiol-17 β : 1,3,5(10)-Estratriene-3,17 β -diol;
 - Estrone: 1,3,5(10)-Estratrien-3-ol-17-one; and
 - Estriol: 1,3,5(10)-Estratriene-3,16 α ,17 β -triol.
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Steroid hormones
- CHEMICAL FORMULAS:
 - Estradiol-17 β : C₁₈H₃₀O₂;
 - \circ Estrone: C₁₈H₂₂O₂; and
 - Estriol: $C_{18}H_{24}O_3$.
- CHEMICAL STRUCTURES:

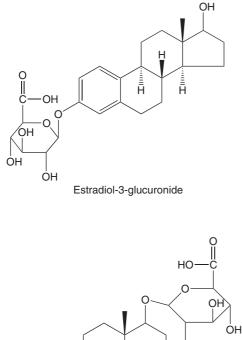


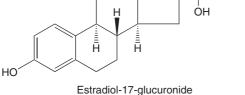


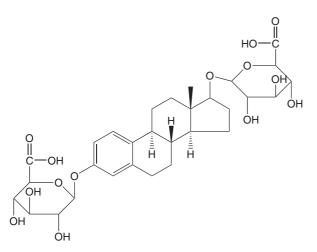
Estrogens Conjugates, Physoplogical

- REPRESENTATIVE CHEMICALS: 17β-Estradiol-3-glucuronide; 17β-Estradiol-17-glucuronide; Estradiol-3,17-disulfate; Estradiol-3,17-diglucuronide; Estrone-3-sulfate
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: 17β -Estradiol-3-glucuronide (CAS 15270-30-1); Estradiol-17-glucuronide (CAS 1806-98-0); Estradiol-3,17-disulfate (CAS 17046-60-5); Estradiol-3,17-diglucuronide; Estrone-3-sulfate (CAS 481-97-0)
- SYNONYMS:
 - 17β-Estradiol-3-glucuronide: 1,2,5(10)-Estratrien-3,17β-diol 3-glucosiduronate;
 - Estradiol-17-glucuronide: 1,2,5(10)-Estratrien-3,17β-diol 17-glucosiduronate;
 - Estradiol-3,17-disulfate: 1,3,5(10)-Estratrien-3,17b-diol disulfate;
 - Estradiol-3,17-diglucuronide: 1,3,5(10)-Estratrien-3,17 β -diol diglucosiduronate; and
 - Estrone-3-sulfate: 1,3,5(10)-Estratriene-3-ol-17-one sulfate.
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Steroid hormones
- CHEMICAL FORMULAS:
- 17β -Estradiol-3-glucuronide: C₂₄H₃₂O₈;
- Estradiol-3-glucuronide sodium salt: C₂₄H₃₁NaO₈;

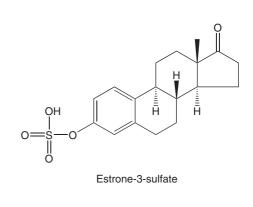
- \circ Estradiol-17-glucuronide: C₂₄H₃₂O₈;
- Estradiol-3,17-disulfate: C₁₈H₂₂O₈S₂;
- Estradiol-3,17-diglucuronide: C₃₀H₄₀O₁₄; and
- \circ Estrone-3-sulfate: C₁₈H₂₂O₅S.
- CHEMICAL STRUCTURES:

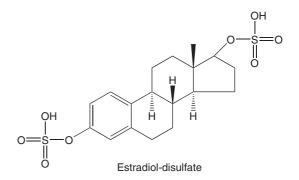






Estradiol-3,17-diglucuronide





Uses

Estrogens have been used for many therapeutic purposes, including as a contraceptive when in conjunction with a progestin. Naturally occurring estrogens, including those of equine origin (PremarinTM), are also employed in hormone replacement therapy to prevent osteoporosis, hot flushes, sweating, atrophic vaginitis and provide some protection against cardiovascular disease by increasing HDL and decreasing LDL cholesterol levels in blood. Estrogens have been used in the treatment of developmental delays or hypogonadism, as well as of some estrogen-dependent neoplasms in women, such as breast carcinomas, and prostate cancer in men in the past. An increasing number of cosmetic creams and preparations contain micronized natural estrogens. Certain types of estrogenic substance (e.g., mycoestrogens) have been used in animal feeds to increase muscle mass in livestock for greater meat production.

Background Information

In the early 1920s, the studies of Allen and the team of Long and Evans demonstrated that removal of ovaries from prepubertal rodents terminated development of the uterus, vagina, and mammary gland. Following this observation, the influence of administered estrogen on stratification and subsequent quantification of vaginal epithelium was developed as a highly sensitive measure of female sex hormone action. From conception and the earliest stages of embryonic development, through adolescence, to other stages of growth and aging, hormones play a vital role in human life. Estrogens are essential for differentiation, development, and functioning of many target organs in women; however, recent evidence indicates that female sex hormones are also important in male growth, particularly of bone and related tissues. Cardiovascular development and health are also influenced by estrogen in males and females.

At various stages of a woman's reproductive cycle, different types of estrogens are synthesized in distinct organ sites, beginning with the formation of estradiol-17 β in the ovary. However in pregnancy, the fetal-placental unit produces the less potent substance, estriol, as a major estrogen. After menopause, the androgens, androstenedione, and dehydroepiandrosterone, are synthesized by the adrenal glands, and serve as precursors that are converted to estrone in peripheral tissues by the action of the enzyme aromatase.

Throughout a woman's life, the naturally occurring estrogens are conjugated with either sulfate or glucuronide (see Structures), which increases their solubility for excretion in urine. These conjugates are weakly estrogenic. Interestingly, a preparation of similar compounds from pregnant mares' urine called Premarin[®] is used in the treatment of perimenopausal symptoms. Other naturally occurring estrogens in humans include the catechol estrogens discovered in the central nervous system. Although the concentrations of these highly active estrogenic substances are low in relation to those of the ovarian estrogens, they appear to play an important role in the evolvement of sexual behavior and possibly in the development of cancer.

Physiological Roles and Levels

Estrogens provoke their characteristic responses in hormone-target tissues such as breast and uterus by first associating with specialized intracellular proteins called estrogen receptors. These receptor molecules reside in nuclei and bind estrogen with high affinity and specificity. Upon activation, they associate with defined sequences called estrogen response elements located in the 5'-flanking regions of responsive genes and enhance transcription. The estrogen receptor protein is a cellular prerequisite for response to an estrogen or its mimic. If these receptor proteins are not expressed in a target cell or if their structures are severely altered due to gene mutations, female sex hormones will be unable to promote developmental responses required for normal reproductive processes. Two types of estrogen receptors, α and β , have been described.

Although many of the steps involved in the physiological roles of estrogen are well understood, current investigations are directed in diverse areas such as (1) the development of therapeutic estrogen mimics for management of osteoporosis, cardiovascular disease and cancer and (2) a greater understanding of the molecular basis by which certain environmental substances may disrupt normal hormone response mechanisms (so-called endocrine disruptor compounds).

Premenopausal, nonpregnant women produce 100–300 µg of estradiol-17 β and 100–200 µg of estrone per day, while postmenopausal women exhibit diurnal fluctuations in estradiol-17 β blood levels. Blood levels of estradiol-17 β in prepubescent females are $4-12 \text{ pg ml}^{-1}$ while those of women in the early follicular phase of the menstrual cycle range from 30 to 100 pg ml^{-1} , the late follicular phase the levels are $100-400 \text{ pg ml}^{-1}$ estradiol with concentrations of $50-150 \text{ pg ml}^{-1}$ luteal phase. Although postmenopausal women exhibit circulating levels of 5-18 pg ml^{-1} of estradiol, the principal circulating estrogen is estrone formed in peripheral adipose tissues. Prepubertal males have 2-8 pg ml⁻¹ circulating estradiol, while adult males exhibit blood levels of $10-60 \text{ pg ml}^{-1}$ estradiol.

The excretion profile of estrogens in menstruating women with normal cycles is reflected in either plasma estradiol- 17β or urinary levels. However, with clinical problems such as polycystic ovarian disease, the extraovarian production of estrogens is best evaluated using urine specimens.

Exposure Routes and Pathways

In most mammals, estrogens (female sex steroid hormones) are synthesized from cholesterol using the parent ring structure, cyclopentanoperhydrophenanthrene of the estrane series. The steroidogenic pathway includes the production of the androgenic precursors dehydroepiandrosterone and androstenedione, the latter of which is converted to testosterone, then to estradiol- 17β . This requires aromatization of these andogenic precursors by an aromatase enzyme complex. The major source of estrogen in postmenopausal women is the conversion of androstenedione to estrone by aromatase activity in adipose tissue, liver, and skin. The A-ring is aromatic and *cis-trans* isomerism is not possible at carbon 5 and carbon 10. Natural estrogens are produced in the ovaries, placenta, and in small amounts in the central nervous system of mammals, affecting the reproductive system and secondary sexual characteristics, as well as the health of bone and vascular systems.

Naturally occurring or synthetic estrogens are prescribed to many women for use as oral contraceptives or hormone replacement therapy. Oral contraceptives (birth-control pills) are used to prevent pregnancy. Estrogen and progestin are two female sex hormones. Combinations of estrogen and progestin work by preventing the release of eggs from the ovaries (ovulation) and changing the cervical mucus and the lining of the uterus. Oral contraceptives are a very effective method of birth control, but they do not prevent the spread of AIDS and other sexually transmitted diseases. Plant estrogens (phytoestrogens) are also ingested at relatively low levels with certain foods such as soy. Additional environmental estrogens (xenoestrogens) are found in various pesticides and herbicides, as well as industrial pollutants.

Toxicokinetics

Ovarian thecal cells are stimulated by LH to increase the conversion of cholesterol to androstenedione. The enzyme aromatase catalyzes the conversion of androstenedione to estrone and testosterone to estradiol-17 β , which is then released into the bloodstream where it is bound to the serum proteins, sex-hormone-binding globulin (SHBG, TeBG), and albumin. In cycling females, ovarian secretion rates of estrogens reach a peak in the late follicular phase. In the liver, estrogens are conjugated to sulfate or glucuronate to make them more water-soluble for urinary excretion.

Mechanism of Toxicity

In vivo, estrogen suppresses ovulation by feedback inhibition at the hypothalamus and pituitary. Additionally, estrogens and progestins induce cervical mucosa thickening altering the success of implantation. Abnormal levels of estrogens binding to the estrogen receptors can lead to an increased transcription of target genes, such as that for progestin receptor. This increase in target gene products can lead to symptoms associated with overexposure to estrogens (e.g., feminization). The distribution of estrogen receptors in organs throughout the body in the reproductive tract, neuroendocrine system, and visceral organs clearly suggest the broad possibilities for altering physiologic responses by hyper-estrogenization or administration/ consumption of estrogen mimics.

Toxicity (or Exposure)

Accumulating evidence indicates major roles for estrogens either in diminished or elevated levels in a wide variety of human diseases, such as cardiovascular disease, breast carcinoma, and osteoporosis. For every 10000 individuals, the average annual mortality of women ages 65-74 due to cardiovascular disease is 59, to breast carcinoma is 10, and to osteoporotic hip fractures is six. The risks associated with estrogen use include carcinoma of the uterus and breast, gallbladder disease, and abnormal blood clotting. Some of the side effects of overexposure to estrogens include nausea, vomiting, migraine headaches, and the exacerbation of endometriosis. Other clinical conditions associated with estrogen in balance include anovulation, hiroutism, and primary amenorrhea. In addition, estrogens and estrogen mimics can interfere with normal embryonic development.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods in 1997.

Relative binding affinities for the estrogen receptor vary considerably based on the source of the estrogen receptor. Generally, if the RBA for estradiol- 17β is set at 100, that of estrone is 15–60 and that of estroil is 0.2–30.

Environmental Fate

Both natural estrogens, their conjugates and those used as therapeutics result in their release into the wastewater supply due to it being excreted from the body in urine. Although most wastewater is highly filtered before it is released into the water supply, some estrogens and their mimics are not completely removed. Estrogenic compounds are also present in various pesticides and herbicides. Increased use of these chemicals and xenoestrogens can lead to an increased amount of estrogens present in the crops themselves as well as in the ground water supply.

Ecotoxicology

Reproductive and developmental abnormalities in certain wildlife and the occurrence of compounds which mimic hormones appear to be related. For example, between 1995 and 1997 more than half of the counties in Minnesota reported the presence of malformed frogs in sites with no prior history of mutations. Using a *Xenopus* (FETAX) assay and an evanescent field fluorometric biosensor, substances exhibiting estrogenic activity were detected in pond water samples from which frogs with malformations were collected. Furthermore, downstream of pulp paper mills, the sex ratio of certain fish was altered, presumably due to released agents. Although these compounds are unlikely to be of human origin, they appear to be exhibiting estrogen mimicry.

See also: Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals; Estrogens V: Xenoestrogens.

Further Reading

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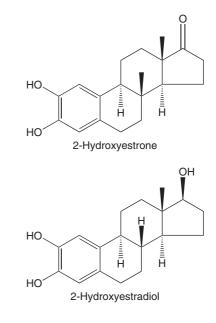
Estrogens II: Catechol Estrogens

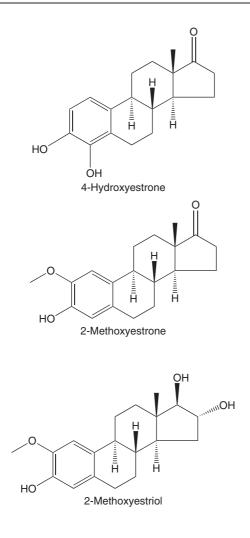
James L Wittliff, Sarah A Andres, and D Alan Kerr II

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- REPRESENTATIVE CHEMICALS: 2-Hydroxyestrone; 2-Hydroxyestradiol; 4-Hydroxyestrone; 2-Methoxyestrone; 2-Methoxyestriol
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 362-06-1 (2-Hydroxyestrone); CAS 362-05-0 (2-Hydroxyestradiol); CAS 3131-23-5 (4-Hydroxyestrone); CAS 362-08-3 (2-Methoxyestrone); CAS 1236-72-2 (2-Methoxyestriol)
- SYNONYMS:
 - 2-Hydroxyestrone: 1,3,5(10)-estratrien-2,3diol-17-one
 - \circ 2-Hydroxyestradiol: 1,3,5(10)-estratriene-2,3, 17 β -triol
 - 4-Hydroxyestrone: 1,3,5(10)-estratrien-3,4diol-17-one
 - 2-Methoxyestrone: 1,3,5(10)-estratrien-2,3diol-17-one 2-methyl ether
 - 2-Methoxyestriol: 1,3,5(10)-estratrien-3,16 α , 17 β -triol 2-methyl ether
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Steroid hormones

- CHEMICAL FORMULAS:
 - \circ 2-Hydroxyestrone: C₁₈H₂₂O₃
 - 2-Hydroxyestradiol: C₁₈H₂₄O₃
 - \circ 4-Hydroxyestrone: C₁₈H₂₂O₃
 - \circ 2-Methoxyestrone: C₁₉H₂₄O₃
 - \circ 2-Methoxyestriol: C₁₉H₂₆O₄
- CHEMICAL STRUCTURES:





Uses

Although the majority of studies on catechol estrogens have focused on their activities *in vivo*, the therapeutic potential of the methoxyestrogens have been explored with preliminary results suggesting an antiproliferative effect that appears to involve disruption of microtubule function, induction of apoptosis and inhibition of angiogenesis. Catechol estrogens act as potent inhibitors of the methylation of catecholamines apparently by competitive binding to catechol-O-methyltransferase (COMT). Furthermore, 2- and 4-hydroxy catechol estrogens are readily O-methylated by COMT. It is suggested that catechol estrogens influence the secretion of luteinizing hormone (LH) and prolactin in rodents and man, as well as sexual behavior in lower mammals.

Background Information

Existence of catechol estrogens was postulated as early as the late 1930s from observations such as that made by Dingemanse and Laqueur who reported the rapid disappearance of estrogen biologic activity *in vivo*. In 1940, Westerfield suggested this resulted from the hydroxylation of the aromatic A ring of the estrogen cyclopentanoperhydrophenanthrene nucleus which formed an O-dihydroxyphenol (a catechol). Catechol estrogens are derivatives of the parent estrane series. Chemical synthesis was completed in the 1950s allowing the first studies of catechol pharmacology.

Catechol estrogens are formed from the naturally occurring estrogens (estradiol- 17β and estrone) in the central nervous system (e.g., hypothalamus and cerebral cortex) and the anterior pituitary and to a lesser extent in other organs. 17β -Oxidoreductase and cytochrome P450 (CYP1A1 and CYP1B1) are enzymes that convert estrogens to catechol metabolites.

Exposure Routes and Pathways

Various catechol estrogens are secreted in high amounts in urine due to formation in measurable quantities in different organs, such as the adrenal, heart, hypothalamus, kidney, liver, pituitary gland, placenta, prostate, and testes, implying the presence of both 2-hydroxylase and 4-hydroxylase. Estrone is metabolized by two alternative pathways of which the 2-hydroxylation pathway leads to the formation of the catechol estrogens, primarily 2-hydroxyestradiol, 2-hydroxyestrone, 2-hydroxyestriol, and their corresponding methoxy derivatives. The 16a-hydroxylation pathway leads predominantly to estriol. Direction of estradiol-17 β metabolism is dependent upon the pathophysiologic state of the individual. Although catechol estrogens may play an antiestrogen role under some circumstances, they evoke a variety of pharmacologic activities.

Physiological Roles and Levels

Because catechol estrogens are highly unstable in solution, being easily decomposed by oxidation especially under alkaline conditions, accurate measurements in sera, urine and tissue extracts have been difficult. In the second half of human pregnancy, concentrations of 2-hydroxyestrone range from 110 to 2100 μ g per 24 h, those of 2-hydroxyestradiol are in the range 20–180 μ g per 24 h, and those of 2-hydroxyestriol are in the range 35–240 μ g per 24 h. Levels of catechol estrogens in post-menopausal women, children, and men are below that required for conventional assays and more sensitive procedures such as a double isotope derivative method based on enzymatic methylation of the catechol estrogen must be employed.

Although the half-lives of catechol estrogens in sera appear to be too short (less than a minute) for consideration as circulating hormones, it appears that they are locally active in certain tissues. It is known that they exhibit a potent influence on gonadatropins in that they induce ovulation and an LH surge in immature rats. Neurophysiologic data suggest that 2- and 4-hydroxylated estrogens also play an important role in the activation of lordosis behavior in rats.

Binding Affinities

Although a wide variety of tissues contain estrogen receptors, it has been proposed that there are neuronal cell receptors that bind catechol estrogens, allowing them to function as neurotransmitters. Affinities of catechol estrogens are highly dependent on the source of the receptor proteins. Relative binding affinities (RBAs) of catechol estrogens compared to that of estradiol-17 β (RBA of 100) were 100–150 for 2-hydroxyestradiol and 4-hydroxyestradiol, and 0.1-0.7 for 4-methoxyestradiol, 2-methoxyestrone and 2-methoxyestradiol using human estrogen receptor. Using rat uterine estrogen receptors, 4-hydroxyestradiol had an RBA of 45, 2-hydroxyestradiol of 24, 4-hydroxyestrone of 11, and 2-hydroxyestrone of 2, while 4-methoxyestradiol and 2-methoxyestrone and 2-methoxyestradiol were less than 0.1-1.

There is growing evidence that catechol estrogens bind to membrane-associated receptors in responsive tissues. Investigations suggest that catechol estrogens may exhibit competitive inhibition of catecholaminergic agonists and antagonists binding to dopaminergic and noradrenergic receptors in brain tissues. Other studies suggest catechol estrogens react directly with specific estrogen receptors. There is also evidence that catechol estrogens associate with sexhormone binding globulin (SHBG, TeBG) with relative binding affinities of 200 for 2-hydroxyestradiol, 75 for 2-hydroxyestrone, and 1 for 2-hydroxyestradiol compared to testosterone.

Toxicokinetics

Catechol estrogens are metabolized by three principal reversible reactions including methylation and demethylation, catalyzed by the enzyme COMT, and conjugation with either glucuronic or sulfuric acid (see structures). Two irreversible reactions involved in metabolism to products with diminished activities, include additional hydroxylations leading to tetrahydroxylated, highly water-soluble compounds and the irreversible binding to amino acids and proteins, including the formation of thioesters.

Mechanism of Toxicity

Carcinogenesis induced by estrogens has been proposed to be mediated by activated catechol metabolites, which are known to react covalently with protein and nucleic acids. Although the reactive 16α-hydroxy, 2-hydroxy and 4-hydroxy estrogens are inactivated by COMT to species that are excreted, these compounds can also be oxidized to semiguinone and o-metabolites, in particular estrogen-3,4-quinone which depurinate DNA. Studies have now shown that the apurinic region of the DNA sequence is often left unchanged by DNA repair enzymes. Mutagenic potential of both oxidative activities and estrogen DNA adducts have been implied. Results such as these suggest the complexity of the mechanism of estrogen carcinogenesis and assessing the risks of estrogen therapy.

Toxicity (or Exposure)

The phenolic A-ring and the oxygen function at C-17 are essential for biological activity and substitutions at other positions in the molecule reduce their estrogenicity (feminizing potency). Thus, 2-methoxyestrone and 2-methoxyestradiol exhibit verv little activity. Actions of catechol estrogens include (1) 2-hydroxyestradiol appears to interact with dopamine receptor, (2) interference of catecholamine synthesis at high local concentrations of the compounds, (3) inhibition of catecholamine degradation via competitive inhibition of COMT, and (4) ability to bind to estrogen receptors in brain and act either as an agonist or antagonist depending upon the pathway.

Chronic alcoholism is associated with alterations with sex-steroid hormone metabolism and clearance. Symptoms of hyperestrogenism in males include gynecomastia, palmar erythema, and spider angiomata. Furthermore, catechol estrogen metabolites are genotoxic in that they appear to cause DNA damage. Metabolites of equine estrogens, such as those administered in therapeutics such as PremarinTM, have been implicated in breast cancer development by associating with the estrogen receptor, which stimulates cell proliferation and gene expression.

In Vitro Toxicity Data

Catechol estrogens are potent inhibitors of tyrosine kinase activity *in vitro*. High concentrations of catechol estrogens are necessary to cause measurable inhibition, but the physiologic role of this interaction remains uncertain. Catechol estrogens have also been shown to be potent inhibitors of the COMT-mediated inactivation of catecholamines.

Environmental Fate

Because of the chemical instability of catechol estrogens, it is unlikely they exist in an active form after excretion. However, this has not been evaluated conclusively.

See also: Estrogens I: Estrogens and Their Conjugates; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals; Estrogens V: Xenoestrogens.

Further Reading

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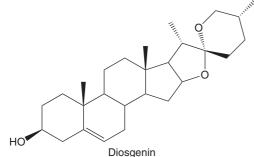
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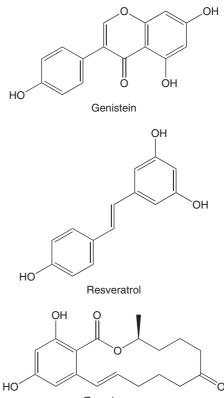
Estrogens III: Phytoestrogens and Mycoestrogens

James L Wittliff, Sarah A Andres, and D Alan Kerr II

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- REPRESENTATIVE CHEMICALS: Diosgenin; Genistein; Resveratrol; Zearalenone
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Diosgenin (CAS 512-04-9); Genistein (CAS 446-72-0); Resveratrol (CAS 501-36-0); Zearalenone (CAS 17924-92-4)
- SYNONYMS:
 - Diosgenin: (25*R*)-Spirost-5-en-3beta-ol
 - Genistein: 4',5,7-Trihydroxyisoflavone
 - Resveratrol: *t*-3,4′,5-Trihydroxystilbene
 - Zearalenone: (E)-3,4,5,6,7,8,9,10-Octahydro-14,16-dihydroxy-3-methyl-7-oxo-1*H*-2-benzoxacyclotetradecin-1-one
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Coumestans; Dihydroxychalcones; Isoflavones and Resorcylic Acid Lactones
- CHEMICAL FORMULAS: Diosgenin, C₂₇H₄₂O₃; Genistein, C₁₅H₁₀O₅; Resveratrol, C₁₄H₁₂O₃; Zearalenone: C₁₈H₂₂O₅
- CHEMICAL STRUCTURES:





Zearalenone

Uses

In general, xenoestrogens in the environment are separated into those occurring naturally (produced by either plants or fungi) and those produced commercially (e.g., insecticides, herbicides, estrogen-like therapeutics, and industrial by-products). Phytoestrogens and mycoestrogens are found in various plants, such as soy (genistein), yams (diosgenin) and grapes (resveratrol), and in fungus species such as Fusarium molds (zearalenone). The compounds associated with these classes of molecules bind to estrogen receptor proteins with a variety of affinities, sufficient to label them as estrogen mimics. Various compounds are present in common foodstuffs and are ingested by humans and animals on a frequent basis. Preliminary epidemiological studies suggest protective effects of certain isoflavones and to a smaller degree as a result of consuming foodstuffs containing lignans (i.e., cereals, flaxseed, and fruits) in altering the development of osteoporosis, cardiovascular disease, and certain cancers. These investigations, as well as traditional and alternative medicine practices, have elevated the interest in using dietary supplements containing these extracts or the purified compounds as part of a healthy diet and for problems associated with menopause, prevention of bone loss, and cardiovascular disease. Furthermore, certain mycoestrogens (e.g., zearalenone) and chemical derivatives have been used as additives to livestock feeds to increase muscle mass for greater meat production.

Background Information

For decades, the consumption of soybean-derived products by Asians living in China, Japan, and Korea has been thought to be related to their lower incidence of certain cancers and coronary artery disease compared to that of western populations. Observations such as the appearance of higher plasma and urine concentrations of isoflavanoids of individuals eating a largely vegetarian diet compared to that of omnivores suggested a role for phytoestrogens in lowering disease incidence, but the contributions of red meat and fat consumption were unclear in these types of comparisons.

More than 20 different compounds have been identified as either phytoestrogens or mycoestrogens in at least 300 unique plant and fungus species, based on their abilities to either bind to estrogen receptors and/or stimulate estrogen-like activities in cell cultures or *in vivo*. There are numerous chemical classes of phytoestrogens, including coumestans, dihydroxychalcones, isoflavones, prenylated flavonoids, stilbenes, and resorcylic acid lactones. Representative examples are coumestrol for coumestans, phloretin for dihydroxychalcones, genistein for isoflavones, 8-prenylnaringenin for prenylated flavonoids, resveratrol for the stilbenes, and zearalenone for resorcylic acid lactones. A list of representative chemicals is shown below with source and relative binding affinities for the estrogen receptors:

Phytoestrogens and mycoestrogens with suspected endocrine-associated effects

Compound	Common source	^a RBA for estrogen receptor
Artemisinin	Wormwood	
Biochanin A	Soy	0.004
Coumestrol	Red clover and alfalfa	0.7–5
Curcumin	Turmeric	
Daidzein	Soy	0.02–1
Diosgenin	Yams	
Diosmin		
Formononetin	Soy	NA
Genistein	Soy	0.5–45
Glycitein	Soy	0.001
Naringenin	Grapefruit and citrus	0.01–0.2
Phloretin		0.07–0.7
Prunetin	Soy	0.0001-0.002
Resveratrol	Muscadine grapes	0.001-0.05
Sarsasapogenin	Sarsaparilla root	
Zearalanone	Fusarium molds	2–15
Zearalenone	Fusarium molds	5–18
α -Zearalanol	Fusarium molds	25–36
α -Zearalenol	Fusarium molds	36–70
β -Sitosterol	Soy	NA
β -Zearalanol	Fusarium molds	0.6–16
β -Zearalenol	Fusarium molds	0.2–23

^aRBA, relative binding affinity (% of estradiol-17β activity). Refer to ICCVAM report for most RBAs (see report for various estrogen receptor sources).

Exposure Routes and Pathways

Human and animal exposure to phytoestrogens usually occurs by two routes, ingestion of food products containing the agents or as over-the-counter nutritional supplements. However, a number of cosmetic preparations contain certain of these natural estrogens which may be absorbed through the skin. Medical problems associated with administration of hormone replacement therapy, as described in the report of the Women's Health Initiative, have motivated many women to seek nonmedical means of post-menopausal endocrine replacement, such as phytoestrogen supplements.

Most exposures to mycoestrogens result from consumption of food products that have been contaminated with *Fusarium* molds. Studies of the activities of phytoestrogens clearly indicate that these compounds bind to the estrogen receptor proteins displacing native estradiol- 17β with variable affinities. Details of the signaling transduction pathways are poorly understood, but appear to involve alterations in target gene expression.

Toxicokinetics

It is generally accepted that the assessment of actual risks or benefits of phytoestrogens and mycoestrogens

to humans and animals is controversial. The complexity of the estrogen signaling network, involving multiple forms of the receptor proteins and the cross talk with the other pathways (e.g., growth factor-induced), limits our current understanding regarding the mechanisms by which these estrogen mimics act as agonists or antagonists. Furthermore, the biological role for the presence of phytoestrogens in plants or mycoestrogens in fungi is unknown. Although many of these compounds are metabolized in the mammalian gut by enzymes such as β -glucosidases, significant quantities appear in urine and feces. For example, lignans may be metabolized to enterodiol and enterolactone while isoflavones may be metabolized to equol and O-desmethylangolensin by intestinal microflora prior to urinary excretion.

Mechanism of Toxicity

Ingestion of phytoestrogens and mycoestrogens may result in alterations in physiological processes controlled by estrogens, since these types of compounds either mimic or compete with natural estrogens for the estrogen receptors with relatively low affinity. While these structurally diverse compounds collectively exhibit estrogen mimicry, they also share common properties such as retention in adipose tissues due to their lipophilicity and ability to cross the placental barrier. These properties, particularly estrogen receptor association, may lead to an up-regulation or down-regulation of expression of target genes (e.g., progestin receptor, prolactin receptor) containing an estrogen response element (ERE) sequence. Since estrogens are essential for normal mammalian differentiation, development and function, primarily in females of the species, interference with or enhancement of related pathways may adversely affect or enhance the health of the host and offspring. For example, some phytoestrogens at concentrations consumed in the American-style diet appear to be associated with a reduced risk of endometrial carcinoma.

Certain phytoestrogens have also been suggested to inhibit enzymes involved in estrogen biosynthesis and metabolism, as well as thyroid biosynthesis. Preliminary studies suggest they inhibit protein kinases and topoisomerases, as well as influence the cell cycle and subsequent proliferation, differentiation and apoptotic pathways. Interest in phytoestrogens, such as isoflavones, has increased since they are reported to exhibit some nonhormonal effects, such as antioxidation.

Chronic Toxicity (or Exposure)

Livestock ingestion of large quantities of phytoestrogens in certain clovers, alfalfas, and in moldy grains with infants fed soy-based formulas. Studies on populations that traditionally consume diets rich in phytoestrogens (i.e., Japanese and Chinese) suggest that they may have a beneficial effect regarding the prevention and development of osteoporosis, cardiovascular disease, and some cancers.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in 1997.

In summary, the body of experimental and epidemiological evidence suggesting phytoestrogens may alter human health continues to expand to cover a wide range of exposures. Effects of transgenerational exposure to unrecognized agents that may be present in foodstuffs, drinking water, and other consumables including medications and cosmetics are of particular concern. Using hormone receptor-based technologies (i.e., recombinant human estrogen receptors) and highly purified preparations of these compounds as standards, exposure and risk assessment may be improved for environmental estrogen mimics, and the quantitative analysis of their occurrence in the environment.

Environmental Fate

Similar to the routes of release of mammalian estrogens, ingested phytoestrogens and mycoestrogens may appear in the environment as a result of intestinal metabolism and excretion in urine and feces. In addition decomposition of botanicals containing these compounds may be released in soil and water. Little is known regarding their biotransformation by soil and water organisms. It is generally accepted that the concentrations of these compounds existing free in the environment pose few health concerns.

Exposure Standards and Guidelines

Dietary supplements containing various combinations of phytoestrogens are neither controlled by federal agencies such as the Food and Drug Administration nor have exposure standards and guidelines been established. This is due to the paucity of data regarding their clinical safety and efficacy. See also: Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens IV: Estrogen-Like Pharmaceuticals.

Further Reading

- Blair R, *et al.* (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. *Toxicological Sciences* 54: 138–153.
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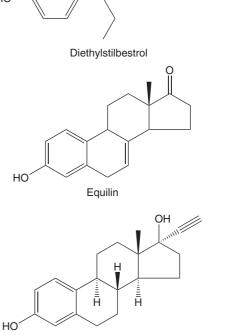
http://iccvam.niehs.nih.gov – ICCVAM/NICEATM Final Report, Expert Panel Evaluation of the Validation Status of *In Vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays, 2002.

Estrogens IV: Estrogen-Like Pharmaceuticals

James L Wittliff, D Alan Kerr II, and Sarah A Andres

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- \circ Ethynyl estradiol: C₂₀H₂₄O₂
- \circ Raloxifene: C₂₈H₂₇NO₄S
- \circ Tamoxifen: C₂₆H₂₉NO
- CHEMICAL STRUCTURES:
- REPRESENTATIVE CHEMICALS: Diethylstilbestrol; Equilin; Ethynyl estradiol; Raloxifene; Tamoxifen
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Diethylstilbestrol (CAS 56-53-1); Equilin (CAS 474-86-2); Ethynyl estradiol (CAS 57-63-6); Raloxifene (CAS 84449-90-1); Tamoxifen (CAS 10540-29-1)
- SYNONYMS:
 - Diethylstilbestrol: 3,4-Bis(4-hydroxyphenyl) hex-3-ene
 - Equilin: 1,3,5(10),7-Estratetraen-3-ol-17-one
 - \circ Ethynyl estradiol: 17α-Ethynyl-1,3,5(10)-estratriene-3,17β-diol
 - Raloxifene: 6-Hydroxy-2-(p-hydroxyphenyl)benzo[b]thien-3-yl-p-(2-piperidinoethoxy)phenyl ketone hydrochloride, EvistaTM
 - Tamoxifen: (Z)-2-[4-(1,2-Diphenyl-1-butenyl) phenoxy]-N,N-dimethylethanamine, NolvadexTM
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Steroid hormones
- CHEMICAL FORMULAS:
 - \circ Diethylstilbestrol: C₁₈H₂₀O₂
 - \circ Equilin: C₁₈H₂₀O₂



OН

Ethynyl estradiol

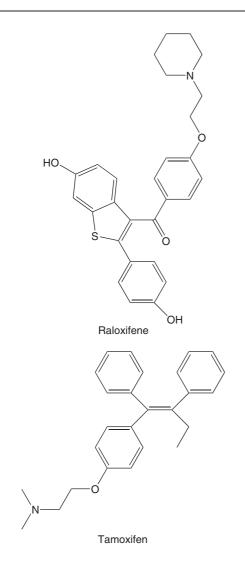


Table 1	Common estrogen-like pharmaceuticals
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Uses

Estrogen-like pharmaceuticals have a variety of clinical uses including as fertility therapeutics (clomiphene), contraceptives (ethynyl estradiol), prevention and treatment of osteoporosis (equilin, Raloxifene) and cancer therapeutics (Tamoxifen). These pharmaceuticals have a variety of affinities for estrogen receptor proteins α and β , and may either activate (agonist) or inactivate (antagonist) estrogen receptorinduced signaling pathways. Representative examples of these pharmaceutics with their relative binding affinities (RBAs) for estrogen receptors are listed in **Table 1**; however, the RBA values are highly dependent upon the source of the receptor protein used in the *in vitro* binding assay.

Background Information

Oral contraceptives have been used widely by women since the 1960s and most contraceptive pills contain various ratios of estrogenic and progestomimetic substances. Hormone replacement therapy (HRT) has evolved considerably in the composition and doses utilized in the treatment of postmenopausal women, during the past four decades. While pharmaceutical compounds exhibiting estrogen mimicry are structurally diverse, they share common properties such as their retention in body fat deposits, their ability to cross the placental barrier, their transport in blood usually bound to serum proteins, and their affinity for the estrogen receptor protein.

Compound	Clinical use	*RBA for ER		
Clomiphene	Fertility therapeutic	**0.1-12		
Diethylstilbestrol	Sustaining pregnancy (discontinued) and cancer therapeutic (discontinued)	400		
Droloxifene	Cancer therapeutic	**0.2-15.2		
Ethynyl estradiol	Oral contraceptive and reproductive medicine therapy	100-200		
Equilenin	Osteoporosis prevention and therapeutic (HRT)	**8		
Equilin	Osteoporosis prevention and therapeutic (HRT)	**24		
17α-Dihydroequilenin	Osteoporosis prevention and therapeutic (HRT)			
17β-Dihydroequilenin	Osteoporosis prevention and therapeutic (HRT)			
ICI 164,384	Cancer therapeutic	14.5		
ICI 182,780	Cancer therapeutic	37.5		
Idoxifene	Cancer therapeutic			
Nafoxidine	Oral contraceptive and cancer therapeutic	0.7		
Norethynodrel	Oral contraceptive	0.2		
Raloxifene	Osteoporosis prevention and therapeutic (postmenopausal women) and cancer **16–69 therapeutic (under investigation)			
Tamoxifen	Cancer therapeutic and cancer chemoprevention	0.06-16		
Toremifene	Cancer therapeutic and HRT (under investigation)	1.4		

Refer to Blair *et al.* and Fang *et al.* (see the Further Reading section) for RBAs (using rat uterine estrogen receptor); and to the ICCVAM report for RBAs listed with ** (see report for various estrogen receptor sources).

*RBA, relative binding affinity (% of estradiol activity); ER, estrogen receptor.

When they associate with estrogen receptor proteins, they either disrupt native hormone action or communicate activities similar to those of estrogen.

Exposure Routes and Pathways

Estrogen therapeutics, prescribed to patients for cancer treatment, menopausal symptom relief and osteoporosis, are ingested orally. Most estrogen-like substances used in therapy are absorbed easily through the gastrointestinal tract, mucous membranes, and the skin.

Toxicokinetics

Overdoses of either contraceptives or hormone replacement therapeutics are uncommon. A variety of dermatologic effects have been observed including photosensitivity, alopecia, and bullous eruption following oral contraceptive overdoses. Neurologic effects of estrogen-containing contraceptives in the presence of a progestin include increased risk of ischemic stroke in generally healthy postmenopausal and occasional exacerbation of migraine headaches. Well-documented serious hematologic effects include increased risk for venous thromboembolism. The primary mode of clinical management is essentially symptomatic and supportive. The primary concern is the ingestion of large doses of estrogen-like pharmaceuticals by children, although few acutely serious ill effects have been reported.

HRT, which reaches a peak level in 4–5 h after oral absorption, is strongly bound to serum proteins. As certain estrogen-like pharmaceuticals enter breast milk, breast-feeding is not recommended and the use of HRT in pregnancy is also not recommended.

Because of the wide chemical diversity of estrogenic pharmaceutics, it is difficult to categorize the pathways for metabolic conversion and excretion. However, the majority of these reactions occur in the liver where they are inactivated by various hydroxylation and oxidation reactions, although the major pathways involve conjugation and excretion in the urine and feces as sulfates and glucuronates.

Mechanism of Toxicity

These compounds have relatively high affinities for estrogen receptors, which can lead to altered regulation of estrogen-responsive genes, thereby altering the proliferation and differentiation of cells in target organs. Detailed studies of their binding specificity for estrogen receptors alpha and beta suggest alternative mechanisms in different tissues.

Toxicity (or Exposure)

Animal

In both rats and mice, the possible toxic side effects of ethynyl estradiol include convulsions/seizure activity, ataxia, and changes in kidney and bladder function. The oral lethal dose is 950 mg kg^{-1} for mice and 960 mg kg^{-1} for rats. The intraperitoneal lethal dose is 250 mg kg^{-1} for mice and 471 mg kg^{-1} for rats.

Human

Since estrogen-like pharmaceuticals exhibit potent activities, many of which are related to naturally occurring estrogens (agonistic), they also exhibit antagonistic activities on a variety of physiologic pathways. Their ability to recognize a diverse group of compounds represents another example of ligand binding promiscuity by the human estrogen receptor proteins. Risks associated with these compounds include endometrial carcinoma, breast carcinoma, gall bladder disease and abnormal blood clotting, as well as a variety of others more specifically related to the agent. Because of the structural diversity of these compounds and their pharmacology, representative examples are described.

Hexestrol and diethylstilbestrol (DES) are synthetic, nonsteroidal estrogens derived from stilbene, which have been used earlier in the treatment of breast cancer in women and prostate carcinoma in men. Furthermore, DES was used in the 1950s and 1960s as treatment of pregnant women who threatened premature delivery. However, a serious medical complication arose in the progeny of these mothers in that daughters were at high risk of developing clear cell adenocarcinoma of the vagina, as well as cervical and uterine deformities. Male offspring of DES-treated mothers also developed genital tract abnormalities. Surprisingly, there appears to be an increase in the incidence of hypospadias in grandsons of DES-treated women. Clinical symptoms of DES administration include arterial and venous thrombosis, fluid retention and nausea in women, as well as gynecomastia and impotence in men.

Currently, ethynyl estradiol represents the most common estrogenic component in combination oral contraceptives (i.e., an estrogen and a progestin). Ethynyl estradiol, a derivative of estradiol-17 β with the substitution of the ethynyl group at C-17, is one of the most potent analogs with estrogenic activity equal to or greater than that of the parent compound. Similar to other compounds in this class, ethynyl estradiol is orally activity being rapidly absorbed by the gut mucosa and liver. Detoxification involves hydroxylation and conjugation to form the glucuronate or sulfate forms prior to excretion in the urine or feces.

Ethynyl estradiol can cause nausea or vomiting, body temperature increase and other menopausal symptoms, and blood clotting. The lowest published toxic dose is 21 mg kg^{-1} (21 day)⁻¹ intermittent. Raloxifene (EvistaTM) is another example of a

Raloxifene (EvistaTM) is another example of a selective estrogen receptor modulator (SERM) that belongs to the triphenylethyene-based group of therapeutics. Its primary pharmacologic activity is as an agonist used in the prevention and treatment of osteoporosis with complementary activities on the liver and serum lipid profiles. The SERM increases bone mineral density, decreases total and low-density lipoprotein (LDL) cholesterol while acting as an estrogen antagonist in uterine and breast tissue via interaction with the estrogen receptor. Raloxifene is primarily absorbed upon oral administration and rapidly metabolized by conjugation with glucuronic acid prior to excretion in the urine and feces.

Raloxifene side effects include bloody or cloudy urine; painful urination; pain in chest, arm or leg (rare); coughing blood (rare); sore/dry throat; trouble in swallowing; body aches; cramping; skin rash; vaginal itching; migraine headache (rare) and loss of speech, vision or coordination (rare). There is also an increased risk of blood clot formation (i.e., deep vein thrombosis, pulmonary embolism, and retinal embolism) in patients with a history of these conditions if treated with Raloxifene.

A recent major clinical trial, published by the National Institutes of Health Women's Health Initiative, was terminated in 2002 because an increased risk of invasive breast cancer was observed from HRT with estrogen and progestin. Furthermore, an increased risk of ischemic stroke was noted. Women participating in this study took either a placebo or a combination hormone therapy that contained conjugated equine estrogens and medroxyprogesterone acetate, most commonly prescribed as PremarinTM or PremproTM. These pharmaceutics contain estrogens extracted from pregnant mare's urine, of which almost one-half are equilin and equilenin. The increased risk of breast cancer was first observed after 4 years of HRT; and after 5 years, the risk of breast cancer showed a 26% increase. Although early results suggested a protective effect of HRT in lowering risk of coronary artery disease, later, more thorough studies have revealed an increased risk of heart disease, stroke, and venous thromboembolism. As a result of the Women's Health Initiative, HRT with a conjugated estrogen alone or an estrogen/progestin combination, previously considered first-line therapy for osteoporosis, is now considered a second-line agent, which should be used only

when benefits outweigh risks. In general, acute and chronic toxicity of HRT is uncommon.

Tamoxifen, prescribed clinically as NolvadexTM, is an estrogen receptor antagonist used in the treatment of estrogen receptor-positive breast cancer, and to a lesser extent in other cancers of the female reproductive system. A therapeutic dose of Tamoxifen is in the range of $0.5-0.6 \text{ mg l}^{-1}$, although the concentration associated with acute toxicity is unknown. Historic studies from the National Surgical Adjuvant Breast & Bowel Project established Tamoxifen as a chemopreventive therapy for breast cancer in women at high risk for the carcinoma. One of the significant side effects of Tamoxifen therapy is an increased risk of endometrial carcinoma.

Tamoxifen side effects include headaches, depression, stomach irritation, vaginal discharge, constipation, shortness of breathe (rare), loss of vision (rare), and swelling of the hands and lower legs.

Clomiphene citrate (ClomidTM or SeropheneTM) is a fertility drug used in the treatment of ovulatory failure in women desiring pregnancy. Clomiphene, a nonsteroidal antiestrogen, is a triphenylethylenederived drug related to Tamoxifen and Raloxifene. Representative clinical doses of 50 mg day^{-1} for 5 days (day 3-day 7 of the cycle), administered orally, are used to induce ovulation by acting upon the hypothalamus. Women with either abnormal or irregular uterine bleeding should not be treated with Clomiphene unless the absence of endometrial or cervical abnormalities has been confirmed. Furthermore, women with either liver disease or ovarian cysts should not be given this therapeutic. Although there is a paucity of information regarding fetal damage, estrogen-like pharmaceuticals should not be administered to women who may be pregnant.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in 1997. RBAs for the estrogen receptor vary considerably based on the source of the estrogen receptor proteins and isoforms (α or β). Generally if the RBA for estradiol-17 β is set at 100, that of estrone is 15–60 and that of estriol is 0.2–30.

Clinical Management

Fluid replacement may be necessary after nausea/ vomiting. There are few published results indicating benefit of gastric lavage, in the case of a massive overdosage. Therapy to enhance elimination will most likely not be effective due to the tissue distribution of these compounds.

Environmental Fate

Wastewater from pharmaceutical industries producing these therapeutics may allow introduction of these endocrine disruptors into the ecosystem if proper filtration is not employed. Moreover, the conjugated products excreted in urine and feces from individuals receiving these pharmaceuticals also can be introduced into the wastewater supply. There are few published results regarding bioaccumulation and biotransformation of estrogen-like pharmaceutics released into the ecosystem.

Exposure Standards and Guidelines

For clinical use, $20-40 \text{ mg day}^{-1}$ of Tamoxifen is a safe range for patients, as is 60 mg day^{-1} Raloxifene. Skin exposure is not dangerous, though some estrogens can be absorbed through the skin. When handling these chemicals in powder form, gloves, eyewear, and facemasks should be worn to avoid contact.

See also: Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens V: Xenoestrogens.

Further Reading

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http://www.whi.org - Women's Health Initiative.

Estrogens V: Xenoestrogens

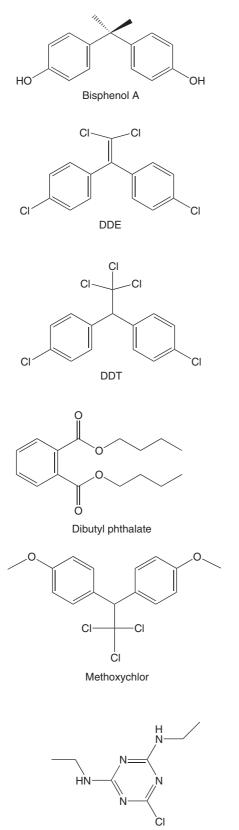
James L Wittliff, D Alan Kerr II, and Sarah A Andres

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- REPRESENTATIVE CHEMICALS: Bisphenol A; DDE; DDT; Dibutyl phthalate; Methoxychlor; Simazine
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Bisphenol A (CAS 80-05-7); DDE (CAS 72-55-9); DDT (CAS 50-29-3); Dibutyl phthalate (CAS 84-74-2); Methoxychlor (CAS 72-43-5); Simazine (CAS 122-34-9)
- SYNONYMS:
 - Bisphenol A: Bis(4-hydroxyphenyl)propane
 - DDE: 2,2-Bis(4-chlorophenyl)-1,1-dichloro ethylene

- DDT: 1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane
- Dibutyl phthalate: 1,2-Benzenedicarboxylic acid dibutyl ester
- Methoxychlor: 2,2,2-Trichloro-1,1-bis(4-methoxyphenyl)ethane
- Simazine: 1-Chloro-3,5-bisethylamino-2,4,6triazine
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Steroid hormones
- CHEMICAL FORMULAS:
 - \circ Bisphenol A: C₁₅H₁₆O₂
 - \circ DDE: C₁₄H₈Cl₄
 - \circ DDT: C₁₄H₉Cl₅
 - Dibutyl phthalate: C₁₆H₂₂O₄
 - Methoxychlor: $C_{16}H_{15}Cl_3O_2$
 - Simazine: C₇H₁₂ClN₅

• CHEMICAL STRUCTURES:





Uses

Xenoestrogens, exhibiting a wide molecular diversity, are found in a number of cosmetic products, such as plasticizers, perfume fixatives, and solvents (e.g., dibutyl phthalate), industrial chemicals and pollutants such as insecticides (e.g., methoxychlor, DDT, and DDE), epoxy resins, and polycarbonate (e.g., bisphenol A), and herbicides (e.g., simazine). This group of chemicals has been classified as environmental endocrine disruptor compounds (EDCs), defined as exogenous agents that interfere with the synthesis, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior. A list of representative chemicals is shown in Table 1 based on commercial usage.

Background Information

While those compounds exhibiting estrogen mimicry are structurally diverse, they share common properties such as retention in body fat deposits (highly lipophilic), ability to cross the placental barrier, transport in blood usually unbound to specialized serum proteins (e.g., steroid hormone binding globulin, SHBG/TeBG), and their affinity for the estrogen receptor protein. If the environmental compound impersonates estrogen sufficiently, it associates with the estrogen receptor protein and either disrupts the action of the native hormone or communicates activities similar to estrogen (i.e., antagonistic or agonistic activities). In addition to the phenotypic expression of gender, estrogens and their mimics may influence development and physiological processes in many organs of the body, particularly the reproductive tract, as well as the central nervous system and skeleton. It is obvious that fragile, biological events occurring during ovulation, pregnancy, fetal development, and lactation could easily be influenced by EDCs, which mimic naturally occurring hormones.

With a variety of sensitive, rapid assays, EDCs now may be recognized by estrogen receptor proteins. The range of techniques available includes:

- 1. cell-free preparations of receptor proteins using both ligand titration and ligand competition assays;
- 2. antibody-based assays in enzyme immunoassay (EIA) or ELISA formats;
- 3. electrophoretic mobility shift assays, conducted in the presence (supershift) and absence of monoclonal antibodies to estrogen receptors; and

Table 1	Examples	of environmental	chemicals	with	suspected
endocrine-disrupting effects					

endocrine-disrupting effects	
Enviromental Chemical	RBA for ER*
Pesticides Herbicides 2,4-D 2,4,5-T Alachlor Amitrole Atrazine Metribuzin	N/A 0.0003**
Nitrofen Simazine Trifluralin	N/A
Fungicides Benomyl Hexachlorobenzene Mancozeb Maneb Metiram-complex Tributyl tin Zineb Ziram	
Insecticides β-HCH Carbaryl Chlordane	N/A N/A
Dicofol Dieldrin DDT and metabolites ∞-Endosulfan Heptachlor and H-epoxide Lindane (γ-HCH) Methamul	0.0005** 0.001 0.012** N/A
Methomyl Methoxychlor Mirex	0.01–0.1 (0.001 – Blair)** N/A
Oxychlordane Parathion Synthetic pyrethroids Toxaphene Transnonachlor	0.00032**
Nematocides Aldicarb DBCP	
Industrial chemicals Bisphenol A Cadmium Dioxin (2,3,7,8-TCDD) Lead Mercury	0.008
PBBs PCBs	0.0002–0.228
Pentachlorophenol (PCP) Penta- to nonylphenols	0.019–0.037 (4-nonylphenol)
Phthalates (dimethyl, diethyl, and dibutyl) Styrenes	(, , ,

Refer to Blair *et al.* and Fang *et al.* (see Further Reading Section) for RBAs (using rat uterine estrogen receptor) and to the ICCVAM report for RBAs listed with

- *RBA = relative binding affinity (% of estradiol activity), ER = estrogen receptor.
- **See report for various estrogen receptor sources.

4. cell-based bioassays using either intact mammalian target cells or yeast cells containing a twoplasmid system with a reporter gene.

Additionally, certain investigations are focused on differential recognition of EDCs by estrogen receptor isoforms separated by high-performance liquid chromatography.

In summary, the body of experimental and epidemiological evidence suggesting many substances in the environment may disrupt human health continues to expand to cover a wide range of exposures. Of greatest concern are the effects of transgenerational exposure to unrecognized agents, which may be present in food stuffs, drinking water, and other consumables including medications and cosmetics. Using hormone receptor-based technology and highly purified preparations of EDCs as standards, there is an opportunity to improve exposure and risk assessment for environmental estrogen mimics, as well as the quantitative analysis of their occurrence in the environment.

Exposure Routes and Pathways

Xenoestrogens are particularly dangerous to animal and human health because they are persistent, ubiquitous chemicals in the environment that bioaccumulate and may even be activated further as a result of biotransformation. An environmental endocrine disruptor is defined as a man-made compound that interferes with one or more steps in the signal transduction pathway of natural hormones in the body responsible for maintenance of homeostasis, reproduction, development, and/or behavior. The enormous chemical complexity of xenoestrogens (e.g., more than 200 possible congeners of polychlorinated biphenyls (PCBs)) as well as variations in the degree of modification (e.g., extent of chlorination) preclude the establishment of common routes of accumulation and mechanisms of both biotransformation and biodegradation. Exposure to xenoestrogens occurs mainly by ingesting contaminated foods and liquids, although small amounts may be inhaled or absorbed through the skin and mucous membranes in the body.

Toxicokinetics

Because of the broad chemical diversity of compounds in this group, metabolism, detoxification, and excretion pathways are quite variable; therefore, the reader should refer to information for a particular compound. As an example, methoxychlor and bisphenol A in low doses have been shown to be rapidly eliminated from the body as conjugated forms by the liver and have efficient metabolic clearance. Only excessive doses may lead to accumulation if the detoxification pathways are saturated. Once absorbed, they are readily distributed via the lymph and blood to all body tissues and are stored in these tissues generally in proportion to organ tissue lipid content. Excretion of DDT in the form of its metabolites (e.g., DDE and its conjugates) is largely via the urine, regardless of route of exposure, but DDT excretion may occur via feces, semen, and breast milk.

Mechanism of Toxicity

With regard to estrogen-associated toxicity, the primary mechanism appears to be via association with the estrogen receptor and subsequent alteration in the signal transduction pathway. Many studies of toxicokinetics suggest the difficulty in extrapolating structure–activity relationships of particular compounds with their influence on biological responses (e.g., reproduction, neuroendocrine behavior).

Acute and Short-Term Toxicity (or Exposure)

Due to variability in toxicity of this large group of compounds, no pattern of exposure symptoms has been observed. In animal studies, short-term exposure to large amounts of DDT in food affected the nervous system and may affect reproduction (e.g., embryonic survival in bald eagles). Exposure to endocrine-disrupting chemicals has also been shown to affect thyroid function in birds and fish. Humans exposed to simazine at high levels for a relatively short period of time can experience weight loss and changes in liver enzymes in the serum.

Chronic Toxicity (or Exposure)

The large number of compounds with highly diverse molecular properties precludes listing individual symptoms of chronic toxicity. As an example, animals exposed to high levels of methoxychlor experience tremors, convulsions, and seizures. Exposure to large doses of DDT had a negative affect on the metabolic function in the animal's liver. High doses of methoxychlor may cause damage to the human nervous system. High exposure to simazine can cause tremors, gene mutations, cancer, and damage to testes, kidneys, liver, and thyroid. Exposure to high PCB concentrations may increase heart size and blood pressure, two factors known to elevate the risk of heart disease. Laboratory tests have been developed to detect DDT and DDE in fat, blood, urine, semen, and breast milk. These tests may indicate low, moderate, or excessive exposure to these compounds, but cannot provide results assessing extent of exposure or whether there will be adverse biological effects in subjects.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods in 1997.

Environmental Fate

Xenoestrogens, regardless of their molecular diversity, have the ability to leach into ground water and contaminate the water supply if not removed by various methods of water purification. Before 1973 when it was banned, DDT entered the air, water, and soil during its production and use as an insecticide. DDT is present at many waste sites, and releases from these sites may continue to contaminate the environment. Most of the DDT in the environment is a result of past use, but it still enters the environment because of its current use in other areas of the world where it is not banned. DDE is only found in the environment as a result of contamination or breakdown of DDT.

DDT and DDE are rapidly degraded when exposed to sunlight with a half-life of 2 days, but in soil they are biodegraded much more slowly with a half-life of 2-15 years, depending on the type of soil organisms. Small amounts of DDT and DDE leach into the ground water supply as well as pollute plants and accumulate in the fatty tissues of fish, birds, and other animals.

Exposure Standards and Guidelines

No general set of guidelines has been established for exposure to the general category of xenoestrogens. Therefore, exposure assessment of a particular class of xenoestrogens (e.g., phthalates) requires determinations of the parent compound and derivatives. As an example, the US Environmental Protective Agency (EPA) has set a reference dose for methoxychlor at $0.005 \text{ mg day}^{-1}$. This is the highest daily oral exposure humans can be exposed to without resulting in harmful side effects. The EPA has also set a limit of 0.04 parts per million (ppm) of methoxychlor in water. Children should not drink water containing more than 0.05 ppm for more than 1 day, while adults should not drink water containing more than 0.2 ppm for up to 7 years. The Occupational Safety and Heath Administration (OSHA) has established a work place exposure limit for methoxychlor at 15 mg m⁻³ for an 8 h work day and 40 h work week.

The EPA has set a maximum contaminant level for simazine at 4 parts per billion (ppb), because it is believed that exposure to this level of herbicidal compound has not induced health problems.

The OSHA has set a limit of 1 mg m^{-3} of DDT in the workplace for an 8 h shift, 40 h work week.

See also: Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals.

Further Reading

- Blair R, Fang H, Branham WS, *et al.* (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. *Toxicological Sciences* 54: 138–153.
- Colborn T, Vom Saal F, and Soto A (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* 101: 378–384.
- Davis D, et al. (1993) Medical hypothesis; xenoestrogens as preventable causes of breast cancer. *Environmental Health Perspectives* 101: 372–377.
- Fang H, Tong W, Shi LM, *et al.* (2001) Structure–activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. *Chemical Research in Toxicology* 14: 280–294.

Relevant Website

http://iccvam.niehs.nih.gov – ICCVAM/NICEATM Final Report (2002) Expert Panel Evaluation of the Validation Status of *In vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays.

Ethane

Stephen R Clough

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- CHEMICAL ABSTRACT SERVICE REGISTRY NUMBER: CAS 74-84-0
- SYNONYMS: Bimethyl; Dimethyl; Ethyl hydride; Methyl methane; Ethane, copressed (UN1035, DOT); Ethane, refrigerated liquid (UN 1961, DOT)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aliphatic hydrocarbon
- CHEMICAL FORMULA: C₂H₆
- CHEMICAL STRUCTURE:



Uses

Ethane is used as a fuel and as a raw material in the manufacture of synthetic organic chemicals (e.g., pharmaceutical and chemical industry).

Exposure Routes and Pathways

Because ethane exists as a gas at normal temperature and pressure, exposure occurs by inhalation. Concentrations of ethane in natural gas range from 5 to 10%. It is also found in the exhaust of diesel (\sim 1.8%) and gasoline (1.3–2.0%) engines. Small amounts of ethane, along with other C1 and C4 alkanes and alkenes, have been detected in mined coal samples. Ethane emissions from cigarettes have been measured at 1600 µg per cigarette. Typical background air concentrations in major US cities range from 0.05 to 0.5 ppm. Because it is lighter than air, a major spill would not be expected to migrate and affect adjacent properties or neighborhoods. It is possible to spill liquid ethane from a refrigerated tank, causing frostbite upon contact with the skin due to rapid evaporation and loss of heat.

Mechanism of Toxicity

Ethane acts as an asphyxiant at concentrations that are high enough to displace oxygen.

Acute and Short-Term Toxicity (or Exposure)

Animal

Guinea pigs exposed to 2.2–5.5% of the gas for 2 h have shown slight signs of irregular respiration that were readily reversible on cessation of exposure. As in humans, ethane acts as a simple asphyxiant at high concentrations.

Human

Ethane is not toxic to humans; studies have shown no adverse effects at air concentrations of up to 50 000 ppm. Ethane is, however, a simple asphyxiant. Concentrations that are high enough to displace oxygen would be expected to cause lightheadedness, loss of consciousness, and possibly death.

Chronic Toxicity

No information could be found on the chronic toxicity of ethane.

Clinical Management

Persons who are exposed to high concentrations should vacate or be removed from the source of the gas and seek fresh air.

Environmental Fate

Ethane is unlikely to undergo photolysis or hydrolysis or to be bioconcentrated in both soil and water. Volatilization is the most important fate process in these environmental media. Ethane is most likely to be found in the atmosphere.

Other Hazards

Ethane is highly flammable and is therefore an explosion and/or fire hazard (lower explosion limit is 3–12.5% by volume). Extreme care must be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity. Explosion-proof equipment should also be used in these areas.

Exposure Standards and Guidelines

Industrially, ethane is handled similarly to methane, and a threshold limit of 1000 ppm is commonly assumed. Many states regulate ethane as a hazardous substance based on its flammable properties (typically over 10 000 lbs).

Miscellaneous

Ethane has been shown to be a product of lipid peroxidation. Some studies have shown that certain microorganisms are able to use ethane as a nutrient while other types of bacteria are inhibited by its presence.

See also: Lipid Peroxidation.

Further Reading

Snyder R (ed.) (1987) Ethel Browning's toxicity and metabolism of industrial solvents. *Hydrocarbons*, 2nd edn., vol. 1, p. 260. Amsterdam: Elsevier.

Ethanol

Bradford H Strohm and Leonard I Sweet

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 64-17-5
- SYNONYMS: Ethyl alcohol; Grain alcohol; Methyl carbinol; Ethyl hydrate; Cologne spirit; EtOH; Potato alcohol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alcohol
- Chemical Formula: C_2H_6O
- CHEMICAL STRUCTURE:



Uses

Ethanol is one of the largest volume organic chemicals used in industrial and consumer products. The primary industrial uses of this aliphatic alcohol are as an intermediate in the production of other chemicals and as a solvent. Ethanol is used in the manufacture of drugs, plastics, lacquers, polishes, plasticizers, and cosmetics. Ethanol is used in medicine as a topical anti-infective, and as an antidote for ethylene glycol or methanol overdose. Commercial products containing ethyl alcohol include beverages, perfumes, aftershaves and colognes, medicinal liquids, mouthwashes, liniments, and some rubbing alcohols.

Exposure Routes and Pathways

Most exposures to ethanol for the general population are through ingestion. Occupational exposure to ethanol occurs principally via inhalation and dermal contact. Ethanol is not well absorbed through intact skin, but is well absorbed via inhalation.

Toxicokinetics

Ethanol is readily absorbed upon inhalation or ingestion. Absorption from the gastrointestinal tract is by simple diffusion with ~80% of an oral dose being absorbed in the small intestine. About 80–90% of ethanol is absorbed within 30–60 min, although food may delay complete absorption for 4–5 h. Inhalation of ethanol vapors in the range of 5000–10000 ppm by human volunteers indicates absorption from lungs to be ~62%.

Ethanol is both water- and lipid-soluble, and therefore distributes into total body water and readily penetrates the blood-brain barriers and placenta. Ethanol has been found in the amniotic fluid of animals after a single oral dose.

Once peak blood ethanol levels are reached, disappearance is linear, with a 70 kg man metabolizing 7–10 g of alcohol per hour.

The metabolism of ethanol occurs predominantly in the liver. The metabolism of ethanol is carried out in the liver by several enzymes, including alcohol dehydrogenase, aldehyde dehydrogenase, microsomal ethanol-oxidizing system or CYP2E1, and peroxisomal catalase. Initially, ethanol is broken down into acetaldehyde by alcohol dehydrogenase, and then it is further broken down to acetic acid by aldehyde dehydrogenase. Acetic acid is released into the blood where it is further oxidized through normal intermediary metabolism in peripheral tissues to carbon dioxide and water.

Normally, 90–98% of the ethanol that enters the body is completely oxidized, predominantly in the liver, eventually entering the citric acid cycle or utilized in anabolic synthetic pathways. The kidney and lungs excrete only 5–10% of an absorbed dose unchanged. The rate of ethanol metabolism varies between individuals, by age, and may be under genetic control.

Mechanism of Toxicity

Ethanol is a central nervous system (CNS) depressant that initially and selectively depresses some of the most active portions of the brain (reticular activity system and cortex). The mechanism of action most likely involves interference with ion transport at the axonal cell membrane rather than at the synapse, similar to the action of other anesthetic agents. Ethanol can bind directly to the gamma-aminobutyric acid (GABA) receptor in the CNS and cause sedative effects. Ethanol may also have direct effects on cardiac muscle, thyroid tissue, and hepatic tissue.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral, inhalation, and dermal toxicity of ethanol in animals is low. Oral $LD_{50}s$ in rats, mice, guinea-pigs, rabbits, and dogs range from ~6 to 18 g kg^{-1} . Inhalation $LC_{50}s$ range from 12 000 to ~50 000 ppm in studies of mice, guinea pigs, and rats. Animals exposed to ethanol in air may manifest the following signs of intoxication including irritation of the mucous membranes, drowsiness, CNS depression, and possibly respiratory failure. Ethanol is not significantly irritating to the skin of rabbits, although it does produce eye irritation in rabbits. A drop of concentrated ethanol placed on the eyes of rabbits causes reversible injury, whereas repeated applications to rabbit eyes may cause loss of corneal tissue function.

Human

Ethanol is an irritant of the eyes and mucous membranes and causes CNS depression at very high levels of exposure. The major acute toxic effect of ethanol is neuronal dysfunction. Ethanol acts principally on the brain whether ingested or inhaled, first as an inhibitor of the higher functions and then as an anesthetic. Animals as well as humans develop tolerance.

In general, inhalation concentrations up to 3500 ppm cause neither irritation nor any subjective symptoms. Exposures of humans to $\sim 5000-10\,000$ ppm cause transient eye and nose irritation as well as cough. Exposures at 15000 ppm produce continuous lacrimation and cough, and levels of 25000 ppm and above were judged as intolerable.

Mild ethanol intoxication is observed at blood alcohol levels in the range of 0.05–0.15%. Symptoms of exposure include impairment of visual acuity, muscular incoordination, decreased reaction time, and changes in mood, personality, or behavior. At blood alcohol levels of 0.15–0.3%, visual impairment, sensory loss, muscle incoordination, slowed reaction time, and slurred speech is observed. At levels of 0.3-0.5% blood alcohol, there is severe intoxication characterized by muscular incoordination, blurred or double vision, and sometimes stupor, hypothermia, vomiting, nausea, and occasionally, hypoglycemia and convulsions. At 0.4% and above, symptoms include coma, depressed reflexes, respiratory depression, hypertension, hypothermia, and possibly death from respiratory or circulatory failure, often as a result of aspiration of stomach contents in the absence of a gag reflex. The fatal concentration in whole blood is usually considered to be $>400 \text{ mg dl}^{-1}$. The lethal dose for man is 8–10 ml kg⁻¹ body weight.

Chronic Toxicity (or Exposure)

Animal

Subchronic and chronic toxicity testing in animals indicate that the liver is the primary site of action. Effects upon the liver observed in animals parallel those observed in humans and include fatty degeneration, focal necrosis, inflammation, and fibrosis leading to cirrhosis.

Ethanol has been studied in rats, mice, and hamsters for carcinogenicity. While some results are inconclusive, there are data from animals indicating that ethanol consumption may enhance the carcinogenic activity of other known carcinogenic agents. However, studies on male and female mice conducted by the National Toxicology Program (NTP) indicate that the evidence for carcinogenicity is inadequate. According to the American Conference of Governmental Industrial Hygienists (ACGIH), ethanol is 'not classifiable as a human carcinogen'.

Ethanol has been investigated for reproductive toxicity in male mice and rats, and while producing effects upon testes and other reproductive tissues, has generally not been shown to affect reproductive outcome or performance.

Rats, mice, and rabbits have been tested for developmental toxicity upon ethanol exposure. Inhalation of ethanol by pregnant rats at up to 20 000 ppm for 7 h per day on gestational days 1–19 produced no treatment-related effects on uterine implantation or embryonic development. Similarly, 15% ethanol in drinking water of rats, mice, and rabbits, while eliciting maternal toxicity and reducing fetal weights, failed to elicit teratogenic effects. Effects were noted in the offspring of female mice maintained on liquid diets containing 15–35% ethanol dry calories for at least 30 days before and during gestation until day 18.

Human

Alcohol consumption and its relationship to the occurrence of human cancers has been the subject of numerous epidemiological investigations. From these studies, the International Agency for Research on Cancer (IARC Volume 44) has concluded that there is sufficient evidence for the carcinogenicity of alcoholic beverages in humans. Malignant tumors of the oral cavity, pharynx, larynx, esophagus, and liver

have been causally related to the consumption of alcoholic beverages. Alcohol ingestion during pregnancy has been found to lead to congenital malformations that have been collectively termed 'fetal alcohol syndrome'. Fetal alcohol syndrome is characterized by mental deficiency and microcephaly. Affected infants typically are small, demonstrate poor muscle coordination, have impaired immune systems, and exhibit various other abnormalities. These abnormalities may be due, at least in part, to a direct action of ethanol that inhibits embryonic cellular proliferation early in gestation. The severity of the effects is related to the extent and timing of alcohol consumption by the mother during pregnancy. This syndrome has been associated with alcoholic women who drink heavily and chronically during pregnancy. There have been no reports of fetal alcohol syndrome resulting from industrial exposure.

Chronic exposures to ethanol vapors can result in irritation of mucous membranes, headache, and symptoms of CNS depression, such as lack of concentration and drowsiness.

Chronic ethanol ingestion has also been shown to produce liver damage, which can eventually lead to cirrhosis of the liver and possibly death. Signs include enlarged liver, elevated serum enzymes, and jaundice.

Infants and toddlers have a clinical course different from that of adolescents and adults. Ethanol ingestion and intoxication can lead to a marked hypoglycemic state, respiratory depression, and hypoxia, in infants and young children.

Clinical Management

The mainstay of medical treatment of patients with ethanol toxicity is supportive care. In general, a conservative approach is recommended for ethanol intoxication. Supportive therapy for overdose may include treatment for respiratory depression, hypotension, and altered glucose or thiamine levels. If the ingestion occurred within one hour of presentation, placing a nasogastric tube and evacuating the stomach contents can prove helpful. In patients with chronic ethanol abuse, therapy may include administration of thiamine to prevent neurologic injury. The administration of medications to cause emesis is not recommended because of the rapid onset of CNS depression as well as aspiration risks.

Pathologic effects of ethanol on hematopoietic tissue can result directly from alcohol ingestion or from secondary nutritional deficiencies or hepatic disease. The clinician will often confront an array of overlapping syndromes in the alcoholic patient, which involves abnormalities of immune system cells. Hemodialysis efficiently clears ethanol from the blood (removing $\sim 50-100\%$) but as an invasive procedure its use is not routinely recommended unless the patient's condition is deteriorating, or the patient has impaired hepatic function, or is nonresponsive to standard therapeutic intervention.

Environmental Fate

If released to the environment from natural or anthropogenic sources, ethanol is expected to preferentially partition to the soil, water, and air. Bioconcentration and bioaccumulation potential is expected to be low, based on the estimated bioconcentration factor and experimental octanol water partition coefficient. If released into water, it is expected to have a half-life of less than 10 days. When released into the air, it is expected to have a half-life of less than 5 days, and is expected to be removed from the air by wet deposition. Biodegradation and volatilization are expected to be important fate and transport processes for ethanol.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for ethanol include the following:

- American Conference of Governmental Industrial Hygienists (1000 ppm TWA);
- Argentina (1000 ppm TWA);
- Australia (1000 ppm TWA);

- Belgium (1000 ppm TWA);
- Brazil (780 ppm TWA; for a 48 h work week);
- Canada (1000 ppm TWA);
- Chile (800 ppm TWA);
- Denmark (1000 ppm TWA);
- Finland (1000 ppm TWA);
- Germany DFG (1000 ppm peak limitation);
- Mexico (1000 ppm TWA);
- Sweden (500 ppm TLV; LLV);
- United Kingdom (1000 ppm TWA); and
- USA OSHA permissible exposure limit (1000 ppm TWA).

Miscellaneous

Ethanol is a colorless, flammable, volatile liquid that has a characteristic odor and burning taste. Odor is generally detected at concentrations ranging from 100 to 180 ppm.

See also: Developmental Toxicology; Neurotoxicity; Poisoning Emergencies in Humans.

Further Reading

National Toxicology Program (2002) Toxicology and Carcinogenesis Studies of Urethane, Ethanol, and Urethane/ Ethanol (Urethane, CAS No. 51-79-6; Ethanol, CAS No. 64-17-5) in B6C3F₁ Mice (Drinking Water Studies). Research Triangle Park, NC: US Department of Health and Human Services, Public Health Service.

Ethanolamine

William Stott

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Ethanolamine (CAS 141-43-5); Diethanolamine (CAS 111-42-2); Triethanolamine (CAS 102-71-6)
- SYNONYMS: Monoethanolamine, 2-Aminoethanol; Diethanolamine, 2,2'-Iminodiethanol; Triethanolamine, 2,2',2"-Nitrilotriethanol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alcohol amines
- CHEMICAL FORMULAS: C₂H₇NO₂ (Monoethanolamine); C₄H₁₁NO₂ (Diethanolamine); C₆H₁₅NO₃ (Triethanolamine)

• CHEMICAL STRUCTURE:



Uses

Ethanolamines are variously used in the synthesis of ethyleneamines, to remove carbon dioxide and

hydrogen sulfide from natural gases and other gas streams, as corrosion inhibitors in metal removal fluids; to produce surfactants used in a variety of industrial, consumer and personal care products, as dispersing agents for agricultural chemicals, and in the manufacture of cosmetics and pharmaceuticals.

Exposure Routes and Pathways

The uses and relatively low vapor pressures of di- (0.00473 Torr, 0.63 Pa) and tri- (0.00018 Torr, 0.0239 Pa) ethanolamines result in primarily dermal exposure of humans. However, the somewhat higher vapor pressure of monoethanolamine (0.75 Torr, 100 Pa) indicates the potential added exposure for this ethanolamine via inhalation. The inhalation of any of the ethanolamines may also occur when respirable aerosols are generated during the use of consumer products or in occupational settings.

Toxicokinetics

Absorption may occur via the oral, inhalation or dermal routes resulting in systemic toxicity. Dermal absorption can vary greatly dependent upon species and whether applied neat or as a component of a formulation. Acute toxicity data suggest that monoethanolamine, a potentially corrosive chemical, exceeds that of the less irritating and higher molecular weight ethanolamines. In vitro skin studies have demonstrated a relatively high rate of absorption of mono- and diethanolamines through mouse skin relative to rabbits (2-6-fold), rats (13-15-fold), or humans (18-23-fold). Other studies have shown that less than 2% of di- or triethanolamines as aqueous solutions or complex mixtures are absorbed through rat or human skin. Once absorbed, the active uptake of mono- and diethanolamines by tissues, primarily liver, and their metabolic incorporation into phospholipids dictates the pharmacokinetics of these compounds. Monoethanolamine is a naturally occurring precursor of phospholipid metabolism and diethanolamine competes for the same metabolic pathways. More than 50% of monoethanolamine underwent metabolic incorporation into lipids of treated rats while $\sim 12\%$ was excreted as CO₂ in 8 h. Likewise, \sim 70–75% of diethanolamine was retained in tissues, primarily liver and kidneys and remaining carcass, with only 22-35% excreted unchanged via urine in rats 96 h postdosing. In contrast, triethanolamine was excreted by mice almost entirely via the urine unchanged within 24-48 h postdosing. Reflective of this, di- and triethanolamines were eliminated from blood with terminal-phase half-lives of \sim 170 and 10 h, respectively.

Mechanism of Toxicity

The mechanism of toxicity differs significantly between the ethanolamines. The toxicity of monoethanolamine is primarily dictated by its irritant properties as a base ($pK_a = 9.7$), which limits toxicity primarily to portal-of-entry tissues. Systemic effects of diethanolamine appear related to competition with ethanolamine for incorporation into phospholipids and with cellular choline uptake processes which together may result in severe choline deficiency in treated rodents. Chronic choline deficiency is believed to be responsible for diethanolamine-induced tumor formation in mice via this relatively well-characterized nutrition-based mode of tumorigenicity, to which humans are relatively refractory. As a secondary amine, diethanolamine also has the potential to undergo nitrosation to form the carcinogen N-nitrosodiethanolamine in the acidic stomach if ingested with high levels of a nitrosating agent such as nitrite. In contrast, effects of triethanolamine upon tissues appear more related to adaptation to high dosages than frank toxicity. However, triethanolamine may also inhibit cellular uptake of choline resulting in choline deficiency in liver and resultant toxicity, including tumor formation, when administered to mice. Though sharing a choline deficiency mode of action, triethanolamine is less potent than diethanolamine and does not appear to compete with ethanolamine for metabolic incorporation into phospholipids.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, skin and ocular irritation potential of ethanolamines is directly related to their strength as bases and inversely with molecular weight. Neat monoethanolamine can cause a chemical burn within a few hours while triethanolamine is a relatively weak irritant after prolonged contact. Oral and dermal lethal dosages also vary considerably. Oral LD_{50} values reported for mono-, di- and triethanolamine in rats are 1.1–2.7, 0.7–2.8, and $5.5-11.3 \,\mathrm{g \, kg^{-1}}$, respectively. Dermal LD₅₀ values reported for mono-, di- and triethanolamine in rabbits also vary widely; $1.0-2.5, 8.1-12.2, \text{ and greater than } 20 \,\mathrm{g \, kg^{-1}}, \text{ re-}$ spectively. Inhalation acute lethality data (LC_{50}) are not reported and no lethality has been reported at saturated atmospheres of any of the ethanolamines, \sim 520, 0.37 and 0.0047 ppm for mono-, di- and triethanolamine, respectively. Short-term repeated dosing of animals with relatively high dose levels of ethanolamines via inhalation, oral, and/or dermal routes result in effects generally reflected in longerterm testing discussed below.

Human

No reports of significant acute toxic responses to the ethanolamines were noted; however, it would be expected that severe skin and eye irritation could result from contact with concentrated monoethanolamine and, to a much lesser extent, diethanolamine.

Chronic Toxicity (or Exposure)

Animal

The toxicity of monoethanolamine, a product of normal metabolism, is largely limited to its irritant effects, and their sequelae, at the site of contact. Exposure of rats, guinea pigs and dogs to vapors in excess of ~ 50 ppm resulted in significant skin irritation, respiratory distress, and changes in respiratory tract, liver, and kidney tissues. The toxicity of diethanolamine is primarily dictated by its disruption of phospholipid synthesis and an induced deficiency in the nutrient choline. Administration of diethanolamine to rats and mice via oral or dermal route resulted in a species-dependent spectrum of effects, generally at dosages of $\sim 150-200 \text{ mg kg}^{-1} \text{ day}^{-1}$ or higher. Organs affected in rats included liver, kidney, central nervous system, and testes and in mice liver, kidneys, heart, and salivary glands were affected. The most sensitive systemic effect was a microcytic, normochromic anemia, which occurred in female rats at dosages as low as $15 \text{ mg kg}^{-1} \text{ day}^{-1}$. Repeated inhalation of diethanolamine aeorosol by rats resulted in larvngeal irritation at $\sim 8 \text{ mg m}^{-3}$ or higher concentration. Lifetime dermal administration of $40 \text{ mg kg day}^{-1}$ or higher diethanolamine to mice resulted in an increased incidence of liver tumors in males and females and an increased incidence of kidney tumors in $160 \text{ mg kg}^{-1} \text{ day}^{-1}$ males. No tumors were observed in lifetime dermal rat bioassays at up to $250 \text{ mg kg}^{-1} \text{ day}^{-1}$ or in a dermal transgenic mouse (TG.AC) bioassay at $\sim 1000 - 1200 \text{ mg kg}^{-1} \text{ day}^{-1}$.

Administration of triethanolamine to rats and mice via dermal application resulted in only minimal effects upon body weights at 250–2000 mg kg⁻¹ day⁻¹ and changes in liver and/or kidney weights with or without histopathologic changes at 500–1000 mg kg⁻¹ day⁻¹. Aerosolized triethanolamine has also been reported to cause irritation of the larynx of rats exposed to 20–100 mg m⁻³ or higher. Lifetime oral administration of up to 1000–2000 mg kg⁻¹ day⁻¹ triethanolamine to rats and up to 3000 mg kg⁻¹ day⁻¹ to mice or

dermal administration of up to $250 \text{ mg kg}^{-1} \text{ day}^{-1}$ triethanolamine to rats and $2000 \text{ mg kg}^{-1} \text{ day}^{-1}$ to male mice have revealed no evidence of carcinogenic potential. However, lifetime dermal administration of $100 \text{ mg kg}^{-1} \text{ day}^{-1}$ triethanolamine to female mice resulted in an increased incidence of liver tumors. No tumors were observed in a dermal transgenic mouse (TG.AC) bioassay at a dose of ~1000–1200 mg kg^{-1} \text{ day}^{-1}.

Ethanolamines have not been found to be developmental toxins in standard or screening assays nor was diethanolamine found to be neurotoxic in a standard neurotoxicity test following subchronic inhalation exposure up to 400 mg m^{-3} .

Human

Standardized animal and human tests of allergic sensitization to the ethanolamines have been negative, but sporadic case reports of human sensitization and a low incidence of sensitization in specific work groups of dermatitis patients have been reported.

In Vitro Toxicity Data

None of the ethanolamines has been found to be genotoxic in a variety of assays in the absence of added nitrosating substances.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

Ethanolamines released to the environment will partition primarily to the water due their relatively low volatility and high water solubility. Bioconcentration factors range from -1.23 to -1.56 reflecting the very low potential for ethanolamines to bioaccumulate in the environment. All three ethanolamines reportedly undergo near complete degradation in the presence of 'acclimated' microflora under standardized testing conditions and microbial degradation pathways have been elucidated. Environmental persistence and bioaccumulation data do not identify any of the ethanolamines as potentially either persistent or bioaccumulative environmental contaminants under criteria used by the United Nations, European Union and Canadian EPA.

Ecotoxicology

The toxicity of the ethanolamines has been extensively studied in aquatic species. LC_{50} values for

several species of fish range from 150 to $2100 \text{ mg} \text{l}^{-1}$ for monoethanolamine, from greater than 100 to $47\,000 \text{ mg} \text{l}^{-1}$ for diethanolamine, and 1800 to greater than $10\,000 \text{ mg} \text{l}^{-1}$ for triethanolamine. LC₅₀ values for the invertebrate *Daphnia magna* were in the range of $140 \text{ mg} \text{l}^{-1}$ for monoethanolamine, $55-306 \text{ mg} \text{l}^{-1}$ for diethanolamine, and $1390 \text{ mg} \text{l}^{-1}$ for triethanolamine in one set of tests. The most sensitive assay system studied appears to be algae and cyanobacteria whose growth has been reportedly inhibited in the range of $1-10 \text{ mg} \text{l}^{-1}$ for monoethanolamine, $3-20 \text{ mg} \text{l}^{-1}$ for diethanolamine, and $2-715 \text{ mg} \text{l}^{-1}$ for triethanolamine.

Exposure Standards and Guidelines

International occupational exposure limits for most major industrialized regions list 2-3 ppm as an 8 h time-weighted average (TWA) and 6 ppm in Sweden as a short-term exposure level (STEL) (15 min) for monoethanolamine; 15 mg m^{-3} (United Kingdom is 3 mg m^{-3} while United States does not list a value) as a TWA and 30 mg m^{-3} as an STEL for diethanolamine; 5 mg m^{-3} as a TWA and 10 mg m^{-3} in Sweden as an STEL for triethanolamine. Additional occupational exposure values for monoethanolamine include a US Occupational Safety and Health Administration permissible exposure limit of 3 ppm or 6 mg m⁻³, a National Institute of Occupational Safety and Health (NIOSH) recommended exposure limit (REL) of 3 ppm or 8 mg m^{-3} , a German MAK level of 2 ppm or 5.1 mg m⁻³, and an American Conference of Governmental Industrial Hygienists (AC-GIH) 8 h TWA value of 3 ppm or 7.5 mg m⁻³ and an STEL of 6 ppm or 15 mg m^{-3} . Additional values for diethanolamine include an ACGIH threshold limit value (TLV) of 0.46 ppm or 2 mg m^{-3} and a NIOSH REL of 3 ppm or 15 mg m^{-3} . The only additional value for workplace triethanolamine exposure is an ACGIH TLV of 5 mg m^{-3} . Neither an IRIS value nor other ambient, nonworkplace guidance values have been established. The US Food and Drug Administration advises cosmetics manufacturers to avoid using any secondary amines, including diethanolamine, along with nitrosating agents due to the risk of nitrosamine formation. The US Environmental

Protection Agency has promulgated a rule prohibiting the use of nitrites in diethanolamine-containing metal removal fluids for this same reason. Monoand triethanolamines are listed as potential indirect additives in foods.

Di- and triethanolamines are listed by the International Agency for Research on Cancer (IARC) as Group 3, "not classifiable as to its carcinogenicity to humans." None of the ethanolamines is identified as a carcinogen by the US EPA, National Toxicology Program Report on Carcinogens, ACGIH or the MAK Commission. ACGIH and the MAK Commission provide a notation that significant skin absorption is possible while MAK Commission identifies all three ethanolamines as potential dermal sensitizers.

See also: Carcinogenesis; Eye Irritancy Testing; Nitrosamines; Skin.

Further Reading

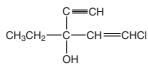
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Ethchlorvynol

S Rutherfoord Rose

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 113-18-8
- SYNONYMS: β-Chlorovinyl ethyl ethinyl carbinol; 1-Chloro-3-ethyl-pent-1-en-4-yn-3-ol; Placidyl[®]; Arvynol[®]; Serenesil[®]
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Tertiary acetylenic alcohol
- CHEMICAL FORMULA: C₇H₉ClO
- CHEMICAL STRUCTURE:



Uses

Ethchlorvynol is used as a sedative/hypnotic. It also possesses some anticonvulsant and muscle relaxant properties. As with most sedative/hypnotics, ethchlorvynol has abuse potential.

Exposure Routes and Pathways

Ethchlorvynol is marketed as a liquid-filled capsule with a polyethylene glycol diluent. Toxicity has resulted from oral overdose and from intravenous injection of the liquid contents of a capsule.

Toxicokinetics

Ethchlorvynol is highly lipid soluble and rapidly absorbed with peak plasma levels occurring within 1–1.5 h. Approximately 90% of a dose undergoes hepatic hydroxylation and glucuronidation, and several metabolites have been identified, including hydroxyethchlorvynol. Both the parent compound and metabolites undergo enterohepatic recirculation. Binding to plasma proteins is $\sim 35-50\%$. The distribution volume is $2.5-41 \text{ kg}^{-1}$, with significant amounts distributed to, and slowly released from, adipose tissue. The elimination of ethchlorvynol appears to be biphasic, with a distribution half-life of 1–5 h, and an elimination half-life of 10–25 h. The elimination phase is prolonged (up to 100 h) following large overdoses. Less than 10% is excreted unchanged in the urine.

Mechanism of Toxicity

The pharmacology of ethchlorvynol is much like that of the barbiturates; thus, an interaction that results in γ -aminobutyric acid-like activity is likely involved. Toxicity results in dose-dependent depression of the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

Pulmonary edema and pleural effusions have been reported following intravenous injection in experimental animals.

Human

Overdosage results in dose-dependent depression of the CNS, ranging from fatigue and lethargy to respiratory depression and coma. Coma may be profound, with a flat EEG, and has been reported to last as long as 17 days. CNS depression may be potentiated by the presence of ethanol or other CNS depressants. Hypothermia is a frequent finding, and hypotension with either tachycardia or bradycardia is common with large doses. Ataxia, nystagmus, and headache may occur. Delayed-onset (24-48 h) noncardiogenic pulmonary edema has occurred following large overdoses and is usually associated with deep coma; however, onset may be rapid following intravenous exposure. Overdoses have also been reported to cause paradoxical excitement and pancytopenia and hemolysis. As with other sedative/ hypnotic drugs, bullous lesions and pressure necrosis have been found on comatose patients, and seizures have occurred during withdrawal. Death has occurred following ingestion of 2.5 g ethchlorvynol plus alcohol, but the usual fatal dosage range is ≥ 10 g. Postmortem blood concentrations ranged from 14 to $400 \text{ mg} \text{l}^{-1}$ in one study and from 22 to 213 mgl⁻¹ in another. Blood levels, however, are not used clinically to guide treatment.

Chronic Toxicity (or Exposure)

Human

Patients chronically using ethchlorvynol may be able to tolerate larger doses and higher serum concentrations compared to drug naive patients.

In Vitro Toxicity Data

Ethchlorvynol has been studied in cultured endothelial cells in order to help explain clinical findings of pulmonary edema seen in some ethchlorvynol overdose patients. Endothelial cells demonstrated retraction within 10 min of addition of ethchlorvynol 1 mg ml^{-1} to cell culture.

Clinical Management

Treatment is generally supportive. All patients should have intravenous access, cardiac monitoring, and should be observed for hypothermia and hypotension. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb ethchlorvynol if used within an hour of the exposure. A complete blood count should be obtained to assess for anemia or thrombocytopenia. Hypotension should initially be treated by elevating the feet and administering an intravenous fluid bolus, followed by administration of vasopressors such as norepinephrine or dopamine if necessary. Pulmonary edema should be managed with positive end

Ethene

Patricia J Beattie

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 74-85-1
- SYNONYMS: Ethylene; Acetene; Bicarburetted hydrogen; Elayl; Olefiant gas
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aliphatic alkene
- CHEMICAL FORMULA: C₂H₄
- Chemical Structure: $H_2C = CH_2$

Uses

Ethene is used primarily as a feedstock in the production of polymers and industrial chemicals. Approximately 80% is used for production of polyethylene, ethylene oxide/ethylene glycols, and ethylene dichloride/vinyl chloride. Additionally, ethene is used for the controlled ripening of citrus fruits, tomatoes, bananas, other fruits, vegetables, and flowers.

Exposure Routes and Pathways

Because ethene is a gas, inhalation exposure is the primary route of entry.

expiratory pressure (e.g., not diuretics or inotropic agents) if needed. There are no antidotes. Drug clearance may be enhanced by resin (15-50% removal) or charcoal (5-10% removal) hemoperfusion, but an affect on morbidity has not been demonstrated. Hemodialysis has not proven to be of benefit.

See also: Barbiturates, Long-Acting; Barbiturates, Short-Acting; Drugs of Abuse.

Further Reading

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- Bertino JS Jr. and Reed MD (1986) Barbiturate and nonbarbiturate sedative hypnotic intoxication in children. *Pediatric Clinics of North America* 33(3): 703–722.
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Toxicokinetics

The inhalation toxicokinetics of ethene have been investigated in human volunteers at atmospheric concentrations of up to 50 ppm (57.5 mg m⁻³). The majority (94.4%) of ethene inhaled into the lungs is exhaled unchanged without becoming systemically available via the bloodstream. The remaining ethene is metabolized to ethylene oxide, which then reacts to form complexes with hemoglobin, *N*-(2-hydroxy-ethyl)histidine, and *N*-(2-hydroxyethyl)valine. The biological half-life is ~0.65 h, with ethene being excreted in urine and feces and exhaled as CO₂. The toxicokinetics of ethene in humans and experimental animals appears to be similar.

Mechanism of Toxicity

Ethene is classified as a simple asphyxiant. In sufficient concentrations, ethene causes central nervous system depression and unconsciousness by displacing oxygen in air, which reduces the oxygen available to support cell function.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of inhaled ethene is low, with very high concentrations causing asphyxia due to oxygen displacement. The LC_{50} for mice is estimated to be 950 000 ppm (1093 g m⁻³). Ethene has been tested in both rats and dogs in short-term inhalation exposure studies. Exposure to skin and eyes does not cause irritation. The anesthetic properties reported in humans have also been observed in experimental animals. Ethene is not a cardiac sensitizer in dogs.

Human

In humans, ethene is a relatively nontoxic gas. No adverse effects are observed at concentrations of less than 2.5%. At higher concentrations, ethene exhibits the anesthetic properties associated with oxygen deprivation. Humans exposed to ethene may experience subtle signs of intoxication, resulting in prolonged reaction time. Exposure to 37.5% ethene for 15 min resulted in memory disturbances, and concentrations at 50% resulted in unconsciousness. If oxygen is deprived for a sufficient amount of time, death can occur. Ethene has been used as an anesthetic and has some advantages over those more typically used in that its effects are rapid in onset and recovery with minimal effect on other organ systems.

Chronic Toxicity (or Exposure)

Animal

The toxicity and carcinogenicity of inhaled ethene was studied in Fischer 344 rats. The animals were exposed to 300, 1000, or 3000 ppm for 6 h day^{-1} , 5 days week⁻¹ for 24 months. These exposures resulted in no toxicity or carcinogenicity. Rats exposed by inhalation to ethene $6 h day^{-1}$, $5 days week^{-1}$ for 13 weeks at 0, 300, 1000, 3000, or 10 000 ppm exhibited no adverse effects, with 10 000 ppm considered to be the no-observed-effect level (NOEL). Ethene was tested in rats for reproductive effects as well as impacts on growth and development of the offspring following head-only inhalation exposure to 200, 1000, or 5000 ppm for $6 h day^{-1}$ 2 weeks prior to mating, during the mating period, and until the day prior to necropsy of the males or until day 20 of gestation for the females. No adverse effects were observed on male or female reproductive performance, fertility, pregnancy, maternal and suckling behavior, and growth and development of the offspring. The highest dose was determined to be the NOEL for reproductive and developmental effects in rats.

Human

The International Agency for Research on Cancer has concluded that there is inadequate evidence in humans and experimental animals for the carcinogenicity of ethene.

In Vitro Toxicity Data

Ethene at atmospheric concentrations up to 20% was not mutagenic to one strain of *Salmonella typhimurium* with and without liver metabolic activation system (S9). In other strains of *Salmonella* in the presence and absence of S9, ethene was also negative. Ethene has shown no genotoxic activity in *Escherichia coli*. In nonbacterial tests, ethene did not induce chromosome aberrations in cultured Chinese hamster ovary cells exposed to $280.5 \text{ mg} \text{ l}^{-1}$ in the presence and absence of S9, and did not induce micronuclei formation in bone marrow cells of rats or mice exposed up to 3000 ppm for 6 h day⁻¹, 5 days week⁻¹ for 4 weeks.

Clinical Management

Overexposure to ethene is treated by simply moving the victim to fresh air. Recovery is usually rapid and complete.

Environmental Fate

Emitted ethene is distributed primarily into the atmosphere and reacts with photochemically reactive hydroxyl radicals, ozone, and nitrate radicals, with half-lives ranging from 1.9, 6.5, and 190 days, respectively. Biodegradation in water occurs with half-lives in the range of 1–28 days, or under anaerobic conditions, 3–112 days. Bioaccumulation in aquatic organisms is not expected to occur, based on ethene's high vapor pressure and log octanol/water partition coefficient.

Ecotoxicology

Ethene is a natural plant hormone and plays a role in flowering, fruit ripening, senescence, and abscission. Exposure to high concentrations, however, can adversely impact photosynthesis and growth, resulting in leaf curling and shedding of flowers and leaves. Commonly impacted plants are peas, potatoes, and oats where retardation effects were observed at concentrations ranging from 8 to 50 mg m^{-3} . Other sensitive plants include African marigolds and Cattleya orchids. Aquatic plants and algae do not exhibit similar sensitivity. Calculated LC₅₀ values for various fish species following 4 days of exposure range from 50 to 120 mg l^{-1} . The calculated no-observedeffect concentration for fish (fathead minnow) after $28 \text{ days of exposure is } 13 \text{ mg l}^{-1}$.

Exposure Standards and Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value: simple asphyxiant – inert gas or vapor. A4: Not classifiable as a human carcinogen.

Switzerland: time-weighted average: 11500 mg m^{-3} .

Miscellaneous

The primary hazard associated with use of ethene, however, is its flammability and explosivity.

See also: Polymers; Propene.

Further Reading

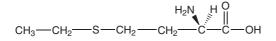
Goodman LS and Gilman A (eds.) (1975) *The Pharma*cological Basis of Therapeutics, 5th edn., p. 84. New York: Macmillan.

Ether See Diethyl Ether.

Ethionine

Fu-Min Menn

- © 2005 Elsevier Inc. All rights reserved.
- CHEMICAL NAME: 1-2-Amino-4-(ethylthio)butyric acid
- REPRESENTATIVE CHEMICAL: L-Ethionine
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: L-Ethionine (CAS 13073-35-3); D-Ethionine (CAS 535-32-0); DL-Ethionine (CAS 67-21-0)
- SYNONYMS: S-Ethyl-L-homocysteine; α-Amino-γ-(ethylmercapto)butyric acid
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Carcinogen
- CHEMICAL FORMULA: C₆H₁₃NO₂S
- Chemical Structure:



Uses

There is no known commercial use for this compound.

Exposure Routes and Pathways

There are multiple routes of entry including skin contact, inhalation, and ingestion.

Toxicokinetics

Ethionine can be absorbed readily through the gastrointestinal tract. This compound can transmit through the placenta from pregnant mother to embryo in rats.

Mechanism of Toxicity

Ethionine is the ethyl analog of the amino acid methionine and has proven to be a useful compound for studying the disturbance in the metabolism of methionine and altered gene expression during cancer development. L-Ethionine is the active form and is highly toxic to most organisms. It acts as an antagonist of methionine that competes with methionine for adenosyl groups from ATP (adenosine triphosphate) to yield S-adenosylethionine instead of S-adenosyl-L-methionine, a universal methyl donor in prokaryotes and eukaryotes. As a consequence of the formation of the S-adenosylethionine, proteins, and RNA become ethylated instead of being methylated. The deficiency of S-adenosylmethionine results in impairing all transmethylation reactions in the various cellular processes of organism, including DNA, RNA, protein, and phospholipid methylation, and causes damage on cellular growth, differentiation, and function. The decrease of S-adenosyl-L-methionine level in animals can cause hypomethylation and DNA damage, which may lead to the development of cancer. However, the L-ethionine induced ATP depletion and protein synthesis impairment can be reversed in rats by the administration of methionine and adenine, the ATP precursor. Ethionine is also known to be a carcinogen and a teratogen.

Acute and Short-Term Toxicity (or Exposure)

Ethionine is an acutely toxic compound that targets the liver and pancreas in animals, and possibly humans. Ethionine inhibits intracellular *S*-adenosylmethionine mediated methylation, and cause widespread liver and pancreatic necrosis.

Animal

Induction of acute hemorrhagic pancreatitis has a 100% mortality rate in young female mice when fed with a choline-deficient diet containing 0.5% ethionine. However, neither mortality nor pancreatitis was reported when ethionine was eliminated from the diet.

Ethionine was detected in the liver, plasma, kidney, small intestine, and red blood cells (in the order of decreasing concentration) of rats after 8 h oral administration with radiolabeled $L-(1-^{14}C \text{ ethyl})$ -ethionine.

Chronic Toxicity (or Exposure)

Ethionine-induced teratogenesis has been reported in rats and chicks. Both mice and rats demonstrate significant strain difference to the carcinogenic effects that are caused by ethionine. In addition to cancer, the ethionine-induced abnormal methylation may also have pathological effects leading to birth defects, neurological disorder, and liver and pancreatic toxicities.

Animal

Tumors were formed in the lung, thorax, respiratory tract, and liver in mice study after 2 year oral administration with high dosages. Low chronic doses of ethionine exposure result in irreversible testicular lesions in rats.

Ethionine-induced hepatocellular carcinoma was reduced from 89% to 36% by adding phenobarbital to the 0.1% ethionine diet in F344 rats during an 18-month carcinogenicity study. A different study showed vitamin E protects rat liver mitochondria from ethionine toxicity.

In Vitro Toxicity Data

Ethionine has been demonstrated to inhibit amylase secretion from the AR42J pancreatic cell line *in vitro*, and it also inhibits amylase secretion *in vivo* from freshly isolated rat acini. Ethionine has been shown to significantly increase sister chromatid exchange frequency in human lymphocytes.

Clinical Management

Treatments for skin exposure include removal of contaminated clothing; the exposed area should be washed with soap and water. For eye exposure, the eyes should be immediately rinsed with plenty of running water for at least 15 min. If swallowed, the mouth should be washed; plenty of water should be taken to induce vomiting. For inhalation exposure, remove victim to fresh air area and provide oxygen or artificial respiration as necessary.

Other Hazards

Ethionine may cause heritable genetic damage.

Exposure Standards and Guidelines

The aerosol should not be breathed and prolonged or repeated exposure avoided. All laboratory work should be conducted in a fume hood, glove box, or ventilated cabinet. Use water to dissolve ethionine if a spill occurs.

Miscellaneous

Ethionine is a white crystalline solid. It is very soluble in water and ethanol, and insoluble in nonpolar solvents. The melting point is 280°C (L-form).

D-Ethionine has been used as an antiinflammatory compound in albino rats.

See also: Butyric Acid; Carcinogen–DNA Adduct Formation and DNA Repair.

Relevant Website

http://toxnet.nlm.nih.gov - TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethionine.

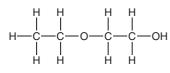
Ethoxyethanol

Brad Stanard

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 110-80-5
- SYNONYMS: Ethanol; 2-Ethoxy; β-Ethoxyethanol; Cellosolve; 2-Ethoxyethanol; Ethyl cellosolve; Ethyl glycol; Ethylene glycol ethyl ether; Hydroxy ether
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Glycol ethers

- CHEMICAL FORMULA: C₄H₁₀O₂
- CHEMICAL STRUCTURE:



Uses

2-Ethoxyethanol is a stable, colorless, flammable liquid, synthetically produced throughout the world. It is produced by the reaction of ethylene oxide with ethanol. A large portion is used in the coatings industry (paints, stains, lacquers) and as solvent for printing inks and dyes, and home and industrial cleans. In 1998, the consumption in Western countries was 571 000 tons. The major function of glycol ethers is to dissolve various components of mixtures to keep them in solution until the last stages of evaporation. The glycol ethers are miscible in polar and nonpolar solutions, which make them unique and add to the quality as a solvent and a cleaner.

Exposure Routes and Pathways

The primary route of exposure occurs via inhalation, ingestion, and eye and skin contact. The potential indoor exposures include paints and other coatings, inks, adhesives, nail polishes, cosmetics, and household cleaning supplies. Exposures can also occur through accidental industrial releases.

Toxicokinetics

2-Ethoxyethanol is readily absorbed through the skin, lungs, and gastrointestinal tract. Once absorbed, it is rapidly metabolized. The metabolic pathway involves dehydroxylation by alcohol dehydrogenase. The resulting aldehyde is quickly reduced to form ethoxyacetic acid, the primary metabolite. Further conjugation occurs in rodents to form the secondary metabolite, ethoxyacetyl glycine; however the glycine; conjugate has not been observed in humans. Ethoxyacetic acid is detected in blood and mucous membranes within minutes. Addition of ethanol will slow the process as a result of competitive inhibition of alcohol dehydrogenase. Finally, the ethoxyacetic acid is dealkylated by P450 monooxygenase and eliminated. Excretion is relatively slow and appears to be biphasic, suggesting evidence of accumulation.

Mechanism of Toxicity

The toxicity associated with 2-ethoxyethanol appears to be caused by the metabolites ethoxyaldehyde and ethoxyacetic acid. The metabolites have a longer half-life implying a higher accumulation following repeated exposures. Both *in vitro* and *in vivo* studies have shown toxic effects from administration of the metabolites that were not seen at higher doses of the parent.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats, mice, dogs, and rabbits have been exposed by the inhalation, dermal, and oral routes. Hepatic changes have been observed at inhalation exposures above 300 ppm. Reduced cytoplasmic density, disruption of lobular structure, elevated plasma fibrinogen, reduced serum proteins, and elevated liver weights have been reported in rats, mice, and rabbits. Most effects seen after acute exposures were reversible.

Human

Acute exposure to high levels of 2-ethoxyethanol results in narcosis, pulmonary edema, and severe liver and kidney damage. Low-level exposure causes conjunctivitis, upper respiratory tract irritation, headache, nausea, and temporary corneal clouding. There are limited human data available in the public domain.

Chronic Toxicity (or Exposure)

Animal

2-Ethoxyethanol is a potent reproductive toxicant. Oral exposure of male rats for six weeks resulted in testicular atrophy, as well as significant decreases in testicular weight, spermatid count, and epididymal sperm count at doses 300 mg kg^{-1} or less. The specific altered sperm morphologies indicate that pachytene spermatocyte is the most sensitive target cell. Inhalation studies in pregnant rats have shown complete resorption of fetuses and reduced fetal weight, as well as skeletal and cardiovascular abnormalities following 733 mg m^{-3} for 7 h per day throughout gestation. Increased resorption rates and fetal deaths, decreased viable fetus weights, and increased cardiovascular defects and skeletal malformations were seen following 0.25 ml applied to skin four times per day. Increased resorptions, incidences of major malformations, minor anomalies, and skeletal variants have been observed following high inhalation doses in both rats and rabbits.

There have been no adequate longer term animal studies to date. There are only minimal data on animal carcinogenicity.

Human

Repeated exposures have resulted in increased oligospermia (low sperm counts) and azoospermia (dead sperm) in male shipyard workers, but there has been some skepticism about these reports due to small sample size. One case controlled study found significant ethoxyacetic acid presence in infertile men. An increased risk of spontaneous abortion and lowered fertility was shown in an epidemiological study of women exposed to mixtures of ethylene glycol ethers.

In Vitro Toxicity Data

Studies on cultured Sertoli cells and germ cells showed both reproductive and genotoxicity following ethoxyacetic acid exposure while lower concentrations of 2-ethoxyethanol showed no morphological changes. Metabolites of 2-ethoxyethanol have been shown to cause chromosomal aberrations and micronuclei in genetic toxicity studies and to induce perturbations in the cell cycle, suggesting DNA lesions as observed on germ cells. Accumulation of other glycol ethers in cell nuclei suggests possible effects on gene regulation, although such studies have yet to be conducted specifically on 2-ethoxyethanol.

Environmental Fate

The environmental fate of ethoxyethanol is relatively short-lived due to degradation by microorganisms in soil, sewer sludge, and water. In the absence of degradation, accumulation in water could occur due to the solubility of 2-ethoxyethanol in water and its relatively low vapor pressure. Degradation to carbon dioxide and water occurs under aerobic conditions. while anaerobic degration yields methane and carbon dioxide. The environmental half-life under aerobic conditions is an estimated 1-4 weeks. It is somewhat more persistent under anaerobic conditions. Atmospheric emissions from use as evaporative solvents result in the greatest environmental exposure, although rapid photolytic degradation occurs. 2-Ethoxyethanol reacts with hydroxyl radicals in the air with a half-life of about 0.2–4 days. The majority remains suspended, although a proportion would

partition to water and to soil. 2-Ethoxyethanol has a very low octanol/water partition coefficient and is therefore not expected to bioaccumulate to any significant degree. Data on toxicity exist for aquatic organisms, including microorganisms, invertebrates, and fish. 2-Ethoxyethanol is not very toxic to these organisms; in a number of studies, the LC₅₀ was above the highest concentration tested.

Exposure Standards and Guidelines

- National Institute for Occupational Safety and Health (NIOSH) immediately dangerous to life or health value is 500 ppm (1843 mg m⁻³).
- NIOSH recommended exposure level is 0.5 ppm (1.8 mg m⁻³).
- Occupational Safety and Health Administration permissible exposure level is 200 ppm (740 mg m⁻³).
- American Conference of Governmental Industrial Hygienists threshold limit value is 5 ppm (18 mg m⁻³) (with skin notation).
- Environmental Protection Agency (EPA) reference concentration is 0.05 ppm (0.2 mg m⁻³)
- EPA reference dose is 0.4 mg kg⁻¹ day⁻¹ (provisional).

See also: Ethylene Glycol Monoethyl Ether; Ethylene Glycol Mono-*n*-Butyl Ether; Methoxyethanol.

Further Reading

- American Conference of Governmental Industrial Hygienists (ACGIH) (2003) 2003 TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents. Biological Exposure Indices. Cincinnati, OH: ACGIH.
- National Institute for Occupational Safety and Health (NIOSH) (2003) *Pocket Guide to Hazardous Chemicals*. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention.

Relevant Websites

- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethoxyethanol.
- http://www.inchem.org-World Health Organization, Environmental Health Criteria 115, Search for 2-methoxyethanol, 2-ethoxyethanol, and their acetates.

Ethyl Acetate

Dale J Marino

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 141-78-6
- SYNONYMS: Acetic acid ethyl ester; Acetic ether acetidin; Acetoxyethane; Ethyl acetic ester; Ethyl ethanoate; Vinegar naphtha
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aliphatic ester
- Chemical Formula: $C_4H_8O_2$
- CHEMICAL STRUCTURE:



Uses

Ethyl acetate is used primarily as (1) a coatings solvent for paints, lacquers, and varnishes; (2) an extraction solvent for various processes, including decaffeination of coffee and tea; (3) a process solvent in the pharmaceutical industry; and (4) a carrier solvent for printing inks, adhesives, and nail polish. It is also used in the manufacture of artificial leather and perfumes, and in certain household products including airplane glue, and paint and nail polish removers.

Ethyl acetate finds particular use as a flavor enhancer in foods and pharmaceuticals because of its fruity taste when diluted. It is included on the US Food and Drug Administration's Generally Recognized As Safe list for use as a synthetic flavoring agent. Ethyl acetate is also approved for use as an indirect food additive in certain packaging materials.

Background Information

Ethyl acetate is a naturally occurring constituent in various fruits, including apples, pears, oranges, and grapefruit; and, because it is a by-product of fermentation, ethyl acetate is found in alcoholic beverages including beer and wine. Given its natural occurrence in fruits and use as a flavor enhancer, ethyl acetate is found in foods, including baked goods, frozen dairy products, fruit juices, candy, beverages, and gum.

Exposure Routes and Pathways

Exposure to ethyl acetate can occur via inhalation, ingestion, or dermal contact. Occupational exposures primarily involve inhalation exposure, given ethyl acetate's use as a solvent. Dermal contact would also be expected.

The general population is primarily exposed to low concentrations of ethyl acetate in food. Higher exposures could occur as a result of inhalation of vapors from lacquers, varnishes, nail polish remover, paint remover, and airplane glue. Dermal exposure to ethyl acetate from use of these products is also possible.

Toxicokinetics

Ethyl acetate is readily absorbed following oral, dermal, or inhalation exposures. However, it is rapidly hydrolyzed to ethanol and acetic acid prior to absorption in the gastrointestinal tract following oral administration, and in the upper respiratory tract following inhalation exposure. Absorbed ethyl acetate is also quickly hydrolyzed in blood to ethanol and acetate. Blood concentrations of ethanol can increase if the production of ethanol, following absorption and hydrolysis of ethyl acetate, exceeds metabolism and elimination of ethanol.

Mechanism of Toxicity

Local effects are thought to be due to the formation of acetic acid resulting from hydrolysis of ethyl acetate. Central nervous system (CNS) depressive effects are thought to be due to a combination of absorbed ethyl acetate and formation of ethanol that results from hydrolysis.

Regional deposition of ethyl acetate in the upper respiratory tract and biochemical differences (higher carboxyesterase activity) is believed to be responsible for damage observed to the olfactory mucosa in laboratory animals following inhalation, compared to that noted in the respiratory epithelium.

Acute and Short-Term Toxicity (or Exposure)

Ethyl acetate is considered to have a low order of acute toxicity by all exposure routes. Its primary effects are sensory irritation and, at higher levels, CNS depression.

Animal

Acute oral LD_{50} values in rats have been reported to range from 5.6 g kg⁻¹ to 11.3 ml kg⁻¹ (10.2 g kg⁻¹).

In mice, rabbits, and guinea pigs, reported acute oral LD_{50} values are 4.1, 4.94, and $5.5 \, g \, kg^{-1}$, respectively. The dermal LD_{50} in rabbits is $> 20 \, ml \, kg^{-1}$ (18.0 g kg⁻¹). Inhalation LC_{50} values in rats have been reported to be 1600 ppm (8 h exposure) and $> 6000 \, ppm$ (6 h exposure). Ethyl acetate is mildly irritating to the eye and minimally irritating to skin.

Exposure of mice to nonlethal concentrations of 2000 ppm ethyl acetate for 20 min produced CNS effects during exposure, including decreased locomotor activity, decreased arousal, and delayed righting reflex. Exposure of rats to either 3000 or 6000 ppm for 6 h decreased motor activity and resulted in CNS effects indicative of sedation at 1 h following exposure. In high-dose animals, decreased motor activity was evident the day following exposure. Body weight loss was also noted in all test groups, that is, 600, 3000, and 6000 ppm.

Human

The most common effects in humans exposed to ethyl acetate are ocular and respiratory tract irritation. Exposures in excess of 400 ppm have been associated with irritation of the eyes and respiratory passages. Subjects have reported mild irritant effects at 400 ppm. At higher concentrations, ethyl acetate exposure is associated with CNS depression, potentially causing headache, nausea, vomiting, drowsiness, and dizziness. Very high concentrations of ethyl acetate as might occur in enclosed spaces with no ventilation, for example, tanks have been reported to cause death from narcosis and anoxia.

Chronic Toxicity (or Exposure)

Animal

A 90 day rat oral gavage study resulted in depressed body and organ weights and depressed food consumption in high dose males $(3600 \text{ mg kg}^{-1} \text{ day}^{-1})$. No effects were noted at $900 \text{ mg kg}^{-1} \text{ day}^{-1}$. From these results, the US Environmental Protection Agency derived an oral reference dose of 0.9 mg kg⁻¹ day⁻¹ for ethyl acetate (uncertainty factor = 1000).

Degeneration of the nasal olfactory mucosa was observed in rats exposed to 350, 750, or 1500 ppm ethyl acetate, 6 h day⁻¹, 5 days week⁻¹ for 90 days. In the mid- and high-dose groups, mild, transient sedation was observed during exposure, and decreased bodyweight, bodyweight gain, and food consumption were observed postexposure. No treatment-related changes were evident in clinical observations, behavioral observations, motor activity, schedule controlled-operant behavior, ophthalmic examinations, urinalysis, organ weights, or the number concentration, motility, or morphology of sperm.

Human

Repeated inhalation exposure of humans to airborne concentrations exceeding 400 ppm is expected to produce irritation of the eyes, nose, and throat. Ethyl acetate can potentially cause drying and cracking of the skin with repeated dermal exposures because of its ability to defat the skin.

In Vitro Toxicity Data

Short-term studies of ethyl acetate in bacteria yielded equivocal results in a *Bacillus subtilis* rec assay for DNA damage/repair and negative (not mutagenic) results in the *Salmonella*/microsome reverse mutation assay.

Ethyl acetate induced aneuploidy in *Sac-charomyces cerevisiae*. It yielded positive results in an *in vitro* sister chromatid exchange assay in Chinese hamster ovary cells. *In vitro* chromosomal aberration assays were positive in Chinese hamster fibroblast cells and negative in Chinese hamster ovary cells. (An *in vivo* bone marrow micronucleus study in Chinese hamsters yielded negative results by both intraperitoneal and oral administrations.)

Clinical Management

There is no specific antidote for ethyl acetate exposure. Treatment should be symptomatic and supportive. Inhalation and ingestion exposures often do not require treatment given the low acute toxicity and rapid hydrolysis of ethyl acetate. If large quantities are ingested, the individual should be monitored for CNS depression, respiratory function, and cardiac contractility. For inhalation exposure, the individual should be moved to fresh air and monitored for respiratory distress. A cough or breathing difficulties may indicate respiratory irritation, bronchitis, or pneumonitis. Artificial respiration should be administered if the individual is not breathing. If ethyl acetate comes in contact with skin or eyes, flush the affected areas with water and monitored for persistent irritation or pain. Contaminated clothing should be removed following dermal exposure. Dermal irritation or dermatitis caused by defatting of the skin from repeated dermal contact should be treated symptomatically.

Environmental Fate

Given its vapor pressure of 73 mmHg at 20°C, ethyl acetate will remain in the vapor phase if released to the atmosphere where it will react with photochemically produced hydroxyl radicals. It is expected to be quite mobile if released to soils ($\log K_{ow} = 0.73$). Volatilization from both dry and moist soils is also expected. If released to water, ethyl acetate will not adsorb to suspended or bed sediments. Volatilization from water is anticipated to be an important loss process (Henry's law constant = 1.34×10^{-4} atm m³ mol⁻¹). Biodegradation is also expected to be an important loss process in both soil and water. Ethyl acetate will not bioconcentrate in aquatic biota.

Ecotoxicology

The reported 96 h, no-observed-effect concentration in algae (*Selenastrum capricornutum*) is 2000 mgl⁻¹. The 48 h EC₅₀ in *Daphnia pulex* is 262 mgl⁻¹, and the 96 h LC₅₀ in fathead minnows (*Pimephales promelas*) is 230 mgl⁻¹.

Other Hazards

High airborne concentrations of ethyl acetate can form in poorly ventilated spaces with the potential for eye, nose, and respiratory tract irritation; CNS depression; and escape impairment. Ethyl acetate is a flammable liquid with a flash point of -4.4° C. Because its vapor density is heavier than air (density = 3.04), vapors can travel a considerable distance to an ignition source and flash back. Explosive concentrations of ethyl acetate can form in air, especially in enclosed spaces (lower explosive limit (LEL) = 2.2%).

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (ACGIH TLV), the National Institute for Occupational Safety and Health recommended exposure limit (NIOSH REL), and the Occupational Safety and Health Administration permissible exposure limit (OSHA PEL) for ethyl acetate are 400 ppm. Both the ACGIH TLV and OSHA PEL are 8h time-weighted average (TWA) while the NIOSH REL is a 10h TWA.

See also: Acetic Acid; Carboxylesterases; Ethanol; Fragrances and Perfumes; Volatile Organic Compounds (VOC).

Relevant Website

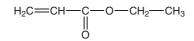
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethyl Acetate.

Ethyl Acrylate

Ralph J Parod

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 140-88-5
- SYNONYMS: Ethyl 2-propenoate; 2-Propenoic acid ethyl ester; Acrylic acid ethyl ester
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Ester
- CHEMICAL FORMULA: $C_5H_8O_2$
- CHEMICAL STRUCTURE:



Uses

Ethyl acrylate monomer is used to make acrylic resins as well as emulsion (water-based) and solution (solvent-based) polymers. Water-based ethyl acrylate polymers are used in latex paints, caulks, leathertreating base coats, floor polishes, and textile finishes. Solvent-based ethyl acrylate polymers are used in lacquers, enamels, and lubricating oils.

Exposure Routes and Pathways

Exposures to ethyl acrylate monomer are most likely to occur in an occupational environment via skin contact and inhalation. However, the closed systems used during manufacture and transportation will limit worker exposures to those that may occur during routine process maintenance, periodic plumbing leaks, and the collection of quality control samples. Under these conditions, exposures are further limited by the use of industrial hygiene controls and personal protective equipment. The acrid odor of ethyl acrylate, which can be detected at 0.001– 0.005 ppm, also serves to limit exposure. Studies of monomer production workers have indicated that mean exposures to ethyl acrylate are typically <1 ppm. The general population does not receive a significant exposure to ethyl acrylate due to low concentrations of residual monomer in consumer products.

Toxicokinetics

Data in experimental animals indicate that ethyl acrylate is readily absorbed from the gastrointestinal tract and the respiratory tract. Absorption of ethyl acrylate through the skin occurs less readily and may be limited by evaporation of ethyl acrylate if the applied dose is unoccluded.

The primary route of ethyl acrylate metabolism is its rapid hydrolysis by tissue and circulating carboxylesterases to acrylic acid and ethanol that undergo further metabolism to CO_2 . In the rat, it has been estimated that ~50% of the ethyl acrylate that passes through the upper respiratory tract is hydrolyzed by carboxylesterase in the nasal mucosa before entering the blood. Another route of ethyl acrylate metabolism is conjugation with the sulfhydryl group of glutathione. Both pathways serve to detoxify ethyl acrylate.

Ethyl acrylate is rapidly distributed throughout the body. Ethyl acrylate and/or its metabolites can be detected in all organ systems, with the highest concentrations being present in the urine, expired air, and organ of entry (i.e., stomach, upper respiratory tract, and skin). Metabolites of ethyl acrylate are excreted primarily via the lungs (as carbon dioxide) and the kidneys. Approximately 60% of an orally administered dose of ethyl acrylate is eliminated from the body as CO_2 within 8 h.

Mechanism of Toxicity

Pretreatment of rats with a carboxylesterase inhibitor enhances the respiratory irritation and lethality produced by the inhalation of ethyl acrylate. This and other observations suggest that the toxicity of ethyl acrylate becomes manifest when local detoxification/defense mechanisms become overwhelmed.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicological studies in animals indicate that ethyl acrylate exposures do not generally result in systemic toxicity at sublethal doses. Although ethyl acrylate concentrations approaching lethal doses may cause histopathological changes in the liver and kidneys, ethyl acrylate toxicity is largely limited to irritant effects, and their sequelae, at the site of application. Ethyl acrylate can produce an allergic contact dermatitis that may cross-react with other acrylic esters. The acute oral and dermal LD_{50} values in rats are 1120 and 3049 mg kg⁻¹, respectively. The 4 h LC₅₀ for ethyl acrylate vapor is 2180 ppm (rat). Ethyl acrylate was not clastogenic in *in vivo* mouse micronucleus assays.

Human

Ethyl acrylate can be highly irritating to the skin, eyes, gastrointestinal tract, and the respiratory tract. Ethyl acrylate may cause an allergic contact dermatitis and may cross-react with other acrylate esters.

Chronic Toxicity (or Exposure)

Animal

In a 13 week gavage study, rats were exposed to ethyl acrylate at doses of 0, 7, 14, 28, 55, and 110 mg kg^{-1} for 5 days week⁻¹. Toxicity was limited to the high dose and consisted of changes in the stomach and duodenal mucosa. The histopathology of other organs, including the sex organs, was normal. The study of no-observed-adverse-effect level (NOAEL) was 55 mg kg⁻¹. In an inhalation study, rats and mice were exposed to ethyl acrylate concentrations of 0, 25, 75, and 225 ppm for 6 h day^{-1} , 5 days week $^{-1}$ for either 6 months (high dose) or 27 months (mid and low doses, and control). The high dose was terminated due to significant loss in body weight. In both species, body weight was reduced at 75 ppm and dose-dependent degenerative changes to the olfactory epithelium and nasal turbinates were noted at all three concentrations. The histopathology of the sex organs was normal. Tumor incidences were not increased. The National Toxicology Program (NTP) studies reported forestomach tumors in rats and mice gavaged for a lifetime with 100 or $200 \,\mathrm{mg \, kg^{-1}}$ ethyl acrylate. The NTP subsequently determined that these tumors resulted from localized irritation and cellular proliferation produced by high tissue concentrations of ethyl acrylate and did not provide an adequate basis for classifying ethyl acrylate as a potential human carcinogen.

In a developmental study, pregnant rats were exposed to ethyl acrylate at concentrations of 0, 25, 50, 100, or 200 ppm on days 6–20 of gestation. The highest dose produced a decrement in maternal body weight gain. Decrements in fetal body weight were observed only at 200 ppm; decreases in embryonic survivals and increases in fetal malformations were not observed at any concentration. The NOAELs for

maternal toxicity, developmental toxicity, and teratogenicity were 100, 100, and 200 ppm, respectively.

The normal sex organ histopathology noted in animals combined with the occurrence of rat fetotoxicity only in the presence of maternal toxicity suggests that ethyl acrylate does not pose a significant reproductive and developmental hazard to humans.

Human

Limited epidemiology data exist for exposure to ethyl acrylate. Mortality from cancer of the colon and rectum was elevated in workers from plants manufacturing and polymerizing ethyl acrylate; however, the findings were confounded by coexposure to other chemicals. Currently, there is inadequate evidence to link human exposures to ethyl acrylate with cancer.

In Vitro Toxicity Data

Data on the *in vitro* mutagenicity (*Salmonella* reverse mutation assay) are negative; positive responses in some *in vitro* assays occurred only in the presence of significant cytotoxicity.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

Ethyl acrylate is a volatile (38 hPa at 20°C) liquid under normal environmental conditions. At equilibrium in the environment, ethyl acrylate will partition primarily to air (94%) with lesser amounts to water (5.6%), soil (<1%), and sediment (<0.1%). In air, ethyl acrylate will be removed by reaction with photochemically produced hydroxyl radicals (11.8 h half-life) and ozone (33 h half-life). When released to water, ethyl acrylate will volatilize to air (Henry's law constant of 25 Pa m³ mol⁻¹) or be biodegraded (57% removal in 28 days). Based on its relatively low octanol–water partition coefficient (log K_{ow} of 1.18), ethyl acrylate does not pose a significant bioaccumulation hazard.

Ecotoxicology

Ethyl acrylate is acutely toxic to aquatic organisms. In a series of studies with analytically measured concentrations, ethyl acrylate exhibited a 96 h LC_{50} of 4.6 mgl⁻¹ in freshwater fish (rainbow trout), a 48 h

 LC_{50} (immobilization) of 7.9 mgl⁻¹ in an aquatic invertebrate (*Daphnia magna*), and an 96 h EC₅₀ (growth rate) of 5.5 mgl⁻¹ in algae (*Selenastrum capricornutum*).

Other Hazards

Ethyl acrylate is flammable with lower explosive limit of 1.8% by volume in air.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) for ethyl acrylate range from 5 to 20 ppm as an 8 h time-weighted average (TWA), with 5 ppm being the predominant value as in the case of the TWA OEL established by the American Conference of Governmental Industrial Hygienists (ACGIH). International short-term exposure limits (STELs) range from 10 to 80 ppm, with 15 ppm being the predominant value as in the case of the STEL established by the ACGIH. The US Occupational Safety and Health Administration lists a permissible exposure limit of 28 ppm for ethyl acrylate (TWA). The National Institute of Occupational Safety and Health indicates 300 ppm ethyl acrylate is immediately dangerous to life or health. Ethyl acrylate is classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer. Ethyl acrylate has been delisted by the US NTP as reasonably anticipated to be a human carcinogen because (1) rat forestomach tumors were seen only when ethyl acrylate was administered by gavage at high concentrations that resulted in marked local irritation and cellular proliferation, (2) animal studies by other routes of exposure, including inhalation, were negative, and (3) chronic exposure of humans to such high concentrations of ethyl acrylate is unlikely.

See also: Carboxylesterases; Respiratory Tract.

Further Reading

Sweeney LM, Andersen ME, and Gargas ML (2004) Ethyl acrylate risk assessment with a hybrid computational fluid dynamics and physiologically based nasal dosimetry model. *Toxicological Sciences*.

Relevant Websites

- http://www.epa.gov Ethyl Acrylate (from the US EPA's Air Toxics Website).
- http://www.bibra.co.uk Toxicity Profile for Ethyl Acrylate (from BIBRA International Ltd., Carshalton, Surrey, UK).

Ethyl Benzene

William S Utley

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 100-41-4
- SYNONYMS: Phenylethane
- Chemical Formula: C_8H_{10}
- CHEMICAL STRUCTURE:



Uses

Ethyl benzene is used as an industrial solvent, and as a component in automotive and aviation fuels. The majority of ethyl benzene is used in the production of styrene.

Exposure Routes and Pathways

The primary exposure route for ethyl benzene is via inhalation and the skin. Ethyl benzene is known to cross the placental barrier but has not been established as a reproductive hazard.

Toxicokinetics

Ethyl benzene distributes to the adipose tissues. It is metabolized to mandelic acid (64%) and phenyl-glyoxylic acid (25%). The percentage of metabolites may vary according to the route of exposure with mandelic acid formation being favored with inhalation. The primary route of excretion is via the urine. Experimental evidence indicates that the percutaneous absorption rate of ethyl benzene is $37 \,\mu g \, \mathrm{cm}^{-2}$.

Mechanism of Toxicity

Ethyl benzene is an inducer of the cytochrome P450 and cytochrome c reductase enzyme systems. Ethyl benzene acts as a mitochondrial-uncoupling agent. It is believed that ethyl benzene metabolites are capable of interfering with dopamine catabolism in the brain. The tuberoinfundibular dopaminergic system may be a target for these metabolites.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ethyl benzene is extremely irritating in animal studies. Repeated skin application has caused blisters. Inhalation or ingestion of high concentrations has led to central nervous system (CNS) depression with death attributed to depression of the respiratory center. Pathological observations include pulmonary edema and generalized visceral hyperemia. The oral LD_{50} for ethyl benzene is 3500 mg kg⁻¹. The dermal LD_{50} in rabbits is \geq 5000 mg kg⁻¹ and the LC₅₀ is 4000 ppm (4 h exposure).

Human

Ethyl benzene is irritating to the eyes and skin. Concentrations of 200 ppm ethyl benzene are known to be irritating to the eyes of humans. Dermal application has led to erythema and inflammation of the skin. Ethyl benzene is the most irritating of the benzene series of compounds tested. Inhalation of high concentrations may cause CNS excitation followed by depression.

Chronic Toxicity (or Exposure)

Animal

Exposure to high concentrations has led to increase liver and kidney weights in experimental animals.

Human

Prolonged exposure may lead to functional pulmonary changes. These may be expressed as an increase in deep reflexes and irritation to the upper respiratory tract. Prolonged exposure has also led to hepatbiliary complaints. While there have been complaints of leukopenia and lymphocytosis, unlike benzene, ethyl benzene does not appear to cause bone marrow problems.

Clinical Management

General life support should be maintained, symptoms treated, and decontamination undertaken if necessary. Persons at special risk are those with impaired pulmonary functions, particularly obstructive airway diseases. The irritant properties of ethyl benzene may exacerbate these preexisting respiratory conditions. Additionally, predisposed groups include persons with liver, nervous system disorders, blood and hemopoietic disorders, and women with ovulation and menstrual cycle disorders.

Environmental Fate

Ethyl benzene is expected to volatilize from surface water and soils where it can undergo degradation via photooxidation. With a K_{oc} of 520 there will be only moderate binding to the soil giving ethyl benzene a tendency to migrate in the soil column. Ethyl benzene will degrade in an aqueous environment with reported values ranging from 10 to 16 days. Ethyl benzene has a low potential to bioconcentrate with reported bioconcentration factors (BCFs) ranging form 0.67 to 15.

Ecotoxicology

Ethyl benzene is only moderately aquatically toxic expressing LC_{50} values in the range of 40–100 mg l⁻¹ for bluegill, fathead minnow, and grass shrimp.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 100 ppm (435 mg m⁻³) (8 h time-weighted average), based on irritation. The National Institute for Occupational Safety and Health short-term exposure limit (15 min exposure limit) is 125 ppm (543 mg m⁻³), based on irritation. The odor threshold is 8.7 ppm. Hazardous waste number F003. The acceptable daily intake (US Environmental Protection Agency (EPA)) is 1.6 mg day⁻¹. The EPA oral reference dose (Rfd) for ethyl benzene is 0.1 mg kg⁻¹ day⁻¹. The oral Rfd

Ethyl Bromide

Kathryn A Wurzel

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- Chemical Abstracts Service Registry Number: CAS 74-96-4
- Synonyms: Bromoethane; Bromic ether; Halon 2001; Hydrobromic ether; Monobromoethane
- Chemiical/Pharmaceutical/Other Class: Halogenated aliphatic hydrocarbons
- CHEMCIAL FORMULA: C₂H₅Br
- CHEMICAL STRUCTURE:



is based on liver and kidney toxicity observed in a subchronic rodent experiment.

Ethyl benzene is listed under section 111/112 of the Clean Air Act and is listed under section 304/307/ 311 of the Clean Water Act.

International Agency for Research on Cancer has classified ethyl benzene as a category 2B (possibly carcinogenic to humans, based on inadequate human data and sufficient animal data).

American Conference of Governmental Industrial Hygienists classifies ethyl benzene as an A3-confirmed animal carcinogen.

The comprehensive environmental response, compensation and liability act (CERCLA) reportable quantity is 1000 lb.

Ethyl benzene is listed under emergency planning and community right to know act (EPCRA) under section 313.

The European Union classifies ethyl benzene as Xn: R-20 (harmful by inhalation).

See also: Diesel Fuel; Jet Fuels.

Further Reading

Ethylbenzene. IARC Mongr Eval Carcinog Risks Hum 2000; 77: 227–266.

Tang W, Hemm I, and Eisenbrand G (2000) Estimation of human exposure to styrene and ethylbenzene. *Toxicology* 144(1–3): 39–50.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ethyl Benzene.

Uses

Ethyl bromide is used as a refrigerant, an ethylating agent in organic synthesis, a solvent in the chemical and pharmaceutical industries, a grain and fruit fumigant, and in ethylation of gasoline. It was earlier used as an anesthetic, both topically and by inhalation.

Exposure Routes and Pathways

Exposure to ethyl bromide may occur through oral, dermal, and inhalation exposures.

Toxicokinetics

Ethyl bromide may be hydrolyzed to a significant degree resulting in the formation of inorganic bromides. Glutathione S-alkyl transferase catalyzes conjugation in which the halogen group is replaced. Further metabolism then occurs to alkyl mercapturic acid sulfoxides. Ethyl bromide crosses the placenta. Unchanged ethyl bromide accounted for $\sim 70\%$ of the dose in the expired air of rats dosed via gavage.

Mechanism of Toxicity

Ethyl bromide causes irritation and has a tendency to cause fatty degeneration of the liver, renal tissue, and the heart.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ethyl bromide is moderately toxic by ingestion and intraperitoneal routes and mildly toxic by inhalation. It is an eye and skin irritant.

Guinea pigs exposed to 2.4% ethyl bromide in air for 30 min experienced some delayed deaths and pathological changes in lungs, liver, spleen, and kidneys. Ethyl bromide is a central nervous system (CNS) depressant causing pulmonary congestion, centrilobular necrosis of the liver, and diffuse nephritis.

Human

Ethyl bromide is markedly irritating to the respiratory tract and the eyes. It is moderately toxic by ingestion but bromide poisoning following acute ingestion is rare. Acute effects of ethyl bromide include CNS depression, coma, hypotension, tachycardia, respiratory distress, nausea and vomiting, headache, and vertigo. Ethyl bromide can produce acute congestion, edema, and liver and kidney damage. Fever may also occur. Serum bromide concentrations $> 50-100 \text{ mg dl}^{-1}$ are usually associated with signs of toxicity; bromide levels greater than 200 mg dl^{-1} are uniformly associated with signs of toxicity. There is significant interpatient variation in severity of symptoms at a given bromide level. Aftereffects of severe exposure may occur up to 30 h after the exposure has ceased.

Acute or chronic exposure may result in redness of face, dilation of pupils, and a rapid pulse. The former use of ethyl bromide as a human anesthetic produced respiratory irritation and caused some fatalities, either immediately due to respiratory or cardiac arrest or from delayed effects as evidenced at autopsy by pulmonary edema and fatty degeneration of the liver, kidney, and heart.

Chronic Toxicity (or Exposure)

Animal

A 2 year study indicated some evidence of carcinogenicity in male rats (pheochromocytomas of the adrenal gland and neoplasms of brain and lung) and equivocal results in female rats (neoplasms of brain and lung) and male mice (lung neoplasms). Female mice experienced an increase in uterine cancer.

Human

Chronic ingestion of excessive amounts of ethyl bromide may produce a toxic syndrome known as 'bromism'. The symptoms are behavioral changes, irritability, headache, confusion, anorexia, weight loss, lethargy, muscular weakness, and slurred speech. Incontinence may develop with chronic intoxication. Chronic intoxication usually develops over 2–4 weeks or longer and the condition is of long duration with symptoms disappearing slowly.

Individuals with certain medical conditions may be at increased risk from ethyl bromide exposure: skin disease, liver disease, kidney disease, chronic respiratory disease (particularly obstructive airway diseases), and cardiac disease with arrhythmias.

Dermal exposure to ethyl bromide can result in bromoderma, which is an erythematous, nodular, or acneform rash over the face and possibly the entire body.

Bromides cross the placenta and may be detected in the milk of nursing mothers. Case reports suggest that prenatal exposure may cause growth retardation, craniofacial abnormalities, and developmental delay.

No epidemiological data relevant to the carcinogenicity of ethyl bromide are available.

Clinical Management

Acute oral exposure is generally treated by emesis. Emesis is most effective when initiated within 30 min of ingestion.

Chronic overexposure is treated by rehydration and administration of sodium chloride (NaCl; salt) intravenously until symptoms are alleviated. Bromide clearance may be increased by rehydrating in conjunction with administration of diuretics. Severe cases may require hemodialysis.

Environmental Fate

Ethyl bromide is likely to be a vapor in the atmosphere where it may be degraded by reaction with photochemically produced hydroxyl radicals. It is moderately mobile in soil. Volatilization is expected to be the most important fate process in soil and water although ethyl bromide is susceptible to hydrolysis in wet soil. It is not expected to adsorb to soil or sediment, or bioaccumulate to any great extent.

See also: Bromine; Gasoline.

Ethylamine

Dale J Marino

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 75-04-7
- SYNONYMS: Ethanamine; Monoethylamine; Aminoethane; 1-Aminoethane
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Amine
- Chemical Formula: C_2H_7N
- Chemical Structure: C₂H₅NH₂

Uses

Ethylamine is primarily used in the production of triazine herbicides. Ethylamine has also found use in resin chemistry, oil refining, and solvent extraction; as a stabilizer for latex rubber; and in the manufacture of dyestuffs, medicinals, corrosion inhibitors, urethane foam catalysts, and agents used in washand-wear fabrics.

Background Information

Ethylamine has been reported to occur in various fresh fruits and vegetables, grains, coffee, various cheeses, and fish. Ethylamine also occurs in the environment as a result of amino acid decomposition.

Exposure Routes and Pathways

Exposure to ethylamine primarily occurs in occupational settings. Given ethylamine's high vapor pressure of over 1 atm at 25° C, such exposures typically occur via inhalation, although dermal contact (and to a lesser extent ocular contact) with aqueous solutions of ethylamine would also be possible. The general population is potentially exposed to low concentrations of ethylamine by ingestion from food and by inhalation from releases to air.

Further Reading

- Bromoethane. Inter-Organization Programme for Sound Management of Chemicals (IOMC) (2002) Available from the World Health Organization, Distribution and Sales Service, 1211 Geneve 27, Switzerland, 2002. iv, 26P. 71 ref.
- IARC (1999) Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 71, p. 1307.World Health Organization, International Agency for Research on Cancer, Geneva.

Toxicokinetics

Ethylamine is not as readily metabolized as methylamine, and portions are excreted unchanged from the lung and in the urine. The metabolism of ethylamine is believed to occur in two stages. The amino group is initially dehydrogenated to an intermediate imine (ethyl imine), which reacts spontaneously with water, forming the corresponding aldehyde (acetaldehyde) and ammonia. The final metabolic products of acetic acid and urea are excreted in the urine. Ethylamine is a normal constituent of mammalian and human urine.

Mechanism of Toxicity

The toxic effects of ethylamine are due primarily to its corrosive action on tissues.

Acute and Short-Term Toxicity (or Exposure)

Ethylamine is severely irritating to the eyes, skin, and respiratory tract.

Animal

The acute rat oral LD_{50} is 400 mg kg⁻¹ and the acute dermal LD_{50} in rabbits is 390 µg l⁻¹ (270 mg kg⁻¹). The reported 1 h inhalation LC_{50} in rats is 5540 ppm (3010 mg m⁻³). A 70% aqueous solution reportedly produced prompt skin burns, leading to necrosis when applied dermally to guinea pigs. Severe skin irritation with extensive necrosis and deep scarring were noted after a 2 h exposure. Severe eye irritation was observed in rabbits following instillation of 0.5 ml of 1% aqueous ethylamine.

Human

Exposure to elevated concentrations of various amines, and presumably ethylamine, is known to cause transient visual disturbance called glaucopsia, which is often referred to as blue haze or halovision. Exposure to elevated levels of ethylamine is expected to potentially cause severe eye and respiratory tract irritation. Dermal contact with aqueous ethylamine is expected to cause severe skin irritation with possible skin burns. Ingestion of aqueous ethylamine is expected to cause burns of the mouth, throat, esophagus, and stomach with possible perforation of the esophagus and stomach.

Chronic Toxicity (or Exposure)

Animal

Repeated exposure of rabbits to 50 ppm ethylamine for $7 h day^{-1}$, $5 day week^{-1}$ for 6 weeks produced corneal erosion and lung irritation. Pulmonary irritation and kidney effects were noted at 100 ppm.

Ten exposures of rats to 250 or 1000 ppm ethylamine for $6 h day^{-1}$ produced slight or moderate necrotizing irritation of the respiratory tract, respectively.

Repeated exposure of rats to 500 ppm ethylamine for 6 h day $^{-1}$, 5 days week $^{-1}$ for 24 weeks produced inflammatory necrosis and squamous metaplasia in anterior portions of the nasal cavity. No lesions were observed in the nasal cavities of rats in 0, 10, or 100 ppm exposure groups.

Human

Repeated exposures to higher concentrations are expected to produce irritation of the eyes, nose, and throat. Repeated exposures could potentially aggravate existing respiratory diseases.

In Vitro Toxicity Data

In vitro assays with ethylamine yielded negative (not mutagenic) results in the *Salmonella*/microsome reverse mutation assay, and an increase in sister chromatid exchanges in Chinese hamster V79 cells.

Clinical Management

Exposed skin and eyes should be irrigated with copious amounts of water. After inhalation exposures, the victim should be moved to fresh air and monitored for respiratory distress. Humidified, supplemental oxygen (100%) should be administered, with assisted ventilation as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gasses. If pulmonary edema is present, positive-end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, emesis or lavage should be avoided. Use of diluents is controversial. Delayed abdominal pain and tenderness or shock may indicate gastric or esophageal perforation.

Environmental Fate

Given its high vapor pressure of over 1 atm at 25°C, ethylamine will remain in the vapor phase if released to the atmosphere where it will react with photochemically produced hydroxyl radicals ($T_{1/2}$ of ~14 h). Dissolution into rain droplets is also thought to be an important removal process. Other atmospheric removal processes, for example, photolysis and hydrolysis, are not significant.

The predominant form of ethylamine under environmental conditions is the ionized (protonated) species, which is expected to bind to soil constituents, suspended sediments, and bed sediments to a greater degree than the neutral form. As such, migration from soil to groundwater is expected to be less than would be anticipated for the neutral form. Volatilization from moist soils or surface water is not expected to be an important fate process. Biodegradation is expected to be an important loss process in both soil and water. The potential for bioconcentration in aquatic biota is low.

Ecotoxicology

The reported no-observed-effect concentration (NOEC) in algae (*Scenedesmus*) is $10 \text{ mg} \text{l}^{-1}$. The 24 h NOEC and EC₅₀ values in crustaceans (*Daphnia magna*) are 31–52 and 94–110 mg l⁻¹, respectively. A nonlethal concentration of $30 \text{ mg} \text{l}^{-1}$ has been reported in fish (creek chub) with a 96 h LC₅₀ of 40–240 mg l⁻¹ (goldfish).

Other Hazards

High airborne concentrations of ethylamine can form, given its vapor pressure, with the potential for severe eye, nose, and respiratory tract irritation; escape impairment; and possible death. The immediately dangerous to life or health (IDLH) concentration for ethylamine is 600 ppm. Anhydrous ethylamine is a flammable gas; aqueous ethylamine is a flammable liquid. Vapors can travel a considerable distance to an ignition source and flash back because ethylamine vapor density is heavier than air.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for ethylamine include the following:

- USA: Occupational Safety and Health Administration permissible exposure limit (Table Z-1) is 10 ppm (18 mg m⁻³).
- USA: National Institute for Occupational Safety and Health recommended exposure limit is 10 ppm (18 mg m⁻³).
- USA: American Conference of Governmental Industrial Hygienists threshold limit value is 5 ppm (9 mg m⁻³), with a 15 min short-term exposure limit of 15 ppm (27 mg m⁻³) (skin notation).
- Australia: 10 ppm.

- Federal Republic of Germany: 10 ppm (skin notation).
- Sweden: 10 ppm, with a short-term value of 15 ppm (skin notation).
- United Kingdom: 2 ppm with a short-term exposure level of 6 ppm.

See also: Corrosives; Respiratory Tract.

Relevant Websites

- http://www.osha.gov Ethylamine (Health Guidelines from the US Occupational Safety and Health Administration).
- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethylamine.

Ethylene Glycol

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 107-21-1
- SYNONYMS: 1,2-Dihydroxyethane; 1,2-Ethanediol; Ethane-1,2-diol; Ethylene alcohol; Glycol alcohol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Glycols
- Chemical Formula: $C_2H_6O_2$
- CHEMICAL STRUCTURE:



Uses

Ethylene glycol has numerous industrial and commercial applications. A major use is in antifreezecoolant mixtures. It is also used in heat-transfer fluids, airport deicing fluids, hydraulic brake fluids, printers' inks, wood stains, adhesives, pesticides, and as a solvent in various other chemicals.

Exposure Routes and Pathways

The primary risk for ethylene glycol toxicity is through gastrointestinal exposure. Exposure can also occur by dermal, ocular, and inhalation routes.

Toxicokinetics

Following ingestion, ethylene glycol is rapidly absorbed and distributed. The volume of distribution is $0.5-0.81 \text{ kg}^{-1}$. The ethylene glycol elimination halflife $(t_{1/2})$ is 3–9 h. This $t_{1/2}$ is prolonged to 11–18 h during ethanol and fomepizole therapy. Metabolism occurs primarily in the liver and kidneys. Ethylene glycol is first metabolized by alcohol dehydrogenase (ADH) to glycol aldehyde. Glycol aldehyde is then metabolized by aldehyde dehydrogenase to glycolic acid. Glycolic acid is converted to glyoxylic acid, which is the rate-limiting step in the metabolism. Glyoxylic acid is then metabolized to oxalic acid, α -hydroxy- β -ketoadipic acid, or glycine. Ethylene glycol and its metabolites are subsequently excreted in the urine.

Mechanism of Toxicity

Ethylene glycol and glycoaldehyde have an intoxicating effect on the central nervous system that can lead to ataxia, sedation, coma, and respiratory arrest. The metabolic acidosis reported in toxicity is due to the acidic metabolites, especially glycolic acid. Ethylene glycol itself may result in a large osmolar gap. Oxalic acid may combine with calcium to form calcium oxylate crystals. The precipitation of these crystals in tissue may result in renal failure and hypocalcemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ethylene glycol causes toxicity in animals similar to the toxicity seen in humans. The clinical management is similar to that described above.

Human

Acute exposure to ethylene glycol usually occurs when single large doses are ingested either accidentally or after intentional suicide attempts. Ingestion of ethylene glycol may initially lead to a state of intoxication similar to ethanol intoxication. This is associated with ataxia, slurred speech, nystagmus, somnolence, coma, and apnea. Ethylene glycol intoxication may lead to an elevated osmol gap due to ethylene glycol itself. A low or normal osmol gap does not rule out ethylene glycol toxicity. As metabolism occurs, the osmol gap begins to diminish and an anion gap metabolic acidosis becomes more pronounced. Renal effects include oliguria, acute tubular necrosis, and renal failure. Other reported clinical effects include mydriasis, focal nerve paralysis, seizures, cardiac disturbances, and cerebral and pulmonary edema. Hypocalcemia may result in tetany, hyperreflexia, and dysrhythmias. Inhalation or eye contact with ethylene glycol vapor may cause upper respiratory tract and eye irritation.

Chronic Toxicity (or Exposure)

Animal

Rats fed 1–2% ethylene glycol in their diets over 2 years demonstrated decreased life span, calcium oxalate bladder stones, plus renal and hepatic injuries.

Human

Human volunteers exposed to ethylene glycol for 20–22 h a day of 1.4–27 ppm developed only mild symptoms of throat irritation, mild headache, and low backache.

In Vitro Toxicity Data

No mutagenic activity was demonstrated in Ames *Salmonella* tests of ethylene glycol.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Because of the rapid absorption

of ethylene glycol, gastrointestinal decontamination procedures are rarely indicated. Charcoal does not bind ethylene glycol and should not be administered. Administration of ethanol or fomepizole should be considered in any symptomatic patient or any patient with a history of significant ingestion. Both ethanol and fomepizole competitively inhibit ADH and prevent metabolism of ethylene glycol to its toxic metabolites. Careful correction of fluid and electrolyte abnormalities is essential. The clinician should insure adequate urine output, but forced diuresis should be avoided. Administration of sodium bicarbonate infusions should be considered in patients manifesting significant acidosis. Administration of thiamine and pyridoxine should be considered as these agents potentially enhance metabolism of ethylene glycol to α -hydroxy- β -ketoadipic acid and glycine, respectively, both of which are less toxic than oxalic acid. Hemodialysis effectively increases clearance and improves fluid/electrolyte balance. This extracorporeal method of elimination should be considered in patients with acute mental status changes, renal failure, significant metabolic acidosis, or serum levels over 50 mg dl⁻¹. Inhalation exposures to ethylene glycol mist should be monitored for respiratory tract irritation. Humidified supplemental 100% oxygen should be administered. Exposed skin and eyes should be treated with irrigation and supportively.

Environmental Fate

Release of liquid ethylene glycol into the environment would be expected to result in volatilization of the substance. In the atmosphere, ethylene glycol is broken down photochemically to produce hydroxyl radicals with ~ 2 day half-life. Release into soil results in near complete aerobic biodegradation within 4 days. Under anaerobic conditions, complete degradation is expected within 7 days.

See also: Ames Test; Ethanol; Pyridoxine; Thiamine.

Further Reading

- Barceloux DG, Krenzelok EP, and Olson K (1999) American academy of clinical toxicology practice guidelines on the treatment of ethylene glycol poisoning. *Journal of Toxicology – Clinical Toxicology* 36: 537–560.
- Jacobsen D, Sebastian CS, and Barron SK (1990) Effects of 4-methylpyazole, methanol/ethylene glycol antidote, in healthy humans. *Journal of Emergency Medicine* 8: 455–461.

Ethylene Glycol Monoethyl Ether

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 110-80-5
- SYNONYMS: 2-Ethoxyethanol; Cellosolve; Dowanol EE; Ektasolve EE; Ethyl cullosolve; Ethylene glycol ethyl ether; Glycol ethyl ether; Glycol monoethyl ether; Hydroxy ether; Jeffersol EE; Oxitol; Poly-solv EE
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Glycol
- CHEMICAL FORMULA: C₄H₁₀O₂
- CHEMICAL STRUCTURE: HOCH₂CH₂OC₂H₅

Uses

Major uses include its use as a component of natural and synthetic resins; metal solvent for formulation of soluble oils; solvent for lacquers, lacquer thinners, dyeing, textiles, and varnish removers; carrier for printing ink; wafer fabrication process for semiconductor manufacturing; and anti-icing additive for aviation fuels.

Exposure Routes and Pathways

Exposure may occur by inhalation of the vapor, ingestion, and dermal contact.

Toxicokinetics

Ethylene glycol monoethyl ether (EGEE) is a colorless and nearly odorless liquid. It is miscible in aqueous and organic solutions, has a low vapor pressure, and is readily absorbed through skin, lungs, and gastrointestinal tract. It is metabolized by alcohol dehydrogenase to alkoxyacetic acids, primarily ethoxyacetic acid. The metabolites of EGEE are renally excreted. Less than 1% of absorbed EGEE is eliminated unchanged through the lungs. In one study, peak ethoxyacetic acid levels occurred 4 h after inhalation exposure with a terminal half-life of ~ 24 h.

Mechanism of Toxicity

The potential toxicity of EGEE is believed to be due to its metabolites. The exact mechanism has not been clearly defined.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals acutely exposed to large quantities of EGEE developed dyspnea, weakness, paralysis, acute nephrosis, pulmonary edema, gastrointestinal hemorrhage, and intrauterine demise. Studies suggest a teratogenic potential of EGEE, predominantly affecting the cardiac, neurologic, and skeletal systems. The oral LD_{50} was $3 g kg^{-1}$ in rats, $3.1 g kg^{-1}$ in rabbits, and $1.4 g kg^{-1}$ in guinea pigs.

Human

Reports of EGEE toxic exposures in humans are limited. EGEE is low in oral toxicity. It is not significantly irritating to the skin. It is potentially irritating to the eyes and mucous membranes depending upon the concentration. Potential central nervous system effects are similar to effects manifested by other solvents and include headache, drowsiness, weakness, staggering gait, tremor, and blurred vision. Aspiration pneumonitis may occur if EGEE is ingested. Unlike ethylene glycol, metabolic acidosis and urine oxalate crystals do not typically develop. Preliminary studies have suggested a potential for reproductive toxicity. No studies have definitively found carcinogenic activity of EGEE in humans.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in mice over 2 years demonstrated almost no effects in doses up to 5%.

Human

Studies of painters exposed to EGEE have demonstrated increased presence of oligospermia, azospermia, and increased odds ratio for lower sperm count.

In Vitro Toxicity Data

Mutagenicity and carcinogenicity studies using the Ames *Salmonella* and *Escherichia coli* tests have been negative for EGEE.

Clinical Management

Exposure victims should be moved immediately to fresh air. Standard emergency supportive care should be provided. Exposed eyes and skin should be rapidly and copiously flushed. Contaminated clothing should be removed. Charcoal and syrup of ipecac should be avoided as their efficacies in EGEE toxicity have not been demonstrated and these agents may increase the risk of pulmonary aspiration. The role of ethanol or of fomepizole as competitive inhibitors of alcohol dehydrogenase for EGEE toxicity has not clearly delineated. Theoretically, either agent may be of benefit to prevent formation of EGEE metabolites. Hemodialysis may be considered for substantial exposures in patients who have developed profound symptoms (e.g., metabolic acidosis).

Environmental Fate

EGEE is produced in large quantities for industrial purposes. Release of EGEE into soil and water should result in rapid degradation, based on biodegradation studies.

See also: Ethanol; Ethylene Glycol; LD₅₀/LC₅₀ (Lethal Dosage 50/Lethal Concentration 50).

Further Reading

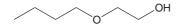
Johanson G (2000) Toxicity review of ethylene glycol monomethyl ether and its acetate ester. *Critical Reviews in Toxicology* 30: 307–345.

Ethylene Glycol Mono-n-Butyl Ether

Bradford H Strohm, Leonard I Sweet, Sharmilee P Sawant, and Harihara M Mehendale

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: 111-76-2
- SYNONYMS: 2-Butoxyethanol; Butyl cellosolve; EGBE; Butyl glycol; Butyl oxitol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Glycol ether
- CHEMICAL FORMULA: C₆H₁₄O₂
- CHEMICAL STRUCTURE:



Uses

Ethylene glycol mono-*n*-butyl ether (EGBE) is a widely used solvent present in many surface coatings, lacquers, enamels, varnishes, varnish removers, inks, and latex paints. It is also used in metal cleaning formulas, commercially available household cleaners, and herbicide components.

Exposure Routes and Pathways

Exposure to EGBE can occur via inhalation, ingestion, or skin absorption. Inhalation is the primary route of human exposure. Exposure of the general population can also occur from dermal absorption during the use of products containing EGBE.

Toxicokinetics

EGBE is rapidly absorbed, distributed, metabolized, and eliminated. The uptake and elimination of EGBE

is expected to be linear. The urine is the primary route of excretion followed by expiration in the form of the metabolite CO_2 . The carboxylic acid, 2-butoxyacetic acid (BAA), is the major urinary metabolite of EGBE. Butoxyacetic acid is formed by oxidation of the alcohol moiety of EGBE through alcohol dehydrogenase and aldehyde dehydrogenase sequentially. Alternate pathways include O-dealkylation to ethylene glycol and conjugation to EGBE glucuronide and/or EGBE sulfate. In human, but not animal studies, the amino acid conjugate of EGBE, *N*-butoxyacetylglutamine, has been identified.

Rats administered EGBE in drinking water excrete 50-60% of the consumed dose in the urine as BAA and exhale 8-10% as CO₂. As dose increases, the proportion of EGBE conjugated with glucuronic acid also increases. Similarly, oxidative dealkylation of EGBE to form ethylene glycol also becomes a more prevalent route of metabolism as dose increases.

The relatively high clearance rate and low mean residence time of EGBE suggests that accumulation potential in the body is low. Elimination of EGBE and BAA following repeated inhalation exposure or dermal uptake appears to be dependent upon species, age, time of exposure, sex, and exposure concentration.

Mechanism of Toxicity

The principal toxicological effect observed upon overexposure to EGBE is the destruction of red blood cells (i.e., hemolysis). BAA, the predominant oxidative metabolite of EGBE, appears responsible for this hemolytic activity. It has been speculated that BAA may interact with red blood cell membranes disrupting erythrocyte osmotic balance, leading to cellular swelling, loss of deformability, and eventually hemolysis. In studies with male rats, treatment with alcohol dehydrogenase inhibitors protected against EGBE-induced hematotoxicity and inhibited EGBE metabolism to BAA. Another event in the mechanism of action is compensatory erythropoiesis, where as a response to the loss of erythrocytes, the bone marrow increases production of young red blood cells.

In vitro studies have indicated that the red blood cells of rats, mice, rabbits, and baboons are susceptible to hemolysis by BAA, whereas blood from pigs, dogs, cats, guinea pigs, and humans are resistant. A number of other studies have confirmed these results in vitro, in red blood cells from a large cross section of the human population, including those with hereditary red cell disease (i.e., sickle cell and spherocytosis) and the aged. These studies indicate that human cells are not as susceptible to hemolysis as rat cells tested under similar conditions. These findings suggest that humans exposed to equivalent doses of EGBE would not be expected to exhibit the same spectrum or severity of hematotoxic-related effects as those produced in rats. In vitro experimental results also suggest that red blood cells are more sensitive to hemolysis by BAA than to hemolysis by EGBE.

Acute and Short-Term Toxicity (or Exposure)

Animal

EGBE acute toxicity has been studied in numerous species via all routes of exposure. In general, overexposed animals exhibited inactivity, weakness, and difficulty breathing, while autopsies revealed congested lungs and kidneys. The principal effect observed leading to death in these acute toxicity studies was damage to the kidneys. Kidney, hematologic, and central nervous system effects have been observed in experimental animals exposed to EGBE, with hemolysis as an initial and sensitive indicator of overexposure. Rats are the most sensitive species and older rats are more sensitive than younger animals to the hemolytic effects of EGBE and its metabolites.

Oral LD_{50} values ranged from 900 mg kg⁻¹ in rabbits to 250 mg kg⁻¹ in rats; dermal LD_{50} values were ~1500 mg kg⁻¹ in rabbits; and inhalation LC_{50} values (4–8 h exposures) were in the vicinity of 500 ppm for cats, guinea pigs, and rats.

Human

EGBE is of low to moderate acute toxicity in humans. Clinical tests and reports from occupational exposures indicate EGBE is an irritant when inhaled. EGBE is considered a skin absorber, with the possibility of significant uptake through the skin. EGBE has not been found to be a sensitizer in clinical tests. Metabolic acidosis, hypokalemia, and hemoglobinuria paralleled by progressive erythropenia, have been reported in individuals poisoned through ingestion of materials containing EGBE. Human volunteers exposed to 98–200 ppm EGBE for 4-8 h reported nasal and ocular irritation and disturbed taste (e.g., metallic taste). No abnormalities were detected in blood pressure, pulse rate, erythrocyte fragility, urinary glucose, or albumen. The estimated immediately dangerous to life or health air concentration is 700 ppm. Severe toxicity has been described in adults who ingested 30-60 ml of pure EGBE; and the estimated lethal oral dose is \sim 1.4 ml kg⁻¹.

Chronic Toxicity (or Exposure)

Animal

The subchronic toxicity of EGBE has been examined in animals via oral, inhalation, and dermal routes of exposure. The lowest no-observed-effect level (NOEL) in an oral subchronic study was 80 mg kg^{-1} for rats administered EGBE in feed over a 90 day period. Inhalation exposure of rats for 13 weeks, 6 h day⁻¹, 5 days week⁻¹ to EGBE vapors at 25–77 ppm indicated an NOEL of 25 ppm. In a 90 day dermal study of rabbits, EGBE was applied 6 h day⁻¹, 5 days week⁻¹ at doses up to 150 mg kg⁻¹. There was no evidence of systemic toxicity or skin irritation at the site of application at any of the dose levels tested.

Studies on male and female rats and mice conducted by the National Toxicology Program have yielded mixed results on carcinogenic potential: male rat (no evidence of carcinogenicity); female rat (equivocal evidence); male mice (some evidence); and female mice (some evidence). The relevance of these studies to humans is uncertain. The American Conference of Governmental Industrial Hygienists (ACGIH) classifies EGBE as a confirmed animal carcinogen with unknown relevance to humans.

The reproductive and developmental toxicity of EGBE in both male and female animals has been the subject of numerous investigations. EGBE has been found to have no-adverse-effects upon the male reproductive systems of mice or rats exposed orally at doses ranging from 222 to 2000 mg kg⁻¹, 5 days week⁻¹ for 5 or 6 weeks. Similarly, inhalation exposure of rats for 3 h produced no-observed-effects upon gross macroscopic postmortem examination.

EGBE has also been tested for effects upon the female reproductive system and the developing

embryo. In general, fetal toxicity has only been observed in animals at maternally toxic doses. Mice, rats, and rabbits have been exposed during gestation at doses of 4000 mg kg⁻¹ day⁻¹ (oral), 424 mg kg⁻¹ day⁻¹ (dermal), and 25–200 ppm 6 h day⁻¹ (inhalation). No teratogenic effects were observed in the litters of dams exposed to EGBE. Signs of maternal toxicity, including decreased body weight and body weight gain, were observed. At the maternal LD₂₀, EGBE did induce fetal deaths in rats. BAA, the metabolite of EGBE, was also studied and found to have no adverse effect upon the developing embryo *in vitro*.

Human

Long-term or repeated exposure may have effects on the hematopoietic system, resulting in blood disorders. The hematotoxicity in humans is characterized by decreased hemoglobin content, progressive erythropenia and hemoglobinuria.

Clinical Management

Management of individuals overexposed to EGBE begins with removing those individuals from the source of exposure, flushing eyes and skin with water, and removing contaminated clothing. The treatment of choice for acute and severe hemolytic anemia, which may result from overexposure to EGBE, is exchange transfusion. If renal failure develops as a consequence of red blood cell hemolysis, hemodialysis is the treatment of choice. In general, monitoring of blood counts, electrolytes, urine hemoglobin, urinary BAA levels, and blood gases may prove useful in assessing overexposure. Administration of ethanol as well as charcoal as a slurry have proven useful in therapeutic intervention after EGBE overexposure.

Environmental Fate

If released to the environment, EGBE is expected to preferentially partition to the soil and water. Bioconcentration and bioaccumulation potential are expected to be low, based on the estimated bioconcentration factor and experimental octanol water partition coefficient. If released to soil or water, aerobic degradation is expected to occur rapidly. Volatilization may be an important fate and transport process based on the Henry's law constant and vapor pressure. When released into the water, EGBE is expected to have a half-life of <10 days. When released into the air, it is expected to have a half-life of <1 day, and is expected to be removed from the air by wet deposition.

Ecotoxicology

Based on the available data, risk to aquatic organisms is low.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for EGBE include the following:

- ACGIH (20 ppm time-weighted average (TWA));
- Argentina (50 ppm TWA);
- Australia (25 ppm TWA);
- Belgium (25 ppm TWA);
- Brazil (39 ppm TWA; for a 48 h work week);
- Canada (25 ppm TWA);
- Chile (20 ppm TWA);
- Denmark (20 ppm TWA);
- Finland (20 ppm TWA);
- Germany DFG (80 ppm peak limitation);
- Mexico (26 ppm TWA);
- Sweden (10 ppm threshold limit value; lowest limit value);
- United Kingdom (25 ppm TWA); and
- United States Occupational Safety and Health Administration permissible exposure limit (50 ppm TWA).

Miscellaneous

EGBE is a colorless liquid with a mild ether odor. Odor is generally detected at concentrations from 0.1 to 0.35 ppm.

See also: Glycol Ethers.

Further Reading

- Agency for Toxic Substances and Disease Registry (1998) Toxicological Profile for 2-Butoxyethanol and 2-Butoxyethanol Acetate. Atlanta, GA: US Department of Health and Human Services, Public Health Service.
- Boatman R, Corley R, Green T, Klannig J, and Udden M (2004) Review of studies concerning the tumorigenicity of 2-butoxy-ethanol in B6C3F1 mice and its relevance for human risk assessment. *Journal of Toxicology and Environmental Health, Part B: Critical Reviews* 7(5): 385–398.
- Gualideri JF, deBoer L, Harris CR, and Corley R (2003) Repeated ingestion of 2-butoxyethanol: Case report and literature review. *Journal of Toxicology: Clinical Toxicology* 41(1): 57–62.

National Toxicology Program (2000) Technical Report on the Toxicology and Carcinogenesis Studies of 2-Butoxyethanol in F344/N Rats and B6C3F₁ Mice. Research Triangle Park, NC: US Department of Health and Human Services, Public Health Service.

Ethyleneimine

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 151-56-4
- SYNONYMS: Azocyclopropane; Dimethylenimine
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alkyl amines
- CHEMICAL FORMULA: C₂H₃N
- CHEMICAL STRUCTURE:

H N

Uses

Ethyleneimine has a broad range of applications that stem from its high reactivity. Ethyleneimine is used in the manufacture of triethylenemelamine (i.e., a precursor in plastic synthesis) and the polymer polyethyleneimine. It is also used in textile chemicals, adhesives, binders, petroleum refining chemicals, fuels, lubricants, coating resins, varnishes, lacquers, agricultural chemicals, cosmetics, ion exchange resins, photographic chemicals, surfactants, and as an alkylating agent.

Exposure Routes and Pathways

Contact with skin or mucous membranes (eyes and nasal) and inhalation are the routes of exposure.

Toxicokinetics

Ethyleneimine is absorbed readily by the oral, dermal, and inhalation routes. It penetrates the skin so quickly that its toxicity is not decreased even if the area of contact is washed 1 min after contact. Urinary excretion accounts for $\sim 50\%$ of administered doses.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for EGBE.

Mechanism of Toxicity

Ethyleneimine is an extremely reactive alkylating agent that undergoes ring opening reactions with cellular nucleophiles.

Acute and Short-Term Toxicity (or Exposure)

Animal

The inhalation LC_{50} (1 h exposure) was 185 ppm in rats, 150 ppm in mice, and 170 ppm in guinea pigs. The oral LD_{50} was 15 mg kg⁻¹ in rats and mice. Ethyleneimine exposure can result in extensive degeneration of the tissues but not sensitization. *In vivo* studies in mammalian systems have shown that ethyleneimine is strongly mutagenic to murine bone marrow cells *in vivo*.

Human

Breathing vapors causes nausea and vomiting, accompanied by a characteristic swelling of the face (mouth, eyelids, and throat). These symptoms disappear when the exposure ceases. Ethyleneimine can be very irritating to the skin, eyes, or mucous membranes. It is mildly corrosive to skin and mucous membranes. Fatal intoxication caused mainly by skin absorption has been observed.

Chronic Toxicity (or Exposure)

Animal

A study of rats receiving subcutaneous injections of ethyleneimine over 540 days found local (i.e., site of injection) sarcomas in some animals. Studies of mice receiving ethyleneimine by stomach tube for 2–4 weeks after birth, and then in the diet for 77–78 weeks found heptatomas and pulmonary tumors in many of the mice, and lymphomas in a small percentage of the mice. Another subcutaneous injection study in mice over 48 weeks of injections found sarcomas at the site of injection, along with some hepatomas, pulmonary tumors, and Harderian gland tumors over the 2 years of total observation.

Human

Exposure may cause cancer. The (US) Occupational Safety and Health Administration has categorized ethyleneimine as a carcinogen. The (US) National Institute for Occupational Safety and Health considers ethyleneimine to be a potential occupational carcinogen.

In Vitro Toxicity Data

Studies in viruses, prokaryotes, fungi, and algae were discussed. These systems have shown ethyleneimine to be strongly mutagenic. For example, studies in *Drosophila* have shown that ethyleneimine induces mitotic or meiotic chromosome aberrations and dominant or recessive lethal mutations. Ethyleneimine is strongly mutagenic to Chinese hamster ovary cells *in vitro*. Further, ethyleneimine has been shown to induce large numbers of chromatid type aberrations *in vitro* in human WI-36 cells and leukocytes. The DNA in cultivated lymphocytes is degraded indicating that ethyleneimine inhibits DNA repair system.

Clinical Management

In acute situations, the skin should be washed thoroughly with soap and water. If ingested an emetic should be administered or gastric lavage performed. Oxygen should be provided if breathing is difficult. If severe blood poisoning occurs, 1% methylene blue solution should be given at 1 ml kg^{-1} intravenously.

Environmental Fate

Ethyleneimine may be released to the environment as emissions or in wastewater connected with its manufacture and use. It is a reactive molecule; however, there are no data on its fate in environmental media. In the atmosphere, it should react with hydroxyl radicals (the estimated half-life is 1.5 days). If released in water, it will hydrolyze at neutral pH in about 5 months but it is apt to be lost much faster by evaporation or chemical reactions with metal ions. While it should rapidly evaporate from soil, it may also leach into the soil or complex with metal ions in the soil.

Ecotoxicology

There is no bioconcentration or bioaccumulation; however, it would not be expected to bioconcentrate in fish.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration (OSHA) has categorized ethyleneimine as a carcinogen, and has stated that worker exposure to ethyleneimine is to be controlled through the required use of engineering controls, work practices, and personal protective equipment, including respirators. Ethyleneimine is listed by the US Environmental Protection Agency as a hazardous air pollutant generally known or suspected to cause serious health problems.

See also: Carcinogenesis; Toxicity Testing, Mutagenicity.

Further Reading

- Bingham E, Corhssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn., vol. 4, pp. 1108–1112. New York: Wiley.
- International Agency for Research on Cancer (IARC) (1999) Aziridine. *IARC Monographs on Evaluation of Carcinogenetic Risks in Humans* 71: 337–344.

Ethylene Oxide

Ralph J Parod

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 75-21-8
- SYNONYMS: 1,2-Epoxyethane; Oxirane
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Epoxides
- Chemical Formula: C_2H_4O

Uses

Ethylene oxide is typically manufactured by the catalytic oxidation of ethylene. Most ethylene oxide is produced and consumed captively in the production of ethylene glycol. It is also used in the production of nonionic surfactants, polyester resins, and specialty solvents. A small percentage of ethylene oxide consumption is attributed to its use as a sterilant for medical devices and pharmaceuticals.

Exposure Routes and Pathways

Ethylene oxide is a gas at room temperature and pressure; therefore, inhalation is the primary route of exposure. Dermal exposures may occur to liquid ethylene oxide that exists at temperatures below 11°C; however, rapid evaporation minimizes the opportunity for absorption. Background exposures to ethylene oxide occur due to its presence in cigarette smoke and automobile exhaust as well as its conversion from ethylene normally present in the body as the result of metabolic processes and the consumption of plants where it is a natural hormone.

Toxicokinetics

Ethylene oxide is rapidly absorbed through the respiratory tract. Due to its high solubility in blood, uptake is largely dependent on the ventilation rate and the concentration of ethylene oxide in the inspired air. Studies in mice indicate that at ~0.5 ppm, ~100% of the inspired ethylene oxide is absorbed. At higher concentrations, the percentage absorbed decreases from 90% (10 ppm) to 68% (100 ppm) and falls to 36% at 1000 ppm. Humans exposed to ethylene oxide at levels ranging from ~0.1 to 10 ppm absorb 75–80% of the inspired ethylene oxide.

Absorbed ethylene oxide is rapidly distributed throughout the body. In mice exposed by inhalation to radiolabeled ethylene oxide, distribution was immediate, with the highest concentrations of ethylene oxide or its metabolites in the lungs, liver, and kidneys. After 4 h, levels in the liver and kidney had decreased and were comparable to those detected in the lungs, testes, spleen, and brain.

Ethylene oxide is metabolized by either conjugation with glutathione or hydrolysis by epoxide hydrolase. Metabolites from both pathways are excreted primarily in the urine, although some are further metabolized to CO_2 and exhaled via the lungs along with a small amount of unmetabolized ethylene oxide. While metabolism of ethylene oxide is qualitatively similar among species, the glutathione pathway appears to predominate in mice and rats while the epoxide hydrolase is the primary metabolic pathway in larger species, including man. The half-life of ethylene oxide in the blood of mice (3–12 min), rats (9 min), and humans (42 min) is relatively short.

Mechanism of Toxicity

The mechanisms of toxicity are not yet understood; however, it is likely that, in general, the toxic effects of ethylene oxide are due to its ability to react with cellular molecules, altering function. The carcinogenicity of ethylene oxide noted in experimental animals is probably due to its direct alkylation of nucleic acids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute 4 h inhalation LC50 values range from 835 ppm in mice to 1460 ppm in rats, with an intermediate value of 960 ppm in dogs. Oral exposures of rats and mice to ethylene oxide in water or corn oil have LD₅₀ values of $250-350 \text{ mg kg}^{-1}$. For both routes of exposure, the dose-response curve for mortality is steep. Effects associated with overexposure via inhalation include eye and respiratory tract irritations, central nervous system (CNS) depression, salivation, vomiting, incoordination, and convulsions. Deaths shortly after exposure are typically associated with pulmonary edema, while later deaths are thought to result from secondary lung infections with potential contribution from systemic toxicity. Study survivors may exhibit bronchitis, pneumonia, dyspnea, and muscle paralysis, particularly of the hindlegs. Histopathological examinations show damage to the lung, liver, kidney, spleen, and brain. Aqueous solutions of ethylene oxide are irritating to the skin but have not been associated with sensitization. Corneal opacities have been noted in some species.

Human

At high concentrations, ethylene oxide acts as an eye and respiratory irritant as well as a CNS depressant. Symptoms of overexposure include nausea, vomiting, and neurological effects. Pulmonary edema may result. Contact with liquid ethylene oxide or its solutions may result in irritation and burns as well as frostbite from evaporative cooling.

Chronic Toxicity (or Exposure)

Animal

Cancer is generally considered the critical endpoint for chronic exposures. In lifetime studies, rats exposed to airborne concentrations of 10, 33, or 100 ppm for 6 h day⁻¹, 5 days week⁻¹ exhibited several treatment-related tumors including mononuclear cell leukemia, peritoneal mesothelioma, and brain tumors. Ethylene oxide was also carcinogenic in mice. Greater exposures (>50 ppm) can cause reproductive toxicity (changes in sperm count, motility and morphology, increased postimplantation losses, decreased litter size) and neurotoxicity (abnormal gait, paralysis, axonal degeneration). Lung damage (edema, pneumonia) is associated with exposures in the range of 100–300 ppm. Very large exposures (>900 ppm) can produce teratogenicity.

Human

Studies of chronically exposed populations suggest that ethylene oxide may cause allergic contact dermatitis and cataracts. Neuropsychological, peripheral, and central nervous system deficits have been reported in hospital workers thought to be exposed to ethylene oxide in the range of 15–250 ppm. There is suggestive but inconclusive evidence from epidemiological studies that ethylene oxide exposures are associated with hematological cancers or reproductive toxicity (i.e., spontaneous abortions).

In Vitro Toxicity Data

Ethylene oxide is regarded as a direct acting mutagen and/or clastogen in a wide range of organisms from bacteria to mammalian cells.

Clinical Management

If contact with the liquid or its solutions occurs, affected areas should be flushed thoroughly with water for at least 15 min. The areas should be observed for burns or resulting irritation. In case of inhalation of ethylene oxide, the victim should be moved to fresh air, an airway should be established, and respiration should be maintained as necessary. The victim should be monitored for irritation, bronchitis, and pneumonitis. If excessive exposure occurs, hospitalization and monitoring for delayed pulmonary edema is recommended.

Environmental Fate

Ethylene oxide released to the environment will partition primarily to the atmosphere due to its high volatility. Although the high water solubility of ethylene oxide indicates it can be extracted from air by rainfall, its rapid volatilization from water (half-life of 1 h) argues against this process being a significant factor in its environmental fate. In the atmosphere, ethylene oxide reacts with hydroxyl radicals resulting in a half-life of 1–12 months. The hydrolysis half-life for ethylene oxide in water and soil is ~1 week. In fresh water, ethylene oxide is hydrolyzed to ethylene glycol; in salt water, it is hydrolyzed to ethylene glycol and ethylene chlorohydrin. In unacclimated aqueous media, ethylene oxide is also subject to biodegradation with estimated half-lives of 1–6 months (aerobic) and 4–24 months (anaerobic).

Ecotoxicology

The 24, 48, and 96 h LC_{50} values for fish are 84–90 mgl⁻¹. For the aquatic invertebrates, the 48 h LC_{50} values are 137–300 mgl⁻¹ (water flea) and 490–1000 mgl⁻¹ (brine shrimp). The 16 h IC_{50} value for ethylene oxide on activated sludge organisms is 10–100 mgl⁻¹.

Other Hazards

Ethylene oxide vapor is extremely flammable at concentrations ranging from 3% to 100% and subject to explosive decomposition. Although liquid ethylene oxide is relatively stable, contact with acids, bases, or heat, particularly in the presence of metal chlorides and oxides, can lead to a violent polymerization.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) generally range between 0.1 and 39 ppm as an 8 h time-weighted average (TWA), with 1 ppm being the most common value. The US Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) have established an 8h TWA OEL for ethylene oxide of 1 ppm. OSHA has also established a 15 min excursion limit of 5 ppm as well as an action level of 0.5 ppm, which if met or exceeded as an 8h TWA for 30 or more days per year triggers additional requirements. Ethylene oxide has been judged a potential/suspected (ACGIH, National Institute for Occupational Safety and Health (NIOSH)) or known (International Agency for Research on Cancer, National Toxicology Program) human carcinogen. For this reason, NIOSH recommends workplace exposure is maintained below 0.1 ppm as an 8 h TWA. NIOSH also lists a concentration of 800 ppm ethylene oxide as immediately dangerous to life or health.

See also: Respiratory Tract; Sensory Organs.

Further Reading

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glycidyl ethers and aromatic monoglycidyl ethers. In: Bingha E, Cohrssen, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 6, pp. 993–1085. New York: Wiley.

Relevant Website

http://www.inchem.org - Concise International Chemical Assessment Document 54, search for Ethylene Oxide

EU See European Union and Its European Commission.

Excretion

Jules Brodeur and Robert Tardif

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Excretion is the process by which chemicals are eliminated from the body. When chemicals gain access to the body, they usually do so as lipid-soluble molecules. In order to be eliminated, most of them must first undergo biotransformation to become more water soluble and, consequently, more easily excreted. Biotransformation and excretion are therefore the main processes involved in the elimination of chemicals.

The most important routes for excretion are urine, feces, and exhaled air; others include milk, sweat, saliva, tears, and hair.

The mechanism responsible for excretion of chemicals is usually (but not exclusively) passive diffusion; lipid-soluble, electrically neutral molecules find a passage through cellular membranes by solubilizing within the lipids of the membrane. The concentration gradient of the chemicals between each side of the membrane acts as a driving force for this process, directing the movement of the molecules from the side of the membrane with a high concentration to the side with a low concentration. Other mechanisms for excretion include filtration through pores in cell membranes and active transport provided by specialized carrier proteins.

Urinary Excretion

This is the most important route for excretion. The kidney is made of several functional units called nephrons. The initial segment of the nephron is a tuftlike structure called the glomerulus, which acts as a filter for plasma; once filtered under the driving force of circulating blood pressure, plasma fluid becomes diluted urine. During the process of filtration, some of the endogenous substances that are dissolved in the plasma can also filter freely altogether with plasma water. The next segment of the nephron is a long tubular structure that allows exchange of water and solutes between the newly formed urine and the blood circulating in the kidney.

Chemicals behave as endogenous solutes; they may filter through the glomerulus and then undergo exchange along the tubular segment to be partially reabsorbed into the blood or excreted. Electrically neutral molecules are subject to reabsorption from urine into blood by simple passive diffusion, moving along a concentration gradient. For drugs that are weak electrolytes, as it is the case for many therapeutic agents, urinary pH has a considerable influence on excretion. At a moderately alkaline pH, weak organic acids are present mostly as ionized or electrically charged molecules; this prevents their diffusion from urine back into blood and facilitates their elimination with the voided urine. The same occurs with weak organic bases at a moderately acidic pH. Altering the pH of urine is currently used to enhance elimination of chemicals in certain drug poisonings (e.g., salicylates and phenobarbital).

Passive diffusion is not the sole mechanism by which chemicals are exchanged between urine and blood in the tubular segment of the nephron. For some agents, active transport, even against a concentration gradient, is another means by which molecules may be transferred from urine to blood, but much more often from blood to urine. There are at least two types of specialized transport systems by which chemicals can be actively secreted in the urine. One is for weak organic acids like penicillin and certain diuretics; another is for weak organic bases like quinine. Like most active transport systems, these systems can be saturated at high concentrations of the transported chemicals and can also be blocked by other chemicals sharing the same transport system; for example, the transport of penicillin into urine can be prevented by concomitant administration of a drug known as probenecid.

In patients with severe renal impairment, kidney function can be effectively substituted by using an artificial kidney. The latter exploits the properties of semipermeable membranes to allow elimination of endogenous waste materials. The same principle can be used to help remove certain freely diffusible chemicals in severe cases of poisoning (e.g., bromides, ethylene glycol, isopropyl alcohol, and lithium). This procedure is called hemodialysis.

Fecal Excretion

This is the second most important route for excretion. Chemicals present in feces are mainly those excreted in the bile but also, to a much smaller extent, those diffusing passively through the intestinal wall. Of course, chemicals that are not completely absorbed during their passage in the gastrointestinal tract are also found in the feces.

All chemicals absorbed in the gastrointestinal tract first reach the liver, where they normally undergo biotransformation to new, more water-soluble molecules (metabolites). Some of these will eventually be excreted in the bile. Thus, in addition to playing an important role in the digestion and intestinal absorption of fats, bile is also involved in the elimination of chemicals from the body.

Bile is formed by liver cells and is collected and transported in the biliary system, which comprises a series of ducts, from extremely small ones to larger ones, branching like a tree throughout the liver. In contrast to what happens in the kidney, the driving force for bile secretion is not the pressure of the circulating blood. It is rather a drawing pressure that is generated within the system of ducts by the presence of various solutes in the bile, creating a passive movement of fluids from liver cells and intracellular spaces (osmotic pressure). Solutes that contribute to create such pressure are bile acids and also smaller molecules like sodium, chloride, and bicarbonate ions. Bile collected at the very smallest ducts, next to each single liver cell, is later modified in larger ducts by processes of reabsorption or secretion of electrolytes and water. Ultimately, bile empties into the first segment of the small intestine, the duodenum.

Some endogenous and foreign chemicals, usually molecules with a molecular weight larger than 325 Da, will appear in bile at concentrations exceeding that in plasma by a factor of 10–1000. Biliary excretion is thus an important route of elimination for such chemicals. Bilirubin is an example of an important end-product of endogenous metabolism of red blood cells that is normally excreted in bile. The excretion of foreign chemicals is supported by various active, carrier-mediated, and saturable transport systems, thus enabling selective removal of organic acids (dyes like sulfobromophtalein and indocyanine green and various glucuronide conjugates of chemicals), organic bases, and neutral substances (ouabain, a cardiac stimulant). Lead (the metal) is also actively transported.

A significant portion of the more water-soluble metabolites secreted in bile is ultimately excreted in the feces. Some metabolites, however, may undergo further enzymatic modification by the intestinal bacterial flora to a state of greater lipid solubility. This metabolic step facilitates reabsorption of such chemicals and extends their life in the body. The process is known as the enterohepatic cycle.

Exhaled Air

Chemicals present in blood that are gases or possess a high degree of volatility diffuse passively into the alveolar air of the lung until they reach equilibrium. The concentration of these chemicals in the air phase is directly proportional to their concentration in blood, and the latter in turn is in equilibrium with the concentration of the chemicals in the tissues. This phenomenon can be applied to noninvasively monitor the presence and the concentration of gases and volatile substances in blood. A practical example of such application is the indirect measurement of alcohol present in blood by analyzing for ethanol in exhaled air with an instrument known as the Breathalyzer.

In industrial settings, exhaled air is used to monitor exposure to volatile organic solvents. A major drawback of this approach is the very high sensitivity of the analysis to rapid changes in exposure concentrations; such changes are rapidly reflected by parallel fluctuations in the concentrations of exhaled air. Under these conditions, point measurements represent exposure poorly over an entire work shift. When exposure concentrations fluctuate, as is usually the case during a work shift, it is recommended to analyze exhaled air the morning after exposure. At this time, blood concentrations of solvents are in equilibrium with concentrations in fatty tissues. The latter present the advantage of slowly and progressively taking up and releasing solvents, thus integrating the previous day exposure independently of the pattern of exposure. Point measurements of solvents in exhaled air the morning after exposure are therefore proportional to exposure during the entire previous work shift.

Milk

Human milk is essentially a solution of sugars and minerals forming a suspension medium for other important nutrients like fat globules and proteins. Normal milk components are derived from maternal blood. Any extraneous chemical that enters blood circulation may also eventually appear in milk.

The transport of foreign chemicals from maternal blood into breast milk can proceed by a number of different mechanisms. Uncharged lipid-soluble molecules may diffuse passively through membranes, whereas small water-soluble and small charged molecules may cross membranes through minute pores or water channels. In addition, lactating cells may secrete nutrients like proteins and fat droplets; both can carry foreign chemicals, either bound to proteins or dissolved into fat droplets.

Nursing mothers taking medications can expect to transfer minute amounts of drugs to their child. However, at maternally therapeutic doses, the amounts transferred are too low to produce pharmacological effects. Over-the-counter analgesics like aspirin and acetaminophen, at usually recommended doses for the mother, should not represent a risk for the nursing infant. The same holds true for nonsteroidal anti-inflammatory agents (like ibuprofen) that are frequently used in self-medication for common aliments like arthritic conditions and musculoskeletal pain. For all prescription drugs, it is strongly recommended that nursing mothers ask a physician or pharmacist about the compatibility of medication with breastfeeding.

Caffeine, a central stimulant found in commonly consumed beverages, like coffee, tea, and certain soft drinks, is excreted in breast milk. Although newborn infants eliminate caffeine very slowly, normal consumption of caffeine is not contraindicated during nursing. Heavy coffee drinking, of course, is not recommended.

Ethanol diffuses readily in the water fraction of breast milk. Nursing mothers should refrain from chronic consumption of alcoholic beverages since such action is conducive to adverse effects on the intellectual and psychological development of the infant.

Drugs of abuse, like cocaine and heroin, are excreted in breast milk in amounts that may be clinically effective; such exposure is formally contraindicated during breastfeeding. Although no adverse effects in the infant have been reported in the case of mothers using marijuana, caution should be exercised.

Finally, lipid-soluble chemicals like the insecticide DDT, polychlorinated biphenyls, and methylmercury are excreted readily as dissolved chemicals into milk fat droplets. Lead is secreted into milk using the same transport system as calcium. Nursing mothers may therefore transfer environmental contaminants to their infants – not to the point, however, of negating the well-established benefits of breastfeeding,

provided the milk is not too heavily contaminated with these chemicals.

Saliva

Saliva is not an important route of excretion since most of the chemicals present in saliva will eventually reach the gastrointestinal tract to be reabsorbed or eliminated in the feces. The unbound fraction of several therapeutic drugs may diffuse passively from plasma into saliva. This provides a noninvasive means of indirectly monitoring plasma concentrations of drugs like lithium, phenytoin, and theophylline. Metals like lead, cadmium, and mercury are also present in saliva.

Hair

Hair is an unexpected and only minor route of elimination for certain chemicals, especially metals. However, the presence of metals in hair has been used as a practical means of monitoring exposure to such chemicals.

Hair is formed from matrix cells present in a bulbshaped follicle located in the dermis. During growth, hair is exposed to circulating blood and extracellular fluids; certain chemicals can then diffuse into cells producing the hair root and eventually the hair strand, where they will be fixed. The interesting aspect about monitoring exposure to metals using hair is the fact that metals will distribute along the hair strand exactly in the sequence of deposition while hair is growing. Knowing that human hair grows at a rate of ~ 1 cm month⁻¹ makes it possible to monitor retrospectively exposure to certain toxic elements, like arsenic, cadmium, mercury, and lead, during the past several months and to establish the duration of exposure.

Part of the evidence that Napoleon Bonaparte suffered poisoning during his exile at St. Helena Island rests upon finding increased concentrations of arsenic in hair samples taken from the emperor's scalp.

In 1971, consumption of homemade bread prepared with flour containing a mercurial fungicide in Iraq led to severe intoxication. The degree of exposure to mercury was monitored using hair as the biological sample. A threshold for neurotoxic effects in children born to exposed pregnant mothers was established at values slightly above $10 \,\mu g \, g^{-1}$ of maternal hair.

Currently, hair is used routinely to monitor exposure to methylmercury in fish-eating native populations of northern Canada. The objective is to adjust consumption of fish, an important, yet contaminated nutritional source, so as not to exceed concentrations of $30 \ \mu g g^{-1}$ of hair in the general adult population and 15 μg in fertile women. Analysis of metals in hair is of limited practical value for monitoring exposure to metals in occupational settings due to the very distinct possibility of hair contamination with exogenous metals present in the ambient air.

See also: Absorption; Biotransformation; Distribution; Kidney; Liver; Metals; Pharmacokinetics/Toxicokinetics.

Further Reading

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Exposure

Gary Whitmyre and Sam Kacew

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Introduction

The biosphere is composed of air, water, and soil media. These media contain chemicals of natural and man-made origin to which people can be exposed. In the broadest sense, the term exposure is defined as the condition of being in contact with or exposed to a chemical, physical, or biological agent. Exposure can be quantified as the amount of chemical available at the exchange boundaries of the organism ('external exposure' or 'potential exposure'). Exposure can also be quantified as an absorbed dose or internal exposure that accounts for the fraction of material that can pass through a biological barrier (e.g., skin, lung surface, gut). The physical course that a chemical takes from the source to the exposed individual is known as the exposure pathway. The manner in which an individual comes into contact with an exposure media (e.g., drinking water, food, air, soil) or a toxic agent is referred to as the route of exposure. Exposure or contact with a toxic agent does not necessarily mean that a harmful or toxic outcome will result (e.g., in the case of exposure to very small amounts of chemicals that are only moderately toxic). In certain situations, a given exposure does not produce an adverse effect, while under other conditions an adverse effect can occur (e.g., in the case of concurrent exposure to a different chemical with the same toxic mechanism of action). There are conditions under which exposure to some chemicals produces a beneficial effect (e.g., dietary exposure to trace metals that are important micronutrients or administration of therapeutic drugs such as antibiotics). Exposure becomes a concern only when contact with a toxic chemical or agent occurs at a

level or amount that results in a harmful consequence to the individual. The agents to which an individual may be exposed include (1) gases (e.g., nitrogen oxide, carbon monoxide, methyl bromide); (2) nonvolatile inorganic chemicals (e.g., cadmium, mercury, lead); (3) biological agents and allergens (e.g., mold, mildew, dust mite allergens); (4) organic chemicals (e.g., solvents); and (5) radiation (e.g., γ -emitters, α -emitters, electromagnetic frequencies (EMF)). Consideration of exposure takes into account the routes of exposure, the dose, the duration of exposure, and the characteristics of the exposed population.

Exposure Routes and Pathways

Individuals may be exposed to toxic agents via a number of possible exposure routes. Exposure to a chemical in the process of the normal breathing of air is termed 'inhalation' exposure. Exposure to a toxic agent in water or food in the diet is termed 'ingestion' exposure. Exposure to a chemical through contact of a media with the skin is referred to as 'dermal' exposure; this exposure route can also be referred to as 'topical' (as in the case of a material intentionally applied to skin) or 'percutaneous' exposure. It should also be noted that the physiological conditions of pregnancy and lactation introduce two other routes of exposure. During pregnancy, there is an exchange of constituents and nutrition from mother to fetus, and removal of wastes from the fetus to the mother through the placenta. Because the placenta is porous to many toxic agents, they may pass from the mother's blood supply into the fetus, resulting in 'transplacental' exposure of the fetus. In addition, because the newborn infant is dependent on maternal milk for nutrition, certain agents may accumulate in the mammary tissue and be passed on to the fetus via human breast milk. Thus, the nursing infant can be exposed to a variety of environmental chemicals that accumulate in the breast milk as well as directly to chemical in the biosphere and contaminants in environmental media (e.g., through inhalation of air, bathing).

Although exposure can occur normally through various routes, an artificial route of exposure allows a toxic agent to reach the blood directly. The administration of certain pharmaceuticals to humans and exposure in some laboratory animal studies involves introduction of the agent via injection through a needle penetrating the skin. This route of exposure is referred to as 'injection route' exposure. A route of exposure involving injection of a chemical or other agent directly into a vein is termed 'intravenous'. 'Subcutaneous' and 'intramuscular' exposure routes involve the injection of a chemical or agent below the skin layer, or into muscle tissue, respectively.

Certain consumer products or medical devices result in unique exposure routes by which individuals may be exposed to chemicals. For example, certain feminine hygiene products result in 'intravaginal' exposure to trace amounts of dioxins and other chemicals. Exposure route possibilities for chemicals in medical devices range from intravenous (e.g., for plasticizers such as DEHP that are leached from intravenous administration tubing sets) to local tissue exposure (e.g., in the case of chemicals leaching from endotracheal tubes and various types of implants). Some complex exposure situations, such as full immersion during swimming, may result in other minor exposure routes such as nose (nasal) and ear canal (aural) exposure. These latter two exposure routes may also be relevant to administration of some pharmaceuticals, such as nasal sprays and eardrops. Another exposure route relevant to ophthalmic formulations and certain irritation testing of chemicals involves administration to the cornea of the eye (occular). The major normal routes of exposure of humans to environmental chemicals and agents typically involve oral, inhalation, and/or dermal exposure routes. Because of the differences in the effectiveness of different biological barriers (e.g., the skin, gut, and lung) a given external exposure may result in a different internal exposure or absorbed dose depending on the route of exposure. Thus, a given external exposure may result in the following descending order of magnitude of absorbed dose for various exposure routes, as an example: injection > inhalation > oral > dermal.

A single individual may be exposed to a given chemical through multiple exposure routes. For example, a worker who is occupationally exposed to a pesticide (e.g., through inhalation and dermal contact during spraying of crops) can be exposed orally to the same pesticide through ingestion of food and contaminated well water, and through dermal contact with contaminants in water during bathing. Another example of multiple exposure routes is for cadmium, whereupon an individual who is occupationally exposed via the dermal and inhalation routes (e.g., smelter worker) can also be exposed via the diet and via inhalation of tobacco smoke (cadmium is a chemical found in tobacco smoke).

Dose

In considering the dose of a toxicant resulting from exposure, 'external' exposure at the biological barriers (e.g., skin) should be distinguished from 'absorbed dose' (e.g., amount of chemical reaching the blood reflecting partial absorption of the chemical), and from the dose at the target tissue (e.g., micrograms per gram organ tissue). The extent to which an exposed individual displays an adverse effect from exposure to a toxic agent depends, in part, on the dose or amount of chemical that reaches the target site. In an industrialized society, individuals are continuously exposed to numerous chemicals by several routes. If one took a sample of the exposed population, different individuals in the population would be receiving widely different doses. If a given exposure is insufficient to produce a toxic dose at the target site, then no adverse effect occurs. In addition, because of interindividual variation, it is likely that different individuals receiving the same external exposure will have different levels of response to the toxicant.

Various factors affect the concentration of a toxicant at the target site. The ability of a substance to be absorbed through the biological barriers (e.g., skin, gut, lung) to reach the blood and then to be distributed to tissues followed by excretion ultimately affects the dose at the target tissue. External exposure to a chemical is, thus, the first step in a multistep chain resulting in target tissue exposure. For example, with oral exposure, the chemical may be absorbed into the blood from the gastrointestinal tract, followed by transit to the liver and other tissues where conversion to a more toxic or less toxic metabolite may occur. The substance, or its metabolites, may be eventually removed from the body by excretion into the urine, feces, or breath (in the case of gases and solvents).

The units of dose are typically expressed in terms of amount of chemical per unit time (e.g., milligrams per day) or amount per unit time normalized to body weight (e.g., milligrams per kilogram per day). Because an individual can be exposed to a given chemical from multiple sources (occupational, drinking water, food, air), the concept of 'aggregate exposure' was developed. In the United States and Canada, the aggregate exposure for a chemical is the sum of all exposures to that chemical by all relevant routes and from all relevant sources. This is different from 'cumulative exposure', which is the combined exposure of two or more chemicals that have the same mechanism of toxic action, summed across all relevant exposure pathways and all relevant sources. The concepts of aggregate exposure and cumulative exposure are widely used in the United States and Canada, for example, in considering exposures to pesticides.

As a first step to estimating exposure or dose, the concentration of a chemical in environmental media (e.g., drinking water, food), in biological fluids (e.g., urine, blood), or in biological tissues (e.g., adipose tissue) can be determined. A number of large-scale human exposure surveys have been conducted that can provide a starting point for estimating chemical exposures to the general population. The design of these studies can be generally characterized by probability-based selection of human subjects, and direct measurement of exposure to multiple chemicals in multiple environmental media and/or multiple body fluids/tissues. The first large-scale study of human exposures to chemicals was the Total Exposure Assessment Methodology (TEAM) study of the early 1980s, conducted by the US Environmental Protection Agency (US EPA). This study conducted personal monitoring of inhalation exposures to 25 volatile organic compounds (VOCs) for 550 individuals. Subsequent US EPA TEAM studies have addressed carbon monoxide, pesticides, and airborne particles. The Non-Occupational Pesticide Exposure Survey (NOPES) focused specifically on pesticide exposures in the United States not involving pesticide exposures in the workplace. Major European studies on indoor levels of VOCs and pesticides have taken place in the Netherlands and Germany. The German Environmental Survey (GerESII) measured selected metals and pesticides in blood urine, hair, house dust, drinking water, and indoor air. In the United States, a major multimedia study of human exposures to chemicals, known as the National Human Exposure Assessment Survey (NHEXAS) was initiated in 1993 by the US EPA and is ongoing. NHEXAS includes measurements of VOCs, pesticides, polycyclic aromatic hydrocarbons (PAHs), and heavy metals in indoor air, outdoor air, drinking water, food, soil, and house dust. Besides measuring chemical concentrations in these environmental media, NHEXAS also measures chemical levels on skin (via wipes), and in urine and blood. Further, in a recently updated study

by the US Centers for Disease Control and Prevention called the National Health and Nutrition Examination Survey (NHANES), selected VOCs, lead dust, phthalates, pesticides, metals, PAHs, and dioxins were measured. Because NHANES has been conducted periodically over the last two decades, the overall data set provides time profiles from which long-term trends in exposures for some of the chemicals may be determined (e.g., declining exposures to for pentachlorophenol).

Duration and Frequency

An exposure is defined not only in terms of dose, but also in terms of the duration and frequency of exposure. Toxicologists usually think of exposure duration in four categories: acute (typically <24 h), subacute (repeated exposure for a month or less), subchronic (1 month to 1 year), and chronic (several years up to a lifetime of repeated exposures). While this general classification scheme was developed primarily in the context of toxicity testing of chemicals in laboratory animals, the same concepts apply to humans who may be exposed to environmental chemicals. Human exposures to many chemicals (e.g., for common air pollutants) may be chronic in nature, occurring daily over a lifetime (although the actual daily dose may vary from day to day and trend down over time as environmental controls improve). Some categories of human exposures (such as professional applicator exposures to specific pesticides over the growing season) may be more subchronic in nature, involving repeated exposures over several months. Some exposures (e.g., to toluene in spray paint) may be episodic, reflecting infrequent exposure; such exposures are more acute in nature. These defined exposures may be in addition to the daily background exposures to very small amounts of the same chemicals from other environmental sources.

Frequency is the temporal characterization of exposures. For humans, frequency can range from daily for air pollutants, to several times weekly for chemicals in certain household cleaning products, to infrequent or intermittent exposures where there are long periods measured in days, weeks, or months between exposure events (e.g., for exposure to an insecticide once a month during monthly treatment of pets for fleas). For chronic toxicants, it is the long-term time-amortized or time-averaged exposure that will determine whether an adverse effect will occur from repeated exposures. For some toxicants, the toxic effects following a single high exposure are quite different from the effects of repeated low exposures. For example, the primary toxic

manifestation of a single high exposure to benzene is central nervous system depression, whereas more moderate repeated exposures to benzene can result in leukemia. For some acute toxicants, dividing an exposure into repeated increments over a period of days generally elicits less of a toxic effect than if the entire amount is given as a single dose, because there is adequate time for biotransformation, excretion, and recovery, resulting in partial or complete reversal of effects prior to the next exposure.

Residential, Occupational, and Other Types of Exposures

It is evident that different members of the population are not exposed to identical doses of a given chemical because all individuals in a population are not exposed to the same environmental conditions. Concentrations of chemicals in environmental media vary throughout the biosphere, and individual differences in lifestyle, employment, housing characteristics, and geographic location result in different exposures to the same chemicals. Human timeactivity studies in the US have shown that people on average spend about 16 h day^{-1} at home, 30 minof which may be in the backyard on average. Exposures in this environment are generally referred to as 'residential exposure'. Exposures to chemicals in the workplace, which can include for example, worker exposures during and after application of pesticides to crops, factory worker exposures to solvents, and office worker exposures to chemicals released from office supplies and equipment, are referred to as 'occupational' exposures. Some exposures that occur not in the home environment or at work can be referred to as 'commuting', 'recreational', 'child daycare', 'school place', or by other terms depending on the location of exposure or the type of exposure being studied or reported.

Sensitive Subpopulations

Sensitive populations may include individuals who, because of predisposing conditions, may be more sensitive to the effects of a toxicant. Sensitive populations may also include individuals who, because of their age, physiological characteristics, lifestyle, location, or dietary habits, may actually receive a higher exposure than typical exposures to the general population. Sensitive populations may include individuals who, because of asthma or prior lung damage (e.g., from smoking or industrial exposures), may be predisposed to respiratory effects being induced by much smaller amounts of respiratory irritants (such as aldehydes in urban air) than individuals who are not predisposed. Another example of a sensitive population is children who, because of eating paint chips contaminated with lead and their low body weight, may receive a significantly higher dose in milligrams per kilogram body weight per day than an average adult. Because of the age of children and their continuing physiological development, they are also more prone to learning deficits from small amounts of lead compared to adults. Subsistence and Native American fishermen may have higher exposures to PCBs, dioxins and other lipophilic chemicals because of bioaccumulation in fish and the higher fish consumption rate (grams per day) of this subpopulation compared to other individuals in the general population.

See also: Exposure Assessment; Exposure Criteria; Medical Surveillance.

Further Reading

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Exposure Assessment

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Exposure assessment is one of the four major steps in the risk assessment paradigm, as defined by the National Academy of Sciences in the United States. Thus, exposure assessment is a key element in any quantitative risk assessment. Exposure assessment is defined as the qualitative or quantitative determination or estimation of the magnitude, frequency, duration, and rate of exposure. The quantitative amount of exposure usually refers to the amount or intake rate (e.g., $mg kg^{-1}$ body weight day^{-1}) of a chemical or physical agent at biological exchange boundaries (e.g., lungs, skin, and gastrointestinal tract); whereas absorbed dose refers to the amount or intake rate of a chemical or physical agent that has penetrated biological exchange boundaries. Thus, exposure can be defined as either an external or internal (absorbed) metric.

The magnitude, frequency, duration, and rate of exposure may be evaluated using measurement or modeling approaches. The determination of which approach is taken, and what level of complexity is appropriate, depends upon a number of factors, such as the purpose of the specific assessment, the quality and quantity of available data, and the experience of the exposure assessor. There are three basic types of exposure assessment: direct, reconstructive, and predictive.

Direct exposure assessment consists of monitoring approaches to actually measure the amount of a chemical in environmental media (e.g., air, water, food, surfaces). Examples of direct monitoring approaches include the radiation dosimeter badge, personal air monitoring approaches to measure breathing-zone concentration of chemicals in air, measurements of chemical residues in foods as consumed, and passive dermal dosimeters (e.g., cotton clothing) for measuring consumer or occupational exposure during the application of products containing pesticides.

Reconstructive exposure assessment uses biological monitoring data, in conjunction with pharmacokinetic data and models, to estimate the levels of absorbed dose (e.g., systemic levels in plasma or whole blood), and in some cases, external exposure to a chemical that resulted in the measured levels in biological tissues and/or fluids. Biological monitoring consists of the measurement of the concentration of a chemical and/or its biotransformation products in biological tissues or fluids (e.g., adipose tissue, blood, urine) or the measurement of the amount of chemical bound to a target molecule (e.g., DNA-bound chemical).

Predictive exposure assessment, which involves estimation of potential exposure using mathematical models, is perhaps the most widely used approach to exposure assessment, and for many exposure assessors is synonymous with the term 'exposure assessment'. Predictive exposure assessments can be of varying sophistication, ranging from simplistic screening-level algebraic equations using worst-case assumptions to more complex models involving random-number based simulations (stochastic or probabilistic models) to define the likely distribution of exposure in a human population of concern. Predictive exposure assessment usually consists of a number of steps, including determination of exposure pathways, construction of likely exposure scenarios, estimation of environmental concentrations (sometimes through more complex environmental

fate modeling), and calculation of the frequency and magnitude of human exposures as a function of time.

Important to the concept of exposure assessment is the profile of temporal exposure that a person experiences. Key elements of a temporal exposure profile include:

- *Exposure duration*, which is the length of time involved in each discrete exposure event (e.g., minutes to days) and, if applicable, the length of time over which two or more discrete exposure events occur (e.g., days to years).
- *Exposure frequency*, which is a measure of how often a discrete exposure event occurs over a defined time period (e.g., a few hours per use for many consumer product exposures, 5 days week⁻¹ for several years in the case of some occupational exposures, or continuous exposure over a lifetime in the case of ambient air pollutants).
- *Exposure chronology*, which may provide a measure of timing (e.g., age interval, such as 13-50 years of age) of the exposure event(s) relative to a time period of toxicological relevance (e.g., life stage, such as females of reproductive age) for a particular adverse (toxic) effect of interest (e.g., fetal developmental toxicity). Thus, the exposure metric should be consistent with the time frame of the dose-response that is particular to the toxicological endpoint of concern. For example, because exposure of a pregnant woman to a teratogen (i.e., a birth-defect causing agent) on a given day of gestation can cause adverse effects in the fetus, the focus is on estimation of daily 'per-event' exposure. Thus, exposure chronology, in combination with knowledge about the toxicological endpoint, can help determine the most appropriate statistical metric for the exposure or absorbed dose estimate and the associated timeaveraging period or integration interval (e.g., maximum daily absorbed dose, versus 90 day moving average absorbed dose) that should be using for calculating an exposure metric for a given chemical.
- *Exposure patterns*, which usually reflect the time (e.g., hours day⁻¹, days per calendar year) and location (e.g., geographical, microenvironment) relationship between sources of exposure (e.g., motor vehicles, home appliances, consumer products, and drinking water) and human activity patterns. Human activity patterns include where and how much time people spend in defined microenvironments during a given day. Further, it can be important to characterize their specific microactivities within a given microenvironment, e.g., the frequency of a 1–2-year-old child's hand-to-mouth

events h^{-1} while playing on a residential lawn. This provides for characterization of exposure patterns that have temporal, spatial, demographic, and behavioral specificity, and allows the exposure assessor to differentiate the relative contribution of different sources (and routes) of exposure (e.g., residential indoor air, versus ambient air, versus inside vehicles such as cars, buses, trains, and airplanes). Thus, a given individual (defined demographically) can be exposed to the same chemical, through multiple routes, and a variety of microenvironments, over the course of a single day or multiple weeks, as a function of their time-activity profile.

Screening-level or initial tier predictive exposure assessment methods typically involve the use of an algebraic equation that expresses exposure or absorbed dose as a function of the concentration of a chemical in relevant media (e.g., air, food, and water) and other important factors. For example, inhalation exposure (E_{inh}) to an airborne chemical can be estimated using some form of the following equation:

$$E_{\rm inh} = (C_{\rm a} \times \rm{IR} \times \rm{ED} \times \rm{EF})/(\rm{BW} \times \rm{AT})$$

where C_a is the concentration of chemical in air $(mg m^{-3})$, IR is the inhalation rate $(m^3 h^{-1})$, ED is the exposure duration (h per day), EF is the exposure frequency (day per year), BW is the body weight of the exposed individual (kg), and AT is the averaging times, or period over which exposure is amortized (days year⁻¹).

In contrast to acute or short-term estimates of exposure, for purposes of chronic or long-term exposure and risk assessments, such as evaluation of potential lifetime excess cancer risk, regulatory agencies typically set the averaging time at that of an average lifetime (e.g., 70 or 75 years times $365 \text{ days year}^{-1}$) to obtain the lifetime average daily dose.

Different methods of human exposure assessment vary with respect to the 'input' data or information required and the degree of uncertainty associated with resulting estimates. For example, the film-thickness approach to dermal exposure assessment is a screening-level methodology that assumes a uniform layer of material (e.g., a liquid consumer product) is on the skin, and that a portion of the material in this layer is absorbed, per the dermal absorption characteristics of the chemical. In contrast, dermal exposure assessment and percutaneous absorption methods can include metrics that account for time-dependent exposure and absorption processes. For example, in the case of secondary dermal contact with chemicals on surfaces (e.g., transfer of pesticide residues from treated carpet to skin or clothing), dermal transfer coefficient methods have been developed from studies involving concurrent measures of transferable residues (e.g., amount per unit time of chemical transferring from a treated surface to a collection medium such as gauze wipe or a cotton cloth rolled with a cylinder exerting a known force) and timebased human passive dosimetry (e.g., amount per unit time of chemical transferred from the treated surface to cotton clothing worn by person during choreographed activities). The resulting dermal transfer coefficient estimates are typically expressed as a 'contact rate' in units of cm^2h^{-1} . Secondary dermal exposure assessment approaches can also be used to estimate dermal exposure to hands (transfer of chemical residues from a surface to hands as a function of time and specific behaviors) and subsequent hand-to-mouth-based incidental ingestion exposures.

Exposure assessment is complicated by the fact that chemical or physical agents can move dynamically, via various pathways, from the source of contamination to human receptors. Historically, these pathways and associated exposure routes have been characterized separately. However, because many human exposures can occur across time through a variety of environmental pathways and by different routes (e.g., inhalation, ingestion, and dermal contact), more recently exposure assessors are using an integrated 'total human exposure assessment' approach. Probabilistic models have been developed to quantify potential multipathway, multiroute exposure distributions to chemicals via multiple sources including diet (food, drinking water), and the residential environment (e.g., exposures during consumer product use and postproduct use or postapplication via the inhalation, dermal, and/or incidental ingestion routes) and to discern source contribution and uncertainty, for specified time periods, geographical locations, and demographic subpopulations. Chemical-specific multipathway/route assessments are also referred to as aggregate exposure assessments. Aggregate exposure assessments have been developed in the United States for various purposes; for example, including pesticides registration and reregistration.

Compilations of exposure factor values and distributions are available through the US Environmental Protection Agency (US EPA) and other organizations. Examples of exposure factors include individual physiological factors (e.g., body weight, inhalation rate, and skin surface area), exposure-related factors (e.g., time–activity data), and building factors (e.g., air exchange rate, room volume, and house volume). In Europe, collections of and recommended values for various exposure factors are available through

the German Exposure Standards document (Standards zur Expositionsabschätzung), Netherlands National Institute for Public Health and the Environment (RIVM) guidance (e.g., 'fact sheet') documents, and through the work of the European Commission's European Information System on risks from chemicals released from consumer products/ articles (EIS-ChemRisks). In addition, experts from many organizations have collaborated via leadership from the Finnish National Public Health Institute (KTL) and funding from Cefic (the European Chemical Industry Council) to develop a multicountry collection of exposure factors called the Exposure Factors Sourcebook for Europe (ExpoFacts).

Because every person in a given population is likely to experience a different exposure from a given source due to, for example, different inhalation rates, different skin surface areas contacted, and different frequencies and durations of exposure due to different time-activity patterns, the recent trend has been to integrate exposure assessment with quantitative methods of uncertainty analysis. Estimates of exposure are bounded by a range of possible values, as a function of inherent variability and uncertainty in exposure parameters, and across alternative methods or modeling approaches. Using probabilistic methods, one can obtain an exposure distribution curve, where each given exposure level has a specific probability of occurring in an exposed population under the defined assessment methods used. In such a distribution, the central tendency value for exposure to a chemical in a population (e.g., 50th percentile) may be orders of magnitude less than the theoretical upper bound estimate of exposure obtained by assuming worst-case values for some exposure parameters (e.g., concentration in air, frequency of exposure, duration of exposure). Thus, probabilistic methods of exposure assessment hold the potential to yield a more realistic cross-section of exposures for the subject population because such methods make full use of all available information, and disclose variability and uncertainty in a manner that informs the risk assessment process.

See also: Exposure; Exposure Criteria; Monte Carlo Analysis; Risk Assessment, Human Health; Uncertainty Analysis.

Further Reading

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- US Environmental Protection Agency (US EPA) (1996) *Exposure Factors Handbook*. Washington, DC: Office of Health and Environmental Assessment, Office of Research and Development, USEPA. Volume I, PB98-124225; Vol. II, PB98-124233; Vol. III PB98-124241; and the set of three volumes is PB98-124217. US EPA has also produced a CD-ROM that contains an interactive version of the Exposure Factors Handbook. The CD-ROM has word search capabilities, downloadable tables, hypertext links to various chapters in the document, and key references.

Relevant Websites

- http://ihcp.jrc.it and http://www.jrc.cec.eu.int European Commission, Joint Research Centre, Institute for Health and Consumer Protection. Websites for accessing the European Information System on risks from chemicals released from consumer products/articles (EIS-Chem-Risks.
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Exposure Criteria

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A central concept in toxicology is that all chemicals have the potential to cause adverse effects at sufficiently high doses. Exposure criteria (exposure limits, toxicity, or risk values) define a dose or exposure level that is likely to be below the level at which adverse effects are expected to occur under particular conditions or that minimize risk to an acceptable level. Most commonly, criteria are expressed either as a dose (e.g., milligram of chemical per kilogram of body weight per day), a chemical concentration in a media (e.g., milligrams of chemical per cubic meter of air, per kilogram of soil, or per liter of water), or as an internal or absorbed dose (concentration in blood or amount excreted), or other units based on the nature of the exposure (e.g., chemical versus physical agents).

Types of Exposure Criteria

Exposure criteria are established by diverse organizations with different areas of responsibility. For example, different federal government agencies in the United States establish exposure criteria for food ingredients and drugs (Food and Drug Administration – FDA), environmental exposures to commodity chemicals and pollutants (Environmental Protection Agency – EPA), and workplace exposures (Occupational Safety and Health Administration - OSHA). Similar separations of activity occur among numerous international organizations and other country governments. The laws that set the statutory authority of these agencies differ and they often regulate different environments, activities, and commodities. Exposure criteria developed for each of these areas are often based on different assumptions and use different procedures and may be designed to protect different populations. For this reason, the exposure criteria developed for the same chemical may differ across the various organizations. Table 1 provides examples of different types of exposure criteria.

Certain exposure criteria serve as health-based guidelines; others are legally binding standards. For example, OSHA Permissible Exposure Limits are enforceable regulatory standards that define acceptable concentrations in air in the work environment. However, National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits (RELs), American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values (TLVs), and American Industrial Hygiene Association (AIHA) workplace environmental exposure levels (WEELs) are health-based guidelines for occupational exposure. Similarly for environmental exposure, US EPA establishes reference doses (RfDs) as guidelines for oral exposure from contaminated media, while establishing different regulatory limits for contaminant concentrations in drinking water. In general, enforceable regulatory standards are based on risk management considerations such as technical feasibility for meeting the standard and cost implications, as well the underlying toxicology.

Table 1 Examples of different types of exposure criteria

Organization	Criteria	Effect	Route	Target population		
US EPA RfD		Noncancer – chronic	Oral (mg kg ^{-1} day ^{-1})	Public		
US EPA	RfC	Noncancer – chronic	Inhalation (mg m $^{-3}$)	Public		
US EPA	MCL	All				
US EPA	NAAQS	All	8 (8)			
ATSDR	MRL	Noncancer – acute, intermediate, chronic	Oral (mg kg ^{-1} day ^{-1}) and inhalation (mg m ^{-3})	Public		
Health Canada	ТΙ	All	Oral (mg kg $^{-1}$ day $^{-1}$) and inhalation (mg m $^{-3}$)	Public		
WHO	TD/TC	All	Oral (mg kg $^{-1}$ day $^{-1}$) and inhalation (mg m $^{-3}$)	Public		
US OSHA	PEL	All	Inhalation (ppm or mg m $^{-3}$)	Workers		
US NIOSH	REL	All	Inhalation (ppm or mgm $^{-3}$)	Workers		
ACGIH	TLV	All	Inhalation (ppm or mgm $^{-3}$)	Workers		
AIHA	WEEL	All	Inhalation (ppm or mgm $^{-3}$)	Workers		
EU SCOEL	Binding Limit	All	Inhalation (ppm or mg m $^{-3}$)	Workers		
US EPA	AEGL	Noncancer	Inhalation (ppm or mg m $^{-3}$)	Public		
AIHA	ERPG	Noncancer	Inhalation (ppm or mg m $^{-3}$)	Public		
US NIOSH	IDLH	Noncancer	Inhalation (ppm or mg m $^{-3}$)	Workers		
ACGIH	BEI	All	Concentration in biological media	Workers		
ACGIH	TLV	All	Various (acoustical energy, temperature, nonionizing radiation, or ergonomic stresses)	Workers		
US OSHA		All	Various (noise, ionizing radiation)	Workers		
ICRP	All Various (nonionizing and ionizing radiation)					

ACGIH – American Conference of Governmental Industrial Hygienists; AEGL – acute emergency guidance level; AIHA – American Industrial Hygiene Association; BEI – biological exposure indices; EPA – Environmental Protection Agency; ERPG – emergency response planning guideline; EU SCOEL – European Union Scientific Committee for Occupational Exposure Limits; ICRP – International Commission for Radiological Protection; IDLH – immediately dangerous to life or health; MCL – maximum contaminant level; MRL – minimal risk level; NAAQS – National Ambient Air Quality Standard; NIOSH – National Institute for Occupational 'Safety and Health'; OSHA – Occupational Health and Safety Administration; PEL – permissible exposure limit; REL – recommended exposure limit; RfC – reference concentration; RfD – reference dose; TD/TC – tolerable dose or tolerable concentration; TI – tolerable intake; TLV – threshold limit value; WEEL – workplace environmental exposure level; WHO – World Health Organization.

Exposure criteria also vary based on the route of exposure. Depending on the anticipated exposure patterns, route-specific exposure criteria can be very valuable for health protection. Many organizations establish exposure criteria for inhalation and oral routes of exposure. Dermal exposure criteria are less common, but are often critical where skin contact is the primary exposure of concern. Care must be taken in applying route-specific criteria for other applications, and should include a thorough evaluation of the underlying toxicological considerations. For example, an exposure criterion for oral dosing may not adequately protect against respiratory effects of a potent respiratory irritant, or an agent that induces dermal sensitization.

The length of time over which the exposure is likely to occur should be considered in evaluating the potential for adverse effects. Many organizations develop exposure criteria for different durations of interest, and multiple criteria for the same chemical may be relevant based on the anticipated duration of exposure. For example, most organizations that establish occupational exposure limits such as the ACGIH or OSHA have procedures for recommending criteria for maximum peak exposures (i.e., ceiling limits), short-term exposure (i.e., 15 min STEL), and full-shift exposure criteria (e.g., 8h time-weighted average). Acute emergency exposure guidelines also are established with a range of durations from as little as 10 min to 8 h. Longer-term environmental exposure criteria also differ by duration of exposure. For example, the US Agency for Toxic Substances and Disease Registry (ATSDR) establishes acute, intermediate, and chronic minimal risk levels (MRLs). Typically, a higher exposure can be tolerated for a shorter period than a lower exposure, and therefore, criteria developed to protect against acute exposure are often higher than for long-term or chronic exposure.

Most exposure criteria are derived to prevent any adverse effect from occurring in the population of interest. However, in some cases, particularly for emergency exposures, thresholds or criteria are needed to evaluate the potential for adverse effects of differing severity. For example, the AIHA Emergency Response Planning Guidelines (ERPGs) provide separate exposure criteria to minimize the potential for minimal, intermediate, or severe effects. NIOSH establishes immediately dangerous to life or health (IDLH) values to prevent severe effects that might impair escape or cause serious irreversible effects.

Target populations of interest also differ among organizations that establish exposure criteria. Many criteria (e.g., EPA RfDs, ATSDR MRLs) are established for the general public and account for potential sensitive populations such as young children, the elderly, or individuals with existing medical conditions. Occupational exposure levels often assume a healthy worker population. Exposure criteria for some drugs consider specific populations of patients who are candidates for the drug. The exposed population intended to be protected by an exposure criterion often impacts underlying assumptions regarding variability in response among the exposed individuals as well as acceptable levels of risk.

Notwithstanding these differences in the underlying assumptions and intended uses among exposure criteria, some general methods and approaches are commonly applied. For many organizations the basic methods differ between noncancer and cancer effects. However, in some cases noncancer effects are the primary effect of concern (e.g., emergency and acute exposure criteria). For most longer-term criteria, an assessment is generally made of both noncancer and cancer effects. Approaches for developing noncancer and cancer criteria are discussed separately below.

Noncancer Exposure Criteria

In general, criteria developed to protect against noncancer effects are based on the assumption that there is a threshold below which no adverse health effects will occur. A critical evaluation of available human health and animal toxicity studies is performed to identify the most sensitive adverse effect relevant to humans. Noncancer exposure criteria are often based on an experimentally defined dose at which no adverse effects were observed (i.e., the no-observedadverse-effect level - NOAEL). If no adequate NOAEL is available, the lowest dose at which adverse effects were observed (lowest-observedadverse-effect level - LOAEL) is used. Another commonly used approach is to fit study data to dose-response models to identify appropriate values (e.g., dose corresponding to the upper bound of the 10% response level or $BMDL_{10}$) as the basis for deriving the exposure criteria.

The resulting critical effect level (NOAEL, LOAEL, or BMDL) is generally divided by additional factors to account for uncertainty in extrapolating from the selected critical effect level to the safe exposure level in human populations. The application of uncertainty factors varies among organizations and on the intended application of the exposure criterion. For example, lower factors are generally used for occupational settings than for environmental exposures intended to protect the general public. Recent efforts have seen increased use of chemical-specific data to replace default applications of uncertainty factors. For example, data on the toxicokinetic differences among species for a specific compound may be used to replace default factors in extrapolating from animal toxicity data to humans. The following general areas of uncertainty are often weighed in developing a composite uncertainty factor:

- Extrapolation from animal toxicity studies to humans.
- Variability in human sensitivity.
- Extrapolation from subchronic to chronic duration.
- Use of a LOAEL rather than a NOAEL as the critical effect.
- Other uncertainties in the overall database.

The factors that apply to a given dataset will vary. Some organizations apply default values for these or other considerations (e.g., US EPA in deriving RfDs), while others do not (e.g., ACGIH in deriving TLVs). For example, the US EPA RfD is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. It is defined in terms of dose in milligrams per kilogram body weight per day (mg kg⁻¹ day⁻¹). A critical effect is determined following a thorough evaluation of the human health and toxicology literature, and uncertainty factors are applied to this value to derive the RfD as follows:

RfDs are commonly used to evaluate whether exposures to contaminants in environmental media are acceptable. Exposure may occur via drinking water or contact with soil (separate criteria called reference concentrations or RfCs are established for inhalation by US EPA). Exposure must be estimated in terms of mg kg⁻¹ day⁻¹ and is then compared to the RfD. If the estimated exposure exceeds the RfD, then the exposure may not be acceptable. While details vary, similar principles are used in deriving and evaluating exposure criteria established by other US organizations, organizations that establish emergency or occupational criteria, and international organizations.

Exposure Criteria for Carcinogens

Many organizations establish exposure criteria for carcinogens in a manner similar to noncarcinogens, by identifying effect levels for the tumorigenic response and accounting for uncertainties by applying uncertainty factors. Other organizations have traditionally applied alternative methods that rely on linear extrapolation from tumorigenic dose levels to low dose exposures. More recently, many organizations determine which of these approaches is most appropriate based on the underlying carcinogenic mode of action. The weight of evidence for alternative modes of action is judged and the implementation of dose–response approaches is then based on the underlying biology.

As an example of evaluating weight of evidence, a number of organizations have developed weight of evidence classification schemes to evaluate the likelihood that a chemical is carcinogenic. Such organizations include the International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP), US EPA, OSHA, and ACGIH, among others.

Depending on the mode of action, some carcinogens may act via threshold mechanisms and, therefore, exposure criteria can be reasonably developed using the same approach as for noncarcinogens. However, other compounds may act through mechanisms such as direct DNA reactivity that may not have determinable thresholds. In these cases, most organizations employ methods that assume that any exposure carries some risk of effect even at low doses. A dose-response curve is generated to identify exposure levels associated with a specified level of excess risk. A number of doseresponse models are used to extrapolate from high doses to low doses, often associated with environmental exposure. Such models include the one-hit, multistage, gamma multihit, probit, and Weibull models among others. Software is available that provides these modeling tools (e.g., Benchmark Dose Modeling software available from the US EPA). The slope of the dose-response curve in the low-dose region is used as an indicator of the potency of a carcinogen. Some models calculate this slope directly as part of the fit to the data. Alternatively, the slope can be calculated from the line extrapolated from a defined tumor response level as determined using Benchmark Dose modeling (e.g., the upper bound estimate of the tumorigenic dose associated with a 10% response level) to zero. An estimate of the upper bound of the slope is often used to define the slope factor. The slope factor can be used to estimate cancer risk as shown below:

The unit risk is defined as the upper bound additional lifetime cancer risk associated with exposure to either $1 \ \mu g \ l^{-1}$ in water or $1 \ \mu g \ m^{-3}$ in air. The dose or exposure concentration associated with a given risk can also be calculated by rearranging terms in the slope factor equation shown above to solve for the dose term. The result is termed the risk specific dose (or concentration). The risk specific dose is often used as the basis for the exposure criteria for carcinogens.

The level of risk that is considered acceptable varies, and may be defined by law, regulation, or policy. In general, only very low risks are used in setting criteria for environmental exposures (e.g., between 1 in 10 000 and 1 in 1 000 000). For occupational settings a higher risk level (e.g., 1 in 1000) is often used in deriving the exposure criteria.

See also: Carcinogen Classification Schemes; Dose-Response Relationship; Emergency Response and

Preparedness; Exposure Assessment; Occupational Exposure Limits; Risk Assessment, Human Health.

Relevant Websites

- http://www.epa.gov US Environmental Protection Agency.
- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Exposure Criteria.
- http://www.hc-sc.gc.ca Health Canada.
- http://www.inchem.org World Health Organization.
- http://www.osha.gov Occupational Safety and Health Administration.
- http://www.cdc.gov National Institute for Occupational Safety and Health.
- http://www.acgih.org American Conference of Governmental Industrial Hygienists.
- http://www.aiha.org American Industrial Hygiene Association.
- http://europe.osha.eu European Union Scientific Committee for Occupational Exposure Limits.
- http://www.icrp.org International Commission for Radiological Protection.

Exxon Valdez

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Introduction

Spills of oil into the world's oceans are common occurrences although very large releases are rare. The spills from the *Torrey Canyon* in the English Channel in 1967 and from the *Amoco Cadiz* off the coast of Brittany, France in 1978 are two of the largest and most serious spills in recent decades. Significant but somewhat smaller spills continue to occur. For example, in 2002, the tanker *Prestige* sank off the northern coast of Spain covering ~ 350 miles of rocks and coves with oil sludge. Public reactions to these spills have mainly been local and the environmental impacts of the spills have been appreciated most by citizens in the regions and countries affected.

The size of the spill is not the only factor that affects the impact. The seriousness of the problems that these spills can cause is also a function of its location and the type and amount of the various chemical constituents in the oil. Those spills closest to shore generally have the greatest adverse effects on the environment since the oil does not have time to disperse before reaching shore and the higher concentrations can significantly impact the sensitive habitats of a variety of organisms. Very large spills farther from the shore can also have serious impacts since a longer stretch of coastline may be affected as the oil spreads out and, even with dilution, levels may remain high enough to have serious effects on aquatic and shoreline ecosystems. With regard to the composition of the oil, one important consideration is the presence and amount of polycyclic aromatic hydrocarbons, which appear to produce toxic effects on some marine species at low concentrations.

Exxon Valdez

The Spill

The ship called *Exxon Valdez* transporting crude oil grounded on March 24, 1989 at Bligh Reef resulting in the rupture of eight of its 11 cargo tanks spilling ~ 11 million gallons of crude oil into Prince William Sound, Alaska. Approximately 1300 miles of pristine shoreline was contaminated with oil to varying degrees, and Prince William Sound was most severely affected. A majority of the spill appears to have been recovered as the result of the cleanup and the natural evaporation process. However, pockets of crude oil remain in some locations, where there is evidence of continuing damage. **Figure 1** shows the spread of the spill in the months following the event.

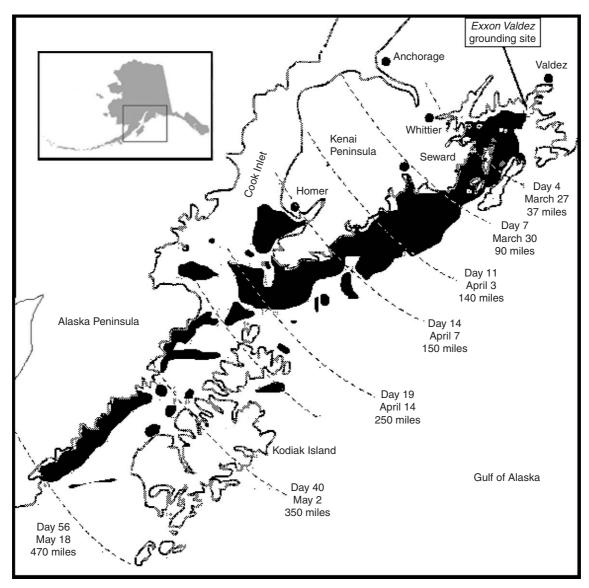


Figure 1 Spread of the spill in the months following the event.

This spill off the coast of Alaska generated the most media attention in the United States of any spill anywhere in the world. It also led to the most comprehensive scientific investigation of such incidents although much less oil was spilled than in many other tanker accidents. For example, the *Exxon Valdez* spilled only one-sixth as much oil as the *Amoco Cadiz* and about one-half as much as the *Prestige*. It is estimated that the *Amoco Cadiz* spilled ~220 000 tons of oil, probably the largest amount spilled by a tanker in history.

Attempts to clean up the *Exxon Valdez* spill were made by a combination of government agencies and the industry. The main methods employed were burning the oil, mechanically removing it from the water and the shore, use of chemical dispersants, and application of hot water to the shore. In addition, many beaches were fertilized to promote growth of microscopic bacteria that degrade hydrocarbons, a process known as bioremediation. However, it was later found that some of the cleanup methods were damaging (e.g., hot water treatment turned out to be harmful since small organisms were literally cooked by the hot water).

Ultimately, the cleanup crews collected ~14% of the oil that was spilled while ~13% sunk to the sea floor. A major portion of the remaining oil evaporated and another portion was naturally degraded over the years. About 2% (~216 000 gal) remained on the beaches. The most recent survey of lingering oil was conducted in the intertidal zone of Prince William Sound in the summer of 2001 by the National Oceanic and Atmospheric Administration. The survey results indicate that oil was present at 58% of the sites assessed and that a total shoreline area of ~ 20 acres in Prince William Sound is still contaminated.

Toxicological Impacts

During the 15 years since the spill, there has been a continuing research effort to evaluate the initial and long-term impacts of the spill, how they were ameliorated by remedial actions and the passage of time, and the status of various organisms affected by the spill. Extrapolation from the data that have been collected suggests that the Exxon Valdez spill led to the deaths of $\sim 250\,000$ seabirds, 3000 sea otters, 300 harbor seals, 250 eagles, and a number of killer whales. Monitoring data indicate that populations of some of these creatures, such as the sea otter, had not recovered as of 2002 - more than a dozen years later. However, other animal populations, such as those of the bald eagle, recovered fully in the intervening years. The fate of sea otters falls into an intermediate category, since populations are recovering but were not fully recovered as of 2002.

There are four general causes of toxicity in animals exposed to oil spill residuals. The first is the adverse impact of the oil on the insulation value of the fur and feathers of animals. The second is acute toxicity from ingesting oil products, often while animal is trying to clean the oil off fur or feathers. The third is long-term or delayed toxicity due to oil residue exposures that are not lethal but which decrease the hardiness or reproductive fitness of the exposed animals. The last is the brain lesions and disorientation caused by inhalation of toxic fumes. In addition, populations of animals can be severely affected if oil toxicity adversely impacts the creatures that they feed on or greatly decreases available habitat. Both of these problems can affect the organism's ability to survive and reproduce.

In addition to effects of the spill on larger animals, there were also impacts on smaller organisms resulting from the spill and the cleanup. Mortality in microalgae and benthic invertebrates occurred due to a combination of chemical toxicity and their physical displacement from natural habitat by pressurized wash-water that was applied after the spill to clean up the contaminated area.

Postspill Research

One of the aims of the research undertaken after the *Exxon Valdez* spill was to understand the impacts of oil spills on the environment. However, assessing the toxicity of oil spills is complicated by a number of variables, such as the presence of oil from other sources; for example, natural seeps, and by the absence of baseline prespill data with which to compare postspill environmental levels and effects. In addition, it has been difficult to determine the natural factors that affect the persistence of the oil in various environmental locations near the spill since the persistence was strongly influenced by the steps taken to clean the oil. Further, the Exxon Valdez spill occurred in the arctic environment and it is not clear how valid it is to extrapolate the data gathered in this environment to other climates. These considerations suggest that any conclusions drawn about persistence would be situation-specific and hard to apply to other spills. A further complicating factor is that in a number of cases, scientific studies were designed to address very specific concerns related to litigation rather than to answer broad environmental toxicology questions which might be applicable to a variety of other locations and situations.

Research into the impact of the spills has been aided by the passage of the US Oil Pollution Act of 1990, which included a provision establishing the Oil Spill Recovery Institute (OSRI). OSRI provides funding to support oil-spill related research as well as education and technology development for dealing with oil spills in the Arctic environment. The results of research it supported and other research, such as that funded as part of litigation activities, has been summarized in the 2002 National Research Council report Oil and the Sea: Inputs, Fates and Effects. This report also puts into perspective the small contribution of tanker and pipeline spills as compared to other sources of ocean oil such as land-based runoff, polluted rivers, small boats and water craft, as well natural seeps from the sea floor.

Summary

Tanker spills are dramatic examples of the adverse impacts of large amounts of oil on aquatic and coastline ecosystems. The *Exxon Valdez* spill led to great public awareness of this problem and subsequent legislation aimed at decreasing the probability and impact of oil spills, improving our understanding of the impacts of such spills, and increasing our capabilities for dealing with spills of oil from tankers and other sources. The continuing occurrence of tanker spills and the difficulties in dealing with the consequences of such incidents suggest that additional research emphasis be placed on ways to prevent such incidents in the future.

Further Reading

- National Research Council (2002) *Oil in the Sea: Inputs, Fates, and Effects.* Washington, DC: National Academy Press.
- Peterson CH, Rice SD, Short JW, *et al.* (2003) Long-term ecosystem response to the Exxon Valdez oil spill. *Science* 302: 2082–2086.
- Wells PG, Butler JN, and Hughes JS (eds.) (1995) Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters. Philadelphia, PA: American Society for Testing and Materials.

Eye Irritancy Testing

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Introduction

Virtually all man-made chemicals have the potential to end up in the eyes of people. In fact, many (e.g., cosmetics and shampoos) are intended to be used in such a manner that ocular exposure is inevitable in a large number of cases.

In the early 1930s, an untested eyelash dye containing *p*-phenylenediamine ('Lash Lure') was brought on the market in the United States. This product (as well as a number of similar products) rapidly demonstrated that it could sensitize the external ocular structures, leading to corneal ulceration with loss of vision and at least one fatality. This occurrence led to the revision of the Food, Drug and Cosmetic Act of 1938. To meet the new provisions of this act, a number of test methods were proposed. Latven and Molitor and Mann and Pullinger were among those to first report on the use of rabbits as a test model to predict eye irritation in humans. No specific scoring system was presented to grade or summarize the results in these tests, however, and the use of animals with pigmented eyes (as opposed to albinos) was advocated. Early in 1944, Friedenwald et al. published a method using albino rabbits in a manner very similar to that of the original (1944) Draize publication but still prescribing the description of the individual animal responses as the means of evaluating and reporting the results. Although a scoring method was provided, no overall score was generated for the test group. Draize (head of the Dermal and Ocular Toxicity Branch at the US Food and Drug Administration (FDA)) modified Friedenwald's procedure and made the significant addition of a summary scoring system.

Relevant Websites

- http://www.oilspill.state.ak.us Exxon Valdez Oil Spill Trustee Council. Oil Spill Facts. State of Alaska, Anchorage, Alaska, 2003.
- http://www.response.restoration.noaa.gov Official website for National Oceanic and Atmospheric Administration: Office of Response and Restoration.
- http://www.fakr.noaa.gov Official website for Office of Exxon Valdez Oil Spill Damage Assessment and Restoration.

During the 40 years since the publication of the Draize scoring system, it has become common practice to call all acute eye irritation tests performed in rabbits 'the Draize eye test'. However, since 1944, ocular irritation testing in rabbits has significantly changed. Clearly, there is no longer a single test design that is used, and there are different objectives that are pursued by different groups using the same test. This lack of standardization has been recognized for some time and attempts have been made to address standardization of at least the methodological aspects of the test (such as how test materials are applied and scoring performed), if not the design aspects (such as numbers and sources of test animals). For the purposes of the remainder of this entry, the term Draize test has been replaced with eye irritancy testing.

Ocular irritation tests are significantly different from the other local tissue irritation tests on a number of grounds. For the pharmaceutical industry, eye irritation testing is performed when the material is intended to be put into the eye as a means or route of application for ocular therapy. There are a number of special tests applicable to pharmaceuticals or medical devices that are beyond the scope of this discussion since they are not intended to assess potential acute effects or irritation. In general, however, it is desired that an eye irritation test be both sensitive and accurate in predicting the potential to cause irritation in humans. Failing to identify human ocular irritants (lack of sensitivity) is to be avoided, but of equal concern is the occurrence of false positives.

The primary eye irritation test was originally intended to predict the potential for a single splash of chemical into the eye of a human being to cause reversible or permanent damage. The common core design of the test, as currently utilized, consists of instilling either 0.1 ml of a liquid or 0.1 g of a powder (or other solid) into one eye of each of six rabbits. The material is not washed out, and both eyes of each animal (the untreated eye acting as a control) are graded according to the Draize scale (**Table 1**) at 24, 48, and 72 h after test material instillation. The resulting scores are summed for each animal. A variation of the test involves the use of three additional rabbits which have their eyes irrigated shortly after instillation of test material. There are, however,

 Table 1
 Scale of weighted scores for grading the severity of ocular lesions

Cornea A. Opacity – degree of density (area which is most dense	
is taken for reading)	
Scattered or diffuse area, details of iris clearly visible Easily discernible translucent areas, details of iris slightly obscured	1 2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
B. Area of cornea involved	
One-quarter (or less) but not zero	1 2
Greater than one-quarter, less than one-half Greater than one-half, less than the whole area	2
Greater than three-quarters up to the whole area	4
luia	
<i>Iris</i> A. Values	
Folds above normal, congestion, swelling, circumcorneal ingestion (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is possible)	1
No reaction to light, hemorrhage; gross destruction (any of these)	2
Scoring equals $A \times B$	
Total possible maximum = 10	
Conjunctivae	
A. Redness (refers to palpebral conjunctival only)	
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not	2
easily discernible Diffuse beefy red	3
B. Chemosis	
Any swelling above normal (includes nictating membrane)	1
Obvious swelling with partial eversion of the lids Swelling with lids about half closed	2 3
Swelling with lids about half closed to completely closed	4
C. Discharge	
Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hair just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3
Scoring $(A + B + C) \times 2$	
Total maximum - 20	

Total maximum = 20

Note: The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae.

Reproduced from Draize JN, Woodard G, and Calvery HO (1944) Methods for the study of irritation and toxicity of substances applied to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics* 82: 377–390. many variations of these two major design subsets (i.e., with and without irrigation groups).

Even though the major objective of the Draize scale was to standardize scoring, it was recognized early that this was not happening; instead, different people were 'reading' the same response differently. To address this, two sets of standards (also called training guide) have been published by regulatory agencies through the years. In 1965, the US FDA published an illustrated guide with color pictures as standards. In 1974, the Consumer Product Safety Commission (CPSC) published a second illustrated guide which provided 20 color photographic slides as standards. The US Environmental Protection Agency (EPA) also supported the development of a guide with color plates/slides.

A second source of methodological variability has been in the procedure utilized to instill test materials into the eyes. The general consensus is that the substance should be dropped into the cul-de-sac of the conjunctiva formed by gently pulling the lower eyelid away from the eye, then the animal should be allowed to blink and the material should be spread across the entire corneal surface. In the past, however, there were other application procedures (such as placing the material directly onto the surface of the cornea).

There are also variations in the design of the 'standard' test. Most laboratories observe animals until at least 7 days after instillation and may extend the test to 21 days after instillation if any irritation persists (in fact, US EPA labeling requires such an extension). These prolonged postexposure observation periods are designed to allow for evaluation of the true severity of damage and for assessing the ability to repair the ocular damage. The results of these tests are evaluated by a descriptive classification scale (Table 2) such as that described in National Academy of Sciences (NAS) publication No. 1138, which is a variation of that reported by Green et al. This classification is based on the most severe response observed in a group of six nonirrigated eyes, and data from all observation periods are used for this evaluation.

Different regulatory agencies within the United States have prescribed slightly different procedures for different perceived regulatory needs. There have also been a number of additional grading schemes, but these will not be reviewed here.

Current In Vivo Test Protocols

Any discussion of current test protocols (or of any proposed *in vitro* alternatives) must start with a review of why tests are performed. What are the

Table 2 Severity and persistence of irritation

- Inconsequential or complete lack of irritation: Exposure of the eyes to a material under the specified conditions caused no significant ocular changes. No staining with fluorescein can be observed. Any changes that do occur clear within 24 h and are no greater than those caused by normal saline under the same conditions.
- Moderate irritation: Exposure of the eye of the material under the specified conditions causes minor, superficial, and transient changes of the cornea, iris, or conjunctivae as determined by external or slit-lamp examination with fluorescein staining. The appearance at the 24 hr or subsequent grading of any of the following changes is sufficient to characterize a response as moderate irritation: opacity of the cornea (other than a slight dulling of the normal luster), hyperemia of the iris, or swelling of the conjunctivae. Any changes that are seen clear within 7 davs.
- Substantial irritation: Exposure of the eye to the material under the specified conditions causes significant injury to the eye, such as loss of the corneal epithelium, corneal opacity, iritis (other than a slight injection), conjunctivitis, parmus, or bullae. The effects clear within 21 days.
- Severe irritation or corrosion: Exposure of the eye to the material under the specified conditions results in the same types of injury as in the previous category and in significant necrosis or other injuries that adversely affect the visual process. Injuries persist for 21 days or more.

objectives of eye irritation testing, and how are these different objectives reflected not just in test design and interpretation but also in the regulations requiring testing and in the ways that test results are utilized?

There are four major groups of organizations that are required to perform eye irritation studies. These are the pharmaceutical, cosmetic and toiletries, consumer product, and industrial chemical groups. There are also minor categories of use (which we will not consider here) such as for military agents.

In the pharmaceutical industry, eye irritation testing is performed when the material is intended to be put into the eye as a means or route of application or for ocular therapy. There are a number of special tests applicable to pharmaceuticals or medical devices which are beyond the scope of this discussion because they are not intended to assess potential acute effects or irritation. In general, however, it is desired that an eye irritation test that is utilized by this group be both sensitive and accurate in predicting the potential to cause irritation in humans. Failing to identify human ocular irritants (lack of sensitivity) is to be avoided, but of equal concern is the occurrence of false positives.

The cosmetics and toiletries industry is similar to the pharmaceutical industry in that the materials of interest are frequently intended for repeated application in the area of the eye. In such uses, contact with the eye is common, though not intended or desirable. In this case, the objective is a test that is as sensitive (as that in the preceding paragraph), even if this results in a low incidence of false positives. Even a moderate irritant would not be desired but might be acceptable in certain cases (such as deodorants and depilatories) in which the potential for eye contact is minimal.

Consumer products which are not used for personal care (such as soaps, detergents, and drain cleaners) are approached from yet a different perspective. These products are not intended to be used in a manner that either causes them to get into the eyes or makes that occurrence likely. However, because of the very large population that uses them and the fact that their modes of use do not include active measures to prevent eye contact (such as the use of goggles and face shields), the aim is to accurately identify severe eye irritants. Agricultural chemicals generally fit in this category, though many of them are covered by specific testing requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Finally, there are industrial chemicals. These are handled by a smaller population (relative to consumer products). Eye contact is never intended and, in fact, active measures are taken to prevent it. The use of eye irritation data in these cases is to fulfill labeling requirements for shipping and to provide hazard assessment information for accidental exposures and treatment information. The results of such tests do not directly affect the economic future of a material. It is desired to accurately identify moderate and severe irritants (particularly those with irreversible effects) and to know if rinsing of the eyes after exposure will make the consequences of exposure better or worse. False negatives for mild reversible irritation are acceptable.

To fulfill these objectives, a number of basic test protocols have been developed and mandated by different regulatory groups. **Table 3** gives an overview of these protocols. Historically, the philosophy underlying these test designs made maximization of the biological response equivalent to having the most sensitive test.

One widely used study design, which begins with a screening procedure as an attempt to avoid testing severe irritants or corrosives in animals, is described in the following section.

Rabbit Eye Irritancy Testing: Widely Used Study Design

Test Article Screening Procedure

1. Each test substance will be screened in order to eliminate potentially corrosive or severely irritating

Agency	Draize	FHSA	NAS	OECD	IRLG	CPSC	TOSCA	<i>FIFRA</i> ^g
Test species	Albino rabbit	Same	Same ^a	Same	Same	Same	Same	Same
Age/weight	NS ^b	NS	Sexually mature/ less than 2 years old	NS	Young adult/2.0	NS	NS	NS
Sex	NS	NS	Either	NC	Either	NS	NS	NS
No. of animals/group	Six	6–18	Four (minimum)	Three (minimum)	Three (preliminary test) ^c ; six	6–18	Six	Six
Test agent volume and method of instillation – liquids	0.1 ml in the eye	Same as for Draize	Liquids and solids: two or more different doses within the probable range of human exposure ^d	Same as for Draize	Same as for Draize	Same as for Draize	Same as for FHSA	Same as for FHSA
Solids	NS	100 mg or 0.1 ml equivalent when this volume weighs less than 100 mg; direct instillation into conjunctival sac	Manner of application should reflect probable route of accidental exposure	Same as for FSHA	Same as for FSHA	Same as for FSHA	Same as for FSHA	Same as for FSHA
Aerosols ^e	NS	NS	Short burst of distance approximating self-induced eye exposure	1 s burst sprayed at 10 cm	1 sec burst sprayed at ~4 in.	NS	Same as for OECD	Same as for OECD

Table 3 Regulatory guidelines for irritation test methods

Irrigation schedule	At 2 s (three animals) and at 4 s (three animals) following instillation of test agent (three animals)	Eyes may be washed after 24 h reading	May be conducted with separate experimental groups	Same as for FHSA; in addition, for substances found irritating; wash at 4 s (three animals) and at 30 s (three animals)	Same as for FHSA	Same as for FHSA	Same as for FHSA	Same as for FHSA
Irrigation treatment	20 ml tap water (body temp.)	Sodium chloride solution (USP or equivalent)	NS	Wash with water for 5 min using volume and velocity of flow which will not cause injury	Tap water or sodium chloride solution (USP or equivalent)	Same as for FHSA	NS	NS
Examination times (postinstillation)	24, 48, and 72 h; 4 and 7 days	24, 48, and 72 h	1, 3, 7, 14, and 21 days	1, 24, 48, and 72 h ^f	24, 48, and 72 h	24, 48, and 72 h	Same as for OECD	Same as for OECD
Use of flourescein	NS	May be applied after the 24 h reading (optional)	May be used	Same as for FHSA	Same as for FHSA	Same as for FHSA	Same as for FHSA	Same as for FHSA
Use of anesthetics	NS	NS	NS	May be used	May be used	NS	May be used	May be used
Scoring and evaluation	Draize <i>et al</i> .	Modified Draize <i>et al.</i> (1944) or a slit-lamp scoring system	CPSC (1976)	CPSC (1976)	CPSC (1976)	CPSC (1976)	CPSC (1976)	CPSC (1976)

Note: FHSA, Federal Hazard Substance Act; NAS, National Academy of Sciences; OECD, Organization for Economic Cooperation and Development; IRLG, Interagency Liaison Group. ^aTests should be conducted on monkeys when confirmatory data are required.

^bNS, not specified.

^c If the substance produces corrosion, severe irritation, or no irritation in a preliminary test with three animals, no further testing is necessary. If equivocal responses occur, testing on at least three additional animals should be performed.

^dSuggested doses are 0.1 and 0.05 ml for liquids.

^eCurrently, no testing guidelines exist for gases or vapors. ^fEyes may be examined at 1 h and at 7, 14, and 21 days (at the option of the investigator).

^gOffice Pesticide Assessment.

materials from being studied for eye irritation in the rabbit.

- 2. If possible, the pH of the test substance will be measured.
- 3. A primary dermal irritation test will be performed prior to the study.
- 4. The test substance will not be studied for eye irritation if it is a strong acid (pH of 2.0 or less) or strong alkali (pH of 11.0 or greater) and/or if the test substance is a severe dermal irritant (with a primary dermal irritation index (PDII) of 5–8) or causes corrosion of the skin.
- 5. If it is predicted that the test substance does not have the potential to be severely irritating or corrosive to the eye, continue to Rabbit Screening Procedure.

Rabbit Screening Procedure

- 1. A group of at least 12 New Zealand White rabbits of either sex are screened for the study. The animals are removed from their cages and placed in rabbit restraints. Care should be taken to prevent mechanical damage to the eye during this procedure.
- 2. All rabbits selected for the study must be in good health; any rabbit exhibiting sniffles, hair loss, loose stools, or apparent weight loss is rejected and replaced.
- 3. One hour prior to instillation of the test substance, both eyes of each rabbit are examined for signs of irritation and corneal defects with a hand-held slit lamp. All eyes are stained with 2.0% sodium fluorescein and examined to confirm the absence of corneal lesions. Fluorescein staining: Cup the lower lid of the eye to be tested and instill one drop of a 2% (in water) sodium fluorescein solution onto the surface of the cornea. After 15 s, thoroughly rinse the eye with physiological saline. Examine the eye, employing a hand-held long-wave UV illuminator in a darkened room. Corneal lesions, if present, appear as bright yellowish-green fluorescent areas.
- 4. Only 9 of the 12 animals are selected for the study. The nine rabbits must not show any signs of eye irritation and must show either a negative or a minimum fluorescein reaction (due to normal epithelial desquamation).

Study Procedure

1. At least 1 h after fluorescein staining, the test substance is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup (conjunctival cul-de-sac) into which the test material is dropped. The upper and lower lids are then gently held together for 1 s to prevent immediate loss of material.

- 2. The other eye remains untreated and serves as a control.
- 3. For testing liquids, 0.01 ml of the test substance is used.
- 4. For solid or pastes, 100 mg of the test substance is used.
- 5. When the test substance is in flake, granular, powder, or other particulate form, the amount that has a volume of 0.01 ml (after gently compacting the particles by tapping the measuring container in a way that will not alter their individual form) is used whenever this volume weighs less than 10 mg.
- 6. For aerosol products, the eye should be held open and the substance administered in a single, 1 s burst at a distance of ~4 in. directly in front of the eye. The velocity of the elected material should not traumatize the eye. The dose should be approximated by weighing the aerosol can before and after each treatment. For other liquids propelled under pressure, such as substances delivered by pump sprays, an aliquot of 0.01 ml should be collected and instilled in the eye as for liquids.
- 7. The treated eyes of six of the rabbits are not washed following instillation of the test substance.
- 8. The treated eyes of the remaining three rabbits are irrigated for 1 min with tap water at room temperature, starting 20 s after instillation.
- 9. To prevent self-inflicted trauma by the animals immediately after instillation of the test substance, the animals are not immediately returned to their cages. After examination and grading of the eyes of the control animals at 1h postexposure, the animals are returned carefully to their respective cages.

Observations

- 1. The eyes are observed for any immediate signs of discomfort after instilling the test substance. Blepharospasm and/or excessive tearing are indicative of irritating sensations caused by the test substance, and their duration should be noted.
- 2. Blepharospasm does not necessarily indicate that the eye will show signs of ocular irritation.
- 3. Grading and scoring of ocular irritation are performed in accordance with **Table 1**. The eyes are examined, and grades of ocular reactions are recorded.
- 4. If signs of irritation persist at Day 7, readings are continued on Days 10 and 14 after exposure or until all signs of reversible toxicity are resolved.
- 5. In addition to the required observation of the cornea, iris, and conjunctiva, serious effects (such as parmus, rupture of the globe, or blistering of the

conjunctivae) indicative of a corrosive action are reported.

6. Whether or not toxic effects are reversible depends on the nature, extent, and intensity of damage. Most lesions, if reversible, will heal or clear within 21 days. Therefore, if ocular irritation is present at the 14 day reading, a 21 day reading is required to determine whether the ocular damage is reversible or nonreversible.

Limitations

Commonly used methodological variations to improve the sensitivity and accuracy of describing damage in these tests are inspection of the eyes with a slit lamp and instillation of the eyes with a vital dye (very commonly, fluorescein) as an indicator of increases in permeability of the corneal barrier.

To assess the adequacy of the currently employed eye irritation tests in fulfilling the objectives behind their use, we must evaluate them in terms of (1) their accuracy (how well they predict the hazard to humans); (2) whether comparable results can be obtained by different technicians and laboratories; and (3) what methods and designs have been developed and are being employed as alternatives to rabbit eye irritation tests. Assessing the accuracy of rabbit eye irritation tests – or indeed, of any predictive test of eye irritation – requires that the results of such tests be compared to what happens in humans. Unfortunately, the human database for making comparisons is not large. The concerns, however, have been present almost as long as the tests have been performed.

Rabbit Eye Irritancy Testing: Alternative Methods

A number of alternatives have been proposed and adapted for the performance of rabbit eye irritation tests. These alternatives have been directed at the twin objectives of making the tests more accurate in predicting human responses and reducing both the use of animals and the degree of discomfort or suffering experienced by those that are used.

Alternative Species

Dogs, monkeys, and mice have all been suggested as alternatives to rabbits that would be more representative of humans. Each of these, however, also has shown differences in responses compared to those seen in humans and poses additional problems in terms of cost, handling, lack of database, and so on.

Use of Anesthetics

Over the years, a number of authors have proposed that topical anesthetics be administered to the eyes of rabbits prior to their use in the test. Both OECD and IRLG regulations provide for such a use. However, numerous published and unpublished studies have shown that such use of anesthetics interferes with test results, usually by increasing the severity of eye irritation findings.

Decreased Volume of Test Material

An alternative proposal (one which a survey showed has been adopted by a number of laboratories) is to use a reduced volume/weight of test material.

In 1984, Freeberg *et al.* reported a study in which they evaluated 21 different chemicals at volumes of 0.1, 0.03, 0.01, and 0.003 ml. These are materials on which human data were already available. It was found that the volume reduction did not change the rank order of responses, and that 0.01 ml (10 μ l) gave results which best mirrored those seen in humans.

In 1985, Walker reported an evaluation of the lowvolume (0.01 ml) test which assessed its results for the correlation with those in humans based on the number of days until clearing of injury and reported that 0.01 ml gave a better correlation than did 0.1 ml.

While it must be pointed out that there may be some classes of chemicals for which low-volume tests may give results less representative of those seen in humans, it seems clear that this approach should be seriously considered by those performing such tests.

Use of Prescreens

This alternative may also be considered a tier approach. Its objective is to avoid testing severely irritating or corrosive materials in many (or in some cases, any) rabbits. This approach entails a number of steps which should be considered independently.

First, a screen based on physicochemical properties should be used. This usually means pH, but also should be extended to materials with high oxidation or reduction potentials (e.g., hexavalent chromium salts).

Although the correlation between low pHs (acids) and eye damage in the rabbit has not been found to be excellent, all alkalis (pH 11.5 or above) tested have been reported to produce opacities and ocular damage. Many laboratories now use pH cutoffs for testing of 2.0 or lower and 11.5 or 12.0 and higher. If a material falls outside these cutoffs (or is so identified due to other physicochemical parameters), then it is (1) not tested in the rabbit eye and is assumed to be corrosive; (2) evaluated in a secondary screen such as an *in vitro* cytotoxicity test or primary dermal irritation test; or (3) evaluated in a single rabbit before a full-scale eye irritation test is performed. It should be kept in mind that the correlation of all the

physicochemical screen parameters with acute eye test results is very concentration-dependent, being good at high concentrations and marginal at lower concentrations (where various buffering systems present in the eye are meaningful).

The second commonly used level of prescreen is the use of PDI test results. In this approach the PDI study is performed before the eye irritation study, and if the score from that study (PDII ranging from 0 to 8) is above a certain level (normally 5.0

Table 4 In vitro alternatives for eye irritation tests

Morphology
Enucleated superfused rabbit eye system
BALB/c 3T3 cells/morphological assays (HTD)
Cell toxicity
Adhesion/cell proliferation
BHK cells/growth inhibition
BHK cells/colony-formation efficiency
BHK cells/cell detachment
SIRC cells/colony-forming assay
BALB/c 3T3 cells/total protein
BCL/D1 cells/total protein
Primary rabbit corneal cells/colony-forming assay
Membrane integrity
LS cells/dual dye staining
Thymocytes/dual fluorescent dye staining
LS cells/dual dye staining
RCE-SIRC-P815-YAC-I/Cr release
L929 cells/cell viability
Bovine red blood cell/hemolysis
Mouse L929 fibroblasts/erythrocin C staining
Rabbit corneal cell cultures/plasminogen activator
Agarose diffusion
Cell metabolism
Rabbit corneal cell cultures/plasminogen activator
LS cells/ATP assay
BALB/c 3T3 cells/neutral red uptake
BALB/c 3T3 cells/uridine uptake inhibition assay
HeLa cells/metabolic inhibition test (MIT-24)
MDCK cells/dye diffusion
Cell and tissue physiology
Epidermal slice/electrical conductivity
Rabbit ileum/contraction inhibition
Bovine cornea/corneal opacity
Proptoses mouse eye/permeability test
Inflammation/immunity
Chorioallantoic membrane (CAM)
CAM
HET-CAM
Bovine corneal cup model/leukocyte chemotactic factors
Rat peritoneal cells/histamine release
Rat peritoneal mast cells/serotonin release
Rat vaginal explant/prostaglandin release
Bovine eye cup/histamine and leakotriene C4 release
Recovery/repair
Chorioallantoic membrane
Other
EYTEX assay
Computer-based structure-activity
Tetrahymena/motility

or greater), the same options already outlined for physicochemical parameters can be exercised.

In Vitro Tests

Sustained efforts to develop a true alternative (*in vitro*) to current ocular irritancy tests have been undertaken during the past 6 years. A complete review of this work is beyond the scope of this entry, and it continues to be in a high state of flux. However, at least a brief outline or summary of the approaches being pursued is appropriate.

As summarized in Table 4, there are six major categories of approaches to alternative eye irritation tests. The first five of these aim at assessing portions of the irritation response (alterations in tissue morphology, toxicity to individual component cells, alterations in cell or tissue physiology, inflammation or immune modulation, and alterations in repair and recovery processes). These methods have the limitation that they assume that one of these component parts can or will predict effects in the complete organ system. A more likely case is that, while each may serve well to predict the effects of a set of chemical structures which have that component as a determining part of the ocular irritation response, a valid assessment across a broad range of structures will require the use of a collection or battery of such tests.

The sixth category contains tests which have little or no empirical basis, such as computer-assisted structure-activity relationship models. These approaches can only be assessed in terms of how well (or poorly) they perform.

See also: Analytical Toxicology; Consumer Product Safety Commission; Federal Insecticide, Fungicide, and Rodenticide Act; Food and Drug Administration, US; Good Laboratory Practices (GLP); *In Vitro* Test; *In Vivo* Test; Sensory Organs; Toxicity, Acute; Toxicity Testing, Alternatives.

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FAO See Food and Agriculture Organization of the United Nations.

Federal Insecticide, Fungicide, and Rodenticide Act, US

Chris F Wilkinson and Michael A Kamrin

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The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) is the main statute under which all pesticides are distributed and sold in the United States. Federal regulation of pesticides started with the Insecticide Act of 1910, which was directed primarily toward protecting consumers from fraudulent pesticide products; it remained the major law governing pesticide products for 37 years. The Insecticide Act was essentially a labeling statute and did not require registration of products or establish any significant safety standards.

The Insecticide Act was replaced by FIFRA in 1947. FIFRA required that pesticides be registered by the Secretary of Agriculture before being distributed or sold in interstate or foreign commerce and required label warnings and instructions for safe use of highly toxic products. Since that time, there have been numerous amendments to FIFRA to bring the statute to its current form, and a number of these have been linked with major federal reorganizational changes. In particular, on December 2, 1970, President Nixon created the US Environmental Protection Agency (EPA). EPA assumed the pesticide regulatory functions of United States Department of Agriculture (USDA) and with the 1972, 1975, 1978, 1980, 1988, and 1990 amendments as well as the passage of the Food Quality Protection Act in 1996, FIFRA has become an increasingly complex statute with greatly increased authority to regulate pesticide products. The regulations governing pesticides are administered by EPA's Office of Pesticide Programs.

FIFRA requires that every pesticide to be sold or distributed in interstate and intrastate commerce be registered. A registration is equivalent to a license to sell or distribute a pesticide in commerce. Registration is based on submission to EPA, by the registrant, of data 'demonstrating that the pesticide will not cause unreasonable adverse effects on human health or the environment when it is used according to approved label directions'; FIFRA is a risk-balancing statute that does not state that pesticides must be free of all potential risk. It also considers economic and social costs and benefits. A pesticide must also be registered with the appropriate agency in each state in which it is to be used and, in some states such as California, pesticide registration requirements may be even more stringent than those of EPA.

An application for an EPA pesticide registration must be accompanied by data establishing that it is efficacious and can be used without causing unreasonable adverse effects. In registering new products, pesticide manufacturers have the responsibility of providing the data necessary to demonstrate that a material will not present unreasonable risks to humans or the environment. This requires the manufacturer to conduct a comprehensive battery of tests to determine acute and chronic mammalian toxicity, potential adverse effects on nontarget species (birds and fish), environmental fate and transport, and other factors. The likelihood that the material will leave residues in food crops or might leach into groundwater is also evaluated. The tests required to get a single new pesticide product on the market may cost as much as \$40 million and the process may take from 6 to 8 years.

Toxicology data are among the most time-consuming and costly to generate. They are designed to establish the potential adverse effects of the pesticide by different routes of exposure (oral, inhalation, and dermal) and include a complete series of acute, subchronic, and chronic animal studies; metabolism and pharmacokinetic studies; and a battery of tests to determine potential mutagenic activity. While certain exemptions for specific uses may be granted, the tests typically required under FIFRA include acute oral, dermal, and inhalation toxicity; skin and eye irritation; skin sensitization; subchronic (90 day feeding); developmental toxicity (teratology); two-generation reproductive toxicity; and chronic oncogenicity. Several of these, such as the developmental toxicity and oncogenicity studies, are usually conducted with two different species. The primary objective of toxicology testing under FIFRA is to establish no-observed-effect levels (NOELs) or lowest-observed-effect levels for noncarcinogenic endpoints and cancer potency factors (q1* values) for pesticides classified as carcinogens. The NOEL values are used to calculate a reference dose (RfD) (once termed the acceptable daily intake). The RfD is considered to be the daily dose of the pesticide that could be consumed by humans each day, for a lifetime, without causing any adverse effects. The RfD is obtained by applying a 'safety factor' or 'uncertainty factor' to the NOEL. The uncertainty factor reflects the degree of uncertainty in the data; if the data are good, the factor may be relatively small (perhaps 10) but if the toxicology data are uncertain, it may be as great as 1000. A typical safety factor is ~ 100 , a factor of 10 being used to express uncertainty in extrapolating from animals to humans and an additional factor of 10 to cover possible differences in susceptibility in the human population.

All tests required for FIFRA must be conducted according to FIFRA Good Laboratory Practices (GLP) that specify the minimum practices and procedures that must be followed to ensure the quality and integrity of the data submitted in support of a pesticide registration. GLP regulations were initially promulgated in 1983. Compliance with GLP standards is monitored through a program of laboratory inspections and study audits coordinated through the EPA.

If, after a pesticide product has been registered and in commerce for some time, new data become available that suggest that criteria for determining unreasonable adverse effects have been exceeded, a special review (formally called a Rebuttable Presumption Against Registration) can be initiated. The special review may result in no change in the registration status of the pesticide (if the criteria for unreasonable risk are found not to have been exceeded) or may lead to restrictions in the use of a pesticide or the complete suspension or cancellation of the registration.

See also: Delaney Clause; Food, Drug, and Cosmetic Act, US; Food Quality Protection Act, US; Good Laboratory Practices (GLP); Levels of Effect in Toxicological Assessment; Pesticides; Pharmacokinetics/Toxicokinetics; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Toxicity Testing, Alternatives; Uncertainty Analysis.

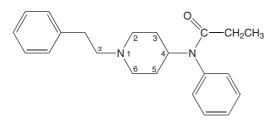
Female Reproductive System See Reproductive System, Female.

Fentanyl

Amanda Lofton

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 437-38-7
- SYNONYMS: Actiq; Phentanyl; Sublimaze; Duragesic
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Synthetic opioid analgesic
- CHEMICAL FORMULA: C₂₂H₂₈N₂O
- CHEMICAL STRUCTURE:



Uses

Fentanyl is a potent analgesic. Intravenous or intramuscular fentanyl is used in the management of acute or postsurgical pain, as a component of balanced anesthesia, or as a preoperative analgesic. Fentanyl is administered via the epidural route in combination with bupivacaine for analgesia. Transdermal fentanyl is indicated for the treatment of chronic pain. Oral transmucosal fentanyl is used for breakthrough acute pain in patients concurrently receiving transdermal therapy. Fentanyl and its derivatives have high dependence liability.

Background Information

In October 2002, the Russian military released a mystery gas to incapacitate Chechen rebels in a theater in Moscow. Hundreds of hostages suffering from 'sleeping gas' poisoning were taken to local hospitals. According to physicians in Moscow, many patients exhibited the classic triad of symptoms consistent with opioid intoxication: miosis, decreased level of consciousness, and respiratory depression. Investigation into the incident suggested that the gas was a mixture of a potent aerosolized fentanyl derivative, like carfentanil, and an inhalational anesthetic, such as halothane.

Exposure Routes and Pathways

Exposure may occur through the parenteral, oral, transmucosal, and dermal routes.

Toxicokinetics

Parenteral fentanyl is rapidly absorbed and effects are observed within minutes. Transdermal absorption is temperature dependent. Febrile patients will more rapidly absorb transdermal fentanyl. Skin in contact with the fentanyl patch absorbs drug and becomes a depot of fentanyl. A concentration gradient between the drug in the patch and the skin layers drives the continued absorption of drug. Steady-state serum concentrations are achieved $\sim 2-24$ h after initial patch application in the naive user. Peak serum concentrations of transmucosal fentanyl are achieved within 30-60 min of administration. Epidural concentrations peak after ~ 30 min. The duration of action of fentanyl varies according to the drug's route of administration. The effects of an intravenous dose last $\sim 30-60$ min. Transdermal fentanyl patches steadily release drug for more than 72 h. The volume of distribution of fentanyl ranges from 3 to 61kg^{-1} . The drug is $\sim 80-86\%$ protein bound in plasma. Fentanyl is primarily metabolized by the liver. Metabolism occurs via N-dealkylation to norfentanyl and other inactive metabolites. Approximately 75% of the drug is renally metabolized with 10% excreted unchanged in the urine. The elimination half-life varies with route of administration. An intravenous dose exhibits a half-life of \sim 219 min, a transmucosal dose 7h, and transdermal administration 17h. The half-life may be increased in patients with hepatic dysfunction.

Mechanism of Toxicity

Fentanyl stimulates mu-opioid receptors in the central nervous system (CNS), altering the body's response to pain. Fentanyl may alter the release of different neurotransmitters, such as β -endorphin, sensitive to pain. Fentanyl can produce profound CNS and respiratory depression through mechanisms common to other opioids. Respiratory depression is mediated through action on the medullary respiratory center. Fentanyl is ~ 50–100 times more potent by weight than morphine. However, unlike morphine, fentanyl appears to cause minimal histamine release. Fentanyl may induce chest wall rigidity, even when administered at therapeutic doses.

Acute and Short-Term Toxicity (or Exposure)

Animal

Fentanyl produces excitatory effects in cats, pigs, and horses. Its effects on dogs mimic its human toxicity. Naloxone can be administered to animals.

Human

Fentanyl overdose leads to the classic triad of symptoms consistent with the opioid intoxication syndrome: miosis, respiratory depression, and CNS depression. Additional toxic effects of fentanyl include bradycardia, hypotension, decreased gastrointestinal motility, euphoria, and acute lung injury.

Chronic Toxicity (or Exposure)

Animal

Studies in rats have demonstrated that fentanyl used in large doses can produce limbic system brain damage.

Human

Fentanyl is used chronically in the management of major pain in humans. One of the common side effects of therapy with opioids is constipation. However, a recent cohort analysis of a large California HMO looking at the incidence of constipation in patients receiving opioid analgesics showed a low incidence of constipation in the patients receiving fentanyl patches (3.7%).

In Vitro Toxicity Data

In an *in vitro* model of opioid dependence using rat pheochromocytoma cells, fentanyl produced an upregulation of adenylate cyclase-cAMP dependent protein kinase.

Clinical Management

Treatment is based on the patient's clinical presentation. Basic and advanced life support measures should be performed as needed. Activated charcoal may be utilized to adsorb orally administered fentanyl, such as the ingestion of a fentanyl patch. Whole bowel irrigation should be considered to speed the elimination of an ingested transdermal patch. Naloxone is the specific pharmacologic antagonist for fentanyl. Naloxone displaces fentanyl at the opioid receptor and reverses its clinical effects.

See also: Charcoal; Morphine.

Further Reading

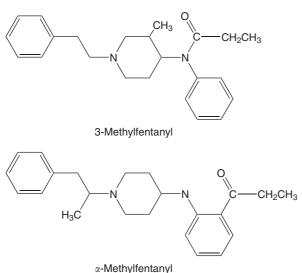
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Fentanyl Derivatives, Illicit

Amanda Lofton

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- SYNONYMS: 3-Methylfentanyl (3MF); α-Methylfentanyl; China White; Designer fentanyl; *p*-Fluorofentanyl; Street fentanyl; Synthetic heroin; Tango and Cash
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Synthetic opioid
- CHEMICAL STRUCTURES:



Uses

Illicit fentanyl derivatives are synthesized in clandestine laboratories solely for substance abuse. In the United States, these agents are classified as restricted Schedule I substances.

Background Information

In 1979, epidemic deaths among heroin users in Orange County, California, were traced to overdoses

of α -methylfentanyl, sold under the name China White. Similar events occurred in Pittsburgh in 1988 and again in Philadelphia in 1992 with the sale of 3-methylfentanyl. In later years, another epidemic took place in New York City when 3-methylfentanyl reappeared on the illicit drug market under the name Tango and Cash.

Exposure Routes and Pathways

Illicit fentanyl derivatives may be nasally insufflated as powder or solubilized and injected intravenously.

Toxicokinetics

The exact kinetics of illicit fentanyl derivatives is uncertain. Kinetics may vary with each manufactured product batch. Fentanyl derivatives are rapidly absorbed across mucous membranes. Addicts report an onset of action or 'rush' within 90 s of administration. Illicit derivatives may be up to 6000 times more potent than morphine. Like their pharmaceutically manufactured counterparts, illicit fentanyl derivatives are likely metabolized by the liver.

Mechanism of Toxicity

Illicit fentanyl derivatives act as agonists at the opioid receptor. Unsuspecting heroin users typically administer their usual 'dose' of heroin, and receive variable amounts of the more potent fentanyl analog. These agents produce profound central nervous system and respiratory depression through mechanisms common to other opioids.

Acute and Short-Term Toxicity (or Exposure)

Animal

The effects of illicit fentanyl derivatives in many animals may be similar to the actions of fentanyl. Swine develop stimulant effects when exposed to fentanyl. Naloxone can be administered to animals as needed.

Human

The toxic effects of illicit fentanyl derivatives include rapid onset respiratory and central nervous system depression. Patients often present comatose and apneic. Other signs and symptoms consistent with opioid intoxication such as bradycardia, hypotension, miosis, and decreased gastrointestinal motility also occur.

Chronic Toxicity (or Exposure)

Animal

Studies in rats have demonstrated that fentanyl used in large doses can produce limbic system brain damage.

Human

Fentanyl is used chronically in the management of major pain in humans. One of the common side effects of therapy with opioids is constipation. However, a recent cohort analysis of a large California HMO looking at the incidence of constipation in patients receiving opioid analgesics showed a low incidence of constipation in the patients receiving fentanyl patches (3.7%).

In Vitro Toxicity Data

In an *in vitro* model of opioid dependence using rat pheochromocytoma cells, fentanyl produced an upregulation of adenylate cyclase-cAMP dependent protein kinase.

Clinical Management

Basic and advanced life support measures should be initiated immediately. Activated charcoal may be utilized to adsorb illicit fentanyl derivatives following ingestion. Naloxone is the specific pharmacologic antagonist for fentanyl derivatives. Naloxone displaces these agents at the opioid receptor and reverses their clinical effects; however, higher than customary doses may be needed to successfully overcome the opioid receptor.

See also: Charcoal; Fentanyl; Heroin; Morphine.

Further Reading

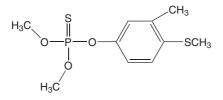
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Fenthion

Andrew M Geller

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- CHEMICAL NAME: O,O-Dimethyl O-[4-(methylthio)-*m*-tolyl]phosphorothioate
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 55-38-9
- SYNONYMS: Baytex; Baycid; Bay 29493; Dalf; DMTP; Entex; Lebaycid; Tiguvon; Mercaptophos; Prentox Fenthion 4E; Queletox; S1752; Spotton; Talodex
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organophosphorothioate pesticide
- CHEMICAL FORMULA: C10H15O3PS2
- CHEMICAL STRUCTURE:



Uses

Fenthion is a broad spectrum insecticide. When applied as a surface spray, it controls adult mosquitoes and other insect pests and spiders in agricultural, horticultural, and home garden use. Recent reports indicate that resistance to fenthion has developed in some species of mosquitoes. Fenthion is used in dermal application for treatment of swine and cattle for control of lice, flies, and ticks, and in flea and tick treatments for pets. It is also used in aqueous applications to kill dragonfly larvae in ornamental fish production ponds. Fenthion is an effective avicide, and has been marketed to control birds considered to be pests.

While fenthion is currently available worldwide, use of fenthion in the United States has greatly decreased. Its use as an avicide was canceled in March, 1999. Its use as a livestock treatment was voluntarily withdrawn by the registrant (Bayer) over a 2 year period from March, 2000. In May, 2003, the registrant requested voluntary cancellation for all of their products containing fenthion. This will likely take effect in the United States in November, 2004.

Exposure Routes and Pathways

When fenthion is used in mosquito control operations that involve adulticide applications over wide areas, exposure to adults and children can occur. Health risk is considered low for homeowners performing yard work or for adults or children taking part in other recreational activities in treated areas because typical applications use ultra-low volumes (0.023–0.046 kg active ingredient (a.i.) per acre aerial, 0.014 kg a.i. per acre ground-based application). There is, however, concern for children if they are exposed to repeated levels at the maximum allowed rate. There is also concern for workers who mix, load, and/or apply fenthion for both aerial and ground mosquito adulticide applications.

Risk of dietary exposure to fenthion is largely due to potential residues in beef meat and fat; while it can be excreted in cow's milk, US Department of Agriculture (USDA) analyses of close to 1300 samples yielded no detections. The US Environmental Protection Agency's (US EPA's) most recent re-registration documents contain data on potential dietary exposure, but these estimates have not been refined because of the change in use of the pesticide.

Drinking water is not considered to be a significant source of exposure because only minor exposure to surface water is likely due to ultra-low volume application rates.

Toxicokinetics

Fenthion is quickly absorbed into the bloodstream through the digestive tract, lungs, and skin. It is eliminated through excretion in the urine and the feces. Studies in rats reported 14 urinary metabolites. The major urinary metabolites are the sulfoxide and sulfone forms of the compound. These are rapidly eliminated primarily via the urine. Four desmethyl metabolites were also identified with the oxygen analogue sulfoxide, constituting a minor component.

Mechanism of Toxicity

The major mechanism of toxicity for fenthion is inhibition of acetylcholinesterase by the oxon metabolite of fenthion. Following extensive cholinesterase inhibition, acetylcholine accumulates in synaptic regions and disrupts cholinergic transmission in the central and peripheral nervous systems. This can result in cholinergic signs and symptoms (see section Clinical Management, for a list of relevant signs and symptoms). Fenthion toxicity does not cause organophosphorus-induced delayed neuropathy (OPIDN) but has been reported to lead to the intermediate syndrome. Recent work with models both *in vivo* and *in vitro* shows that fenthion also has antiandrogenic activity and can produce oxidative stress. More research is needed to establish whether these mechanisms are responsible for significant toxic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD₅₀ values range from ~150–550 mg kg⁻¹ body weight (BW) in mouse, rat, and rabbit. Intraperitoneal and dermal LD₅₀ values range from 125 to 330 mg kg⁻¹ BW and from 330 to 800 mg kg⁻¹ BW, respectively, in these species. An acute no-observedadverse-effect level (NOAEL) of 0.07 mg kg⁻¹ day⁻¹ for dietary exposure for monkeys was derived from a 2 year exposure study.

Human

A 28 day study was conducted using male human subjects. No clinical cholinergic signs (described in the section Clinical Management) were noted during this study. Significant cholinesterase inhibition was noted at a dosage of $0.02 \text{ mg kg}^{-1} \text{day}^{-1}$ one week after initiation of the study.

Chronic Toxicity (or Exposure)

Animal

Chronic toxicity studies on fenthion are available in the rat, dog, and monkey. The dog and monkey studies did not reveal any systemic signs of toxicity due to prolonged exposure to fenthion with the exception of cholinesterase inhibition. In the rat study, however, pathology was noted in the epididymis, the nasolacrimal duct, and in ocular tissue. Fenthion is not considered to be a carcinogen. While one test of carcinogenicity in mice indicated that fenthion may be a carcinogen in male mice, further studies in mice and rats did not support this.

Developmental toxicity studies in rat and rabbit do not show signs of enhanced sensitivity of the developing fetus to fenthion. Dams exhibited clinical signs and decreased body weights at the same dosages that induced fetal effects. In addition, plasma, erythrocyte, and brain cholinesterase inhibition was seen in dams at doses lower than those causing fetal effects. For this reason, the Food Quality Protection Act safety factor for fenthion was reduced from a default $10 \times$ to $1 \times$.

Human

Studies of pesticide applicators, who sprayed mainly fenthion showed some adverse effects on cognitive function and a higher incidence of retinal (macular) degeneration than in controls or in applicators who sprayed other pesticides. Findings of ocular toxicity were also noted in rats dosed subcutaneously with fenthion for a year. This ocular effect may be related to long-lasting effects of fenthion on biochemical function in the retina.

In Vitro Toxicity Data

Fenthion did not show evidence of mutagenicity in the bacterial reverse mutation test or in the chromosome aberration test in Chinese hamster ovary cells. A test of unscheduled DNA synthesis in rat hepatocytes was also negative. Fenthion showed a weakly positive response in 2 of 5 assays for sister chromatid exchange.

Clinical Management

If poisoning is suspected, one should not wait for symptoms to develop. A physician, the nearest hospital, or the nearest Poison Control Center should be contacted immediately. Signs and symptoms of fenthion toxicity include:

- *Mild:* Headache, dizziness, weakness, anxiety, pupillary contraction, blurred vision, and nausea.
- *Moderate:* Nausea, salivation, lacrimation, abdominal cramps, diarrhea, vomiting, sweating, slow pulse, muscular tremors and respiratory compromise.
- Severe: Respiratory difficulty, pinpoint and nonreactive pupils, pulmonary edema, cyanosis, loss of sphincter control, muscle spasms, convulsion, coma, and eventual death due to respiratory failure.

Treatment

Eye and Skin Exposure For exposure to the eyes, the eyelids should be held open and the eyes flushed with copious amounts of water for at least 15 min. In the case of skin contact, the affected areas should be washed immediately with soap or shampoo and water, as appropriate.

Inhalation If fenthion is inhaled, the victim should be removed from the source of contamination to fresh air. If the victim is not breathing, artificial respiration should be administered and medical attention sought as soon as possible.

Ingestion If the victim is alert and respiration is not depressed, vomiting should be induced. Gastric decontamination should be performed within 30 min of ingestion to be most effective.

Atropine sulfate, in conjunction with pralidoxime (2-PAM), can be administered as an antidote. Atropine should be administered by intravenous injection. Intramuscular injection can be used if IV injection is not possible. Atropine dosage: Adults: 0.4–2.0 mg

repeated every 15 min until atropinization is achieved: tachycardia (fast pulse), skin flushing, dry mouth, clearing of bronchial secretions. Atropinization should be maintained by repeated doses for 2–12 h or longer depending on severity of poisoning. Children under 12 years: 0.05 mg kg^{-1} BW, repeated every 15 min until atropinization is achieved. Maintain atropinization with dosage of $0.02-0.05 \text{ mg kg}^{-1}$ BW.

2-PAM should be administered in conjunction with atropine in cases of severe poisoning in which respiratory depression, muscle weakness, and twitching is severe. Adults: 1.0 g IV at no more than 0.5 g min^{-1} . Children under 12 years: $20-50 \text{ mg kg}^{-1}$ (depending on severity of poisoning) IV, injecting no more than half the total dose per minute. This may be repeated at 2–4 h.

Environmental Fate

The persistence of fenthion in the environment is dependent on several factors, including photolysis, metabolism in plants and insects, and microbial degradation. Estimates of the half-life of fenthion in soil vary from <1 day in studies cited by the US EPA for aerobic soil metabolism to 3–6 weeks, cited by Extoxnet. Half-lives for aquatic degradation range from 2.9 to 21.1 days for various ocean, river, swamp, or lake aquatic conditions. Sunlight accelerates degradation of fenthion 20-fold in river water and fivefold in seawater.

The fenthion parent compound is fairly insoluble in water and binds tightly to soil particles. It is, therefore, relatively immobile in most soil types. Its transformation products have higher mobility through the soil.

Fenthion has an octanol:water partition coefficient $(\log K \text{ O:W})$ of 4.8, which indicates that it has the potential to bioaccumulate in fish and nontarget organisms.

Ecotoxicology

Fenthion is very highly toxic to birds. The use of fenthion for control of mosquitos has been implicated in several bird kills, including recent incidents on Marco Island, Florida. The major metabolites, fenthion phenol sulfoxide and fenthion phenol sulfone, have very low toxicity to birds.

Fenthion is also very highly toxic to freshwater, estuarine, and marine invertebrates, and moderately to highly toxic to fish. Fenthion is reported to be toxic to American linden, hawthorn and sugar maple trees, and to certain rose varieties. Germination and vegetative-vigor tests showed that fenthion had little effect on a wide variety of food plants. Toxicity testing using aquatic plants indicated that fenthion is not particularly toxic to these plants.

Exposure Standards and Guidelines

Fenthion is classified as a Toxicity Category II chemical for acute oral, dermal, and inhalation toxicity. It is classified in Toxicity Category III for eye irritation and Category IV for dermal irritation. It is not considered by the US EPA to be a carcinogen, and is therefore classified as a Group E chemical, that is, not likely to be carcinogenic in humans via relevant routes of exposure.

The US EPA Acute Dietary population adjusted dose (PAD) is $0.0007 \text{ mg kg}^{-1} \text{ day}^{-1}$ fenthion. This standard is based on plasma cholinesterase inhibition in monkey (NOAEL = $0.07 \text{ mg kg}^{-1} \text{ day}^{-1}$), divided by a composite uncertainty factor of $100 (10 \times \text{ interspecies}, 10 \times \text{ intraspecies})$, and a $1 \times \text{ factor for Food}$ Quality Protection Act (FQPA).

The US EPA Chronic Dietary PAD of $0.00007 \text{ mg kg}^{-1} \text{ day}^{-1}$ is also based on the monkey NOAEL/LOAEL = $0.02 \text{ mg kg}^{-1} \text{ day}^{-1}$, with a composite uncertainty factor of 300 (as above, with an additional $3 \times$ for lack of a true NOAEL).

See also: Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides.

Further Reading

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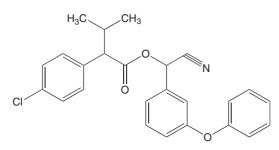
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- http://www.hc-sc.gc.ca Pest Management Regulatory Agency, Health Canada. Re-evaluation of Fenthion. PACR2003-05, March, 2003.
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Fenvalerate

Betty J Locey and Janice Reeves

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 51630-58-1
- SYNONYMS: Sumicidin; Pydrin; Phenvalerate; Cyano(3-phenoxyphenyl)methyl-4-chloro- α -(1-methylethyl) benzeneacetate; (*RS*)- α -Cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate. Esfenvalerate is an isomer of fenvalerate with its own common name
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Type II synthetic pyrethroid insecticide
- CHEMICAL FORMULA: C₂₅H₂₂ClNO₃
- CHEMICAL STRUCTURE:



Uses

Fenvalerate is a restricted-use pyrethroid pesticide used to control insects on food crops (both leaves and fruit), on animal feed, and on cotton. Food crops include peanuts, soybeans, sugarcane, and sunflowers. It is also used to control flies and ticks in stables and barns.

Exposure Routes and Pathways

Fenvalerate may be absorbed through the skin, through the respiratory tract if inhaled, and through the digestive tract if ingested. Dermal contact is the main route of exposure during application.

Toxicokinetics

Fenvalerate is absorbed readily following ingestion, dermal exposure, or inhalation. Plasma levels of pyrethroids are not clinically useful. Fenvalerate undergoes ester cleavage to alcohol followed by rapid hydroxylation. Fenvalerate is distributed to lipid-rich tissues including the brain. Elimination from fatty tissues is slow, with a half-life of 2–7 days. Fenvalerate is eliminated through urine.

Mechanism of Toxicity

Fenvalerate has low toxicity in mammals due to its rapid metabolic breakdown. It acts directly on nerve axons by prolonging sodium channel opening in cell membranes. Insects exposed to fenvalerate are quickly paralyzed: exposure causes quick insect knockdown. In small animals, type II pyrethroids cause salivation, chewing, burrowing, choreoathetosis, and seizures. They also cause lower action potential amplitude, marked membrane depolarization, and eventual total neural activity blockade. Fenvalerate is likely to act both on peripheral and central nervous system. It is also a potent inhibitor of calcineurin (protein phosphatase 2B).

Acute and Short-Term Toxicity (or Exposure)

Animal

Fenvalerate has moderate mammalian toxicity, with an oral LD_{50} in rats >400 mg kg⁻¹. The dermal LD_{50} in rabbits was >2 g kg⁻¹. It is practically nontoxic by inhalation, with an LC_{50} in rats of >2.9 mgl⁻¹. Animals exposed to fenvalerate may exhibit choreoathetosis (abnormal body movements), salivation (CS-syndrome), restlessness, tremors, and piloerection (hair of the skin stands up).

Human

Ingestion commonly results in headaches, dizziness, weakness, nausea, and vomiting. The solvent appears to markedly affect toxicity. A more concentrated dose may cause seizures and coma. Common adverse effects of inhalation are runny nose and scratchy throat. Hypersensitivity reactions that may be noted include wheezing, sneezing, shortness of breath, pneumonitis, pulmonary edema, bronchiospasm, and chest pain. Contact with eyes may cause mild to severe corneal damage. Dermal exposures cause tingling and burning sensations and numbness of the skin. Excitability, tremors, incoordination, numbness, seizures, and coma may result from massive exposure.

Chronic Toxicity (or Exposure)

Animal

Six of 50 male rats exposed to 1000 mg kg^{-1} in a 2 year feeding study showed transient muscular incoordination of the hind limbs, abnormal gait, and ataxia during the third and fourth weeks of the study.

Mice exposed for 12 months or longer reportedly showed evidence of an inflammatory response in the liver, lymph nodes, or spleen. The response was characterized by giant cell infiltration and/or multifocal microgranulomata, typical of a 'foreign-body' type of response. A similar response has been reported in dogs exposed to 1000 mg fenvalerate per kg in the diet for 6 months. There is little or no evidence to suggest that fenvalerate is carcinogenic, mutagenic, or a reproductive/developmental toxicant in animals. Demyelination in peripheral nerves has been reported in experimental animals. Teratogenicity tests have been negative.

Human

Fenvalerate does not pose significant chronic hazard potential.

In Vitro Toxicity Data

Mutagenicity tests with and without metabolic activation have been negative. Testing included *Salmonella typhimurium* assays, testing in *Bacillus subtilis*, and testing in V79 Chinese hamster cells. In addition, bone marrow evaluated after oral exposure was not reported to contain chromosomal damage. It was not reported to cause dominant lethal mutations in mice at $100 \text{ mg kg}^{-1} \text{ day}^{-1}$.

Clinical Management

There is no antidote for fenvalerate. Treatment is primarily supportive. The victim should be monitored for the development of respiratory distress, seizures, and hypersensitivity reactions. Convulsions are often treated with diazepam. Prevention of absorption may be accomplished by gastric lavage followed by activated charcoal. Some formulations include solvents so care should be taken to protect against pulmonary effects during lavage. Basic and advanced life-support measures should be used as necessary.

Environmental Fate

Fenvalerate has low water solubility ($<300 \,\mu g \, l^{-1}$). Its solubility in surface waters is increased with organic matter. Fenvalerate is moderately persistent in soil, with a half-life of 0.5–3 months. Fenvalerate and its degradation products are relatively immobile and not expected to pose leaching problems. Because of this and low water solubility, it has not been found in groundwater sampling. Fenvalerate undergoes photodegradation in water.

Ecotoxicology

Fish are extremely sensitive to fenvalerate. A 48 h LC_{50} of $<0.1 \text{ mgl}^{-1}$ was reported in carp and a 96 h LC_{50} of $3.6 \,\mu\text{gl}^{-1}$ was reported in rainbow trout. Fenvalerate is highly toxic to *Daphnia*, with an LC_{50} of $1 \,\mu\text{gl}^{-1}$. Fenvalerate is only slightly toxic to birds. Oral LD_{50} values reported in hens, bobwhite quail, and mallard ducks were all $> 1 \text{ g kg}^{-1}$. Fenvalerate is highly toxic to honey bees with a contact LD_{50} of $0.23 \,\mu\text{g}$ per bee.

Other Hazards

Avoid contact with acids and bases. In a fire, thermal decomposition will occur, and may produce toxic gases such as carbon monoxide and carbon dioxide.

Exposure Standards and Guidelines

The US Environmental Protection Agency IRIS developed an oral reference dose of 2.5×10^{-2} mg kg⁻¹ day⁻¹ based on a 13 week feeding study in rats. The no-observed-effect level was identified as 125 ppm. The critical effect was identified as neurological dysfunction.

The American Conference of Governmental Industrial Hygienists threshold limit value, timeweighted average, is 5 mg m^{-3} . The minimal lethal dose is probably in the range of 10–100 g.

Maximum residue limits have been recommended by the Joint FAO/WHO Meeting on Pesticide Residues. An acceptable daily intake of $0-0.02 \text{ mg kg}^{-1}$ body weight was established for fenvalerate by JMPR in 1986.

Further Reading

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Fetal Alcohol Syndrome

Kartik Shankar and Harihara M Mehendale

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Fetal alcohol syndrome (FAS) is a set of birth defects caused by maternal consumption of alcohol during pregnancy. The term FAS was coined in 1973 by Jones and Smith in their seminal article that described the constellation of birth defects that characterize FAS in children born to mothers known to have consumed alcohol during pregnancy. FAS is considered the most common preventable cause of mental retardation. The annual cost of FAS according to the 10th Special Report to the US Congress on Alcohol and Health estimated the annual cost of FAS in 1998 to be \$2.8 billion.

Clinical Assessment of FAS

Most experts agree on three diagnostic criteria for the clinical assessment of FAS: (1) prenatal or postnatal growth restriction; (2) central nervous system (CNS) effects including insufficient development of the brain such as microcephaly, agenesis of corpus callosum; and (3) specific craniofacial dysmorphic features. However, it is more difficult to diagnose more subtle effects of fetal alcohol effects in which the more pronounced effects of FAS are not apparent. FAS is estimated to occur at a rate of 5–10 per 10 000 live births in the United States, but it may be higher in certain populations. However, the true incidence of FAS and fetal alcohol effects (FAE) are probably greater because it is difficult to recognize the symptoms and to obtain reliable history of alcohol ingestion. The overall incidence of FAS among infants born to mothers who have a history of drinking alcohol during pregnancy is only 2–3%.

Risk factors for FAS

Several risk factors determine the toxic outcome of *in utero* alcohol exposure. Risk factors suggested include genetic predisposition, marital status, smoking, use of prescribed or over-the-counter drugs and medications, concomitant usage of other drugs of abuse, occupational or environmental exposure to chemicals, socioeconomic status, and adequate nutrition.

Models of FAS

Research using animal models has shown that each of the major characteristics of human FAS, including craniofacial abnormalities, growth deficiency, and abnormalities of the CNS occurs in one of these animal models including mice, rats, chicks, and primates. Because different species and even strains within species show different degrees of vulnerability to alcohol, experimental results must be interpreted with caution. However, most common animal models of FAS (rats and mice) are very similar to humans and their biochemical processes are virtually the same. Research from animal models has also revealed critical time periods of vulnerability that leads to certain FAS abnormalities. For example, in the mouse and chicken embryos exposure to alcohol during cranial neural crest cells development corresponding to the first 3-4 weeks of human gestation resulted in patterns consistent with observed dysmorphologies of cranial structures and craniofacial defects.

How Much Alcohol is Safe in Pregnancy?

There is no safe drinking level of alcohol during pregnancy. Significant controversy surrounds the amount of alcohol that presents a risk to the fetus and whether a single exposure is of greater consequence than a pattern of exposure during development. Results from clinical and animal studies demonstrate that lower levels of alcohol are needed to produce behavioral anomalies than are needed to produce physical effects and that some brain regions are more susceptible than others. FAS is completely preventable by abstinence to alcohol. The American Academy of Pediatrics recommends counseling women of childbearing age about the effects of alcohol on the fetus. In addition, government-required warning labels about the health effects of alcohol are displayed on alcoholic beverages. A recent study found almost 80% of 7334 women interviewed were aware of the detrimental effects of alcohol, including the high-risk drinkers. However, the warning labels had only a modest effect on personal risk perceptions and drinking behaviors, clearly stressing the need for other effective strategies to decrease alcohol consumption during pregnancy. Considering the cost in economic and human suffering imposed by FAS on the child and the family, prevention via abstinence seems to be the most effective way in reducing incidence of FAS. Identifying high-risk drinkers is an important first step in this process.

Mechanisms of Induction of FAS

The mechanisms leading to FAS remain elusive. A single mechanism for the entire spectrum of FAS is unlikely. Influences of genetic susceptibility and nutrition may be of critical importance. Clearly simultaneous or prior exposure to other chemicals may influence the mechanisms and the nature and extent of effects. Several mechanisms involving the direct effects of alcohol on neural development and organogenesis have been explored in animal models. Among them are alcohol-induced changes in neural cell proliferation, reduced growth and neurotropic factors, inhibition of cell adhesion molecule L1, increased oxidative stress and production of free radicals, fetal zinc deficiency, altered vitamin A and folate function, impaired placental function, and disruption of retinoic acid. Of special note is the mechanism of alcohol-induced inhibition of the cell adhesion molecule L1, which is involved in neural cell migration. Recent animal studies have shown that NAPVSIPQ, an active fragment of the glialderived activity-dependent neuroprotective protein, which antagonizes the alcohol-induced inhibition of L1 also protects from alcohol-induced fetal growth retardation and demise. These studies open an exciting area of potential pharmacological intervention for FAS.

See also: Alcoholic Beverages and Alcoholism.

Further Reading

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- Tenth Special Report to the US Congress on Alcohol and Health. US Department of Health and Human Services.

Relevant Website

http://www.niaaa.nih.gov - 10th special report - National Institution on Alcohol Abuse and Alcoholism.

Fexofenadine

Stephen R Clough

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- CHEMICAL NAME: (±)-*p*-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl) piperidino]butyl]-a-methylhydratropic acid, carboxyterfenadine, terfenadine carboxylate, a-dimethyl benzeneacetic acid hydrochloride
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 83799-24-0
- SYNONYMS: Fexofenadine hydrochloride (generic name); ALLEGRA; ALLEGRA-D
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Pharmaceutical; Antihistamine (H1-receptor antagonist)
- Chemical Formula: $C_{32}H_{39}NO_4 \cdot HCl$

Uses

Fexofenadine hydrochloride is a white to off-white crystalline powder. It is soluble in methanol and ethanol, slightly soluble in chloroform and water, and insoluble in hexane. It is the active ingredient of ALLEGRA and acts as a histamine H1-receptor antagonist. Fexofenadine is a selective antihistamine used to relieve allergic rhinitis (seasonal allergy) symptoms including sneezing, runny nose, itching, and watery eyes that come with hay fever. Its effect begins in 1 h and lasts 12 h, peaking around the second or third hour. ALLEGRA is one of the new types of antihistamines that rarely cause drowsiness.

In addition to the antihistamine in ALLEGRA, ALLEGRA-D also contains the nasal decongestant pseudoephedrine.

Exposure Routes and Pathways

ALLEGRA is a drug that is prescribed to be taken in pill form, so exposure would only be expected to occur orally.

Toxicokinetics

In laboratory animals, no anticholinergic, α 1-adrenergic, or β -adrenergic-receptor blocking effects were observed. No sedative or other central nervous system effects were observed. Radiolabeled tissue distribution studies in rats indicated that fexofenadine does not cross the blood-brain barrier.

Fexofenadine hydrochloride was rapidly absorbed following oral administration of a single dose of two 60 mg capsules to healthy male volunteers with a mean time to maximum plasma concentration occurring at 2.6 h postdose. After administration of a single 60 mg capsule to healthy subjects, the mean maximum plasma concentration was 131 ng ml^{-1} . Following single-dose oral administrations of either the 60 or 180 mg tablet to healthy, adult male volunteers, mean maximum plasma concentrations were 142 and 494 ng ml⁻¹, respectively. The tablet formulations are bioequivalent to the capsule when administered at equal doses. Fexofenadine hydrochloride pharmacokinetics is linear for oral doses up to a total daily dose of 240 mg (120 mg twice daily). The administration of the 60 mg capsule contents mixed with applesauce did not have a significant effect on the pharmacokinetics of fexofenadine in adults.

Fexofenadine hydrochloride is 60–70% bound to plasma proteins, primarily albumin and α_1 -acid glycoprotein. The mean elimination half-life of fexofenadine was 14.4 h following administration of 60 mg, twice daily, in normal volunteers. Human mass balance studies documented a recovery of ~80% and 11% of the [¹⁴C] fexofenadine hydrochloride dose in feces and urine, respectively. Because the absolute bioavailability of fexofenadine hydrochloride has not been established, it is unknown whether the fecal component represents unabsorbed drug or the result of biliary excretion. Approximately 5% of the total oral dose was metabolized.

Special population pharmacokinetics (for geriatric subjects, renal, and hepatic impairment), obtained after a single dose of 80 mg fexofenadine hydrochloride, were compared to those for normal subjects from a separate study of similar design. While subject weights were relatively uniform between studies, these adult special population patients were substantially older than the healthy, young volunteers. Thus, an age effect may be confounding the pharmacokinetic differences observed in some of the special populations.

In older subjects (=65 years old), peak plasma levels of fexofenadine were much greater than those observed in normal volunteers (<65 years old). Mean elimination half-lives were similar to those observed in normal younger volunteers.

Cross-study comparisons indicated that fexofenadine hydrochloride distribution in the body of 7–12year-old pediatric allergic rhinitis patients following oral administration of a 60 mg dose was 56% greater compared to healthy adult subjects given the same dose. Plasma concentrations in pediatric patients given a dose that is one-half of what an adult would receive (30 mg fexofenadine hydrochloride in child versus 60 mg for adult) is comparable to adults. In patients with mild to moderate and severe kidney impairment, peak plasma levels of fexofenadine were 87% and 111% greater, respectively, and mean elimination half-lives were 59% and 72% longer, respectively, than observed in normal volunteers. Peak plasma levels in patients on dialysis were 82% greater and half-life was 31% longer than observed in normal volunteers. Based on increases in bioavailability and half-life, a dose of 60 mg once daily is recommended as the starting dose in patients with decreased renal function. The pharmacokinetics of fexofenadine hydrochloride in patients with hepatic disease did not differ substantially from that observed in healthy patients.

Acute and Short-Term Toxicity (or Exposure)

Most drugs are proved to be safe through a series of short- (acute) and long-term (chronic) tests. No drugs that show adverse toxic affects when tested as singledose study will make it into the long-term study programs.

Animal

In acute toxicity studies with laboratory animals, clinical signs of toxicity and effects on body weight or food consumption were not observed in several animal species administered fexofenadine by oral lavage at doses up to 2000 mg kg^{-1} .

In *in vitro* (bacterial reverse mutation, CHO/ HGPRT forward mutation, and rat lymphocyte chromosomal aberration assays) and *in vivo* (mouse bone marrow micronucleus assay) tests, fexofenadine hydrochloride revealed no evidence of mutagenicity.

No deaths occurred at oral doses of fexofenadine hydrochloride up to 5000 mg kg^{-1} in mice (110 times the maximum recommended daily oral dose in adults and 200 times the maximum recommended daily oral dose in children based on mg m⁻²) and up to 5000 mg kg^{-1} in rats (230 times the maximum recommended daily oral dose in adults and 400 times the maximum recommended daily oral dose in adults and 400 times the maximum recommended daily oral dose in children based on mg m⁻²). Additionally, no clinical signs of toxicity or gross pathological findings were observed. In dogs, no evidence of toxicity was observed at oral doses up to 2000 mg kg⁻¹ (300 times the maximum recommended daily oral dose in adults and 530 times the maximum recommended daily oral dose in children based on mg m⁻²).

Human

Most reports of fexofenadine hydrochloride overdose contain limited information. However, dizziness, drowsiness, and dry mouth have been reported. Single doses up to 800 mg and doses up to 690 mg twice daily for 1 month were studied in healthy subjects without the development of clinically significant adverse events.

Chronic Toxicity (or Exposure)

All drug studies require chronic animal tests before they can be tested in primates or humans. Fexofenadine is now on the market and is therefore considered 'safe' by standards set by the US Food and Drug Administration.

Animal

The carcinogenic potential and reproductive toxicity of fexofenadine hydrochloride were assessed using terfenadine studies with adequate fexofenadine hydrochloride exposure (based on plasma area under the concentration versus time (AUC) values). No evidence of carcinogenicity was observed in an 18 month study in mice and in a 24 month study in rats at oral doses up to 150 mg kg^{-1} of terfenadine (which led to fexofenadine exposures that were, respectively, ~3 and 5 times the exposure from the maximum recommended daily oral dose of fexofenadine hydrochloride in adults and children).

In rat fertility studies, dose-related reductions in implants and increases in postimplantation losses were observed at an oral dose of 150 mg kg^{-1} of terfenadine (which led to fexofenadine hydrochloride exposures that were ~3 times the exposure of the maximum recommended daily oral dose of fexofenadine hydrochloride in adults).

There was no evidence of teratogenicity (category C) in rats or rabbits at oral doses of terfenadine up to 300 mg kg^{-1} (which led to fexofenadine exposures that were ~4 and 31 times, respectively, the exposure from the maximum recommended daily oral dose of fexofenadine in adults).

Dose-related decreases in pup weight gain and survival were observed in rats exposed to an oral dose of 150 mg kg^{-1} of terfenadine (~3 times the maximum recommended daily oral dose of fexofenadine hydrochloride in adults based on comparison of fexofenadine hydrochloride AUCs).

Human

There are no adequate and well-controlled studies in pregnant women. Fexofenadine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Clinical Management

Medication is used to cause a desired biological effect. When used, other effects, side effects, may also occur. These may be mild or serious. If side effects develop a physician should be consulted to determine whether the use of the drug should be discontinued. Side effects of ALLEGRA may include colds or flu, drowsiness, fatigue, indigestion, menstrual problems, and nausea. Side effects of ALLEGRA-D may include abdominal pain, agitation, anxiety, back pain, dizziness, dry mouth, headache, heart palpitations, indigestion, insomnia, nausea, nervousness, respiratory tract infection, and throat irritation.

Generally, ALLEGRA-D should not be used by patients who have glaucoma, urination problems, or severe high blood pressure or heart disease. Use is not recommended for children under 12 years. ALL-EGRA-D should not be taken within 2 weeks of using an MAO-inhibitor drug (e.g., Marplan, Nardil, or Parnate). Some individuals may be allergic to ALLEGRA or ALLEGRA-D.

See also: Immune System; Respiratory Tract.

Further Reading

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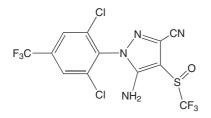
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Fipronil

Xilong Zhao

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- CHEMICAL NAME: 5-Amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((1,*R*,*S*)-(trifluoromethyl)sulfinyl)-1-*H*-pyrazole-3-carbonitrile
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 120068-37-3
- SYNONYM: Fiprole
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Fiprole; Phenylpyrazole insecticide; Chloride channel blocker
- Chemical Formula: $C_{12}H_4Cl_2F_6N_4OS$
- CHEMICAL STRUCTURE:



Uses

Fipronil is a highly active, broad-spectrum insecticide from the phenylpyrazole family and has been on the market since 1993 under different brands. It is widely used for crop protection against major lepidopterous and orthopterous pests on a wide range of field and horticultural crops. Fipronil is used against coleopterous larvae in soils and on golf and commercial turf grass. Fipronil is also commonly used as a public hygiene insecticide to control fleas, ticks and mites on domestic animals under the trade name Frontline, Topspot, or Combat, for control of cockroaches and ants under the brand name Maxforce FC Baits, Goliath, and Nexa, to control termites under the name Termidor, and for control of fire ants under the name Over'n Out!.

Exposure Routes and Pathways

The potential routes for occupational exposure to fipronil are through inhalation, skin contact, and ingestion during production, packaging, and application of fipronil products. Potential for nonoccupational exposure to fipronil, its major metabolite fipronil sulfone or its major photodegradation product desulfinyl fipronil is expected to be very low. Since fipronil has an extremely low vapor pressure and low dermal penetration, nonoccupational exposure to fipronil through inhalation and skin is minimal. Exposure through contacting pet animals applied with Frontline fipronil product is also expected to be low.

Toxicokinetics

Fipronil is well absorbed from the gut but relatively poorly absorbed from the skin. Once absorbed, fipronil is rapidly oxidized to the sulfone derivative by the cytochrome P450 NADPH-dependent monooxygenases in liver and other tissues. Fipronil is also metabolized via hydrolysis of nitrile to yield an amide metabolite. Both fipronil and its metabolites are well distributed in tissues where significant amounts of residues can remain, particularly in fatty tissues. The long half-life (150-245 h in some cases) of fipronil in blood may reflect slow release of residues from fat and might suggest potential bioaccumulation of metabolic products of fipronil. The major routes of elimination of fipronil and its metabolites are feces (45-75% in rat) and urine (5-25% in rat).

Mechanism of Toxicity

Fipronil has a higher toxicity to insects than to mammals. The putative mode of insecticidal activity of fipronil is to block the GABA- and glutamateregulated chloride ion channels, which are responsible for inhibition of normal neural activity. When the inhibitory function is suppressed by fipronil, neural overexcitation ensues leading to death of the insect. The mammalian toxicity of fipronil is related to its blocking action on GABAA receptor chloride channels in the nervous system. The high selective toxicity of fipronil to insects may be due to its higher potency to block the insect GABA-regulated chloride channel than the mammalian GABA-regulated chloride channel, and its potent inhibitory action on invertebratespecific glutamate-regulated chloride channels not present in higher organisms.

Acute and Short-Term Toxicity (or Exposure)

Animal

Fipronil is classed as a World Health Organization Class II moderately hazardous pesticide and is less toxic to mammals than to some birds, fish and most invertebrates. Fipronil has moderate acute toxicity by the oral and inhalation routes in rats with acute LD_{50} values of 97 mg kg⁻¹ and 0.39–0.68 mg l⁻¹, respectively. The acute oral LD₅₀ of fipronil in mouse is 95 mg kg⁻¹. Dermal absorption in rats is less than 1% after 24 h and acute toxicity is considered to be low with an LD₅₀ of more than 2000 mg kg^{-1} . In contrast, it has moderate dermal toxicity to rabbits with an LD_{50} of 354 mg kg⁻¹. Fipronil may cause mild irritation to the eyes and slight skin irritation, but it is not a skin sensitizer in guinea pigs. Signs of toxicity in rats include reduced food consumption, anuria, increased excitability and seizures. Affected organs may include the liver, thyroid and kidney. Desulfinyl fipronil is ~ 10 times more toxic to mammals than fipronil itself.

Human

No confirmed human intoxication cases have been reported.

Chronic Toxicity (or Exposure)

Animal

Fipronil is neurotoxic in both rats and dogs. Rats receiving 300 ppm fipronil in males $(12.68 \text{ mg kg}^{-1} \text{ day}^{-1})$ and females $(16.75 \text{ mg kg}^{-1} \text{ day}^{-1})$ showed an increased incidence of thyroid follicular cell tumors. Similar studies in mice and dogs did not show an increased incidence of thyroid tumors, however. Fipronil can induce reproductive toxicity in rats at high dietary exposure levels (300 ppm). Clinical signs include decreased fertility, decreased body weights in litters, and resorptions. Fipronil may cause a delay in body development at high doses, but there is no evidence of teratogenicity.

Human

Fipronil is classified as a group C (possible human) carcinogen based on findings in rats. Human data on cancer, reproductive and development toxicity are not available.

In Vitro Toxicity Data

Fipronil was not mutagenic in the *Salmonella* or HGPRT gene mutation assay at concentrations up to $500 \,\mu\text{g}$ per plate and $386 \,\mu\text{g} \,\text{ml}^{-1}$, respectively. No evidence of a clastogenic or aneugenic effect of fipronil was found in the micronucleus assay or human lymphocyte cytogenic assay *in vitro* at concentrations up to $300 \,\mu\text{g} \,\text{ml}^{-1}$.

Clinical Management

Fipronil can be harmful or fatal with overexposure through skin, ingestion, or inhalation routes. It may cause skin irritation, rash, edema, shortness of breath, drowsiness, excitement, involuntary shaking, and convulsions. Eye contact may cause redness and tearing. Inhalation of fipronil may aggravate existing chronic respiratory problems such as asthma, emphysema, or bronchitis.

If exposed on skin or clothing, the contaminated clothing should be removed and the skin rinsed with copious water for 20 min. Exposed eyes should be immediately irrigated with a steady, gentle stream of water for 20 min. In case of inhalation or ingestion exposure, move the person to fresh air and call a poison control center or doctor immediately for treatment advice.

There is no specific antidote for fipronil intoxication. Clinical treatment should be based on observed signs and symptoms of distress in the patient. Recommendations for treatment of overexposure cases are based on routine anticonvulsant therapy. Phenobarbital or diazepam may be useful in controlling convulsions induced by fipronil. For phenobarbital, start with $10-20 \text{ mg kg}^{-1}$ of phenobarbital in rapid intravenous perfusion and continue according to the patient's response. For diazepam, start with 10-30 mg diazepam by intravenous injection according to body weight. This dose is to be repeated every 10-30 min according to the patient's response. Even when symptoms of fipronil intoxication are rapidly reversed by treatment, the treatment must be continued for several days, gradually decreasing the dose of anticonvulsant based on the patient's clinical response. This is necessary due to the slow elimination of the compound.

Environmental Fate

Fipronil is photolyzed into several degradates represented by desulfinyl fipronil and fipronil sulfone in the field. Fipronil is also rapidly metabolized to fipronil sulfone in plants. The half-life of fipronil on treated vegetation ranges from 3 to 7 months depending on the substrate and the habitat where it is applied. Its photodegradation, volatilization and hydrolysis are the contributors to fipronil field dissipation. Fipronil has low soil mobility. It binds to the soil and has little potential for groundwater contamination. In water and sediment that lack oxygen, fipronil degrades with a half-life of 116-130 days. Fipronil remains stable to breakdown in water at mildly acid to neutral pH and degrades in basic solutions with a half-life of 28 days. In studies where fipronil was exposed to light, fipronil had a half-life of 3.6 h in water and 34 days in loamy soil.

Ecotoxicology

Nontarget toxicity of fipronil to wildlife varies with species. Fipronil is highly toxic to fish with LC_{50} (96 h) of $42 \,\mu\text{gl}^{-1}$ for African tilapia, $85 \,\mu\text{gl}^{-1}$ for bluegill sunfish, $248 \,\mu g l^{-1}$ for rainbow trout, and $430 \,\mu g \, l^{-1}$ for European carp. Fipronil is also highly toxic to aquatic invertebrates including oysters, shrimps and other crustacea, and highly toxic to bees and upland game birds, but is almost nontoxic to earthworms, soil microorganisms, aquatic plants, and certain groups of waterfowl and birds such as field sparrow $(LD_{50} = 1120 \text{ mg kg}^{-1})$ and Mallard duck $(LD_{50}>2150 \text{ mg kg}^{-1})$. Its tendency to bind to sediments and its low water solubility may reduce the potential hazard to aquatic wildlife. The metabolites of fipronil have a higher toxicity to birds and freshwater invertebrates than fipronil itself. It is

appropriate to use fipronil-based insecticides with accompanying environmental, ecotoxicological, and human health monitoring.

Other Hazards

Flammable properties of fipronil product (e.g., Termidor[®] SC Termiticide): flash point >93°C (199°F); flammability class: will burn. Closed containers may explode (due to the build-up of pressure) when exposed to extreme heat. Suitable extinguishing media include water spray, foam, CO₂, and dry chemical media. Firefighters should be equipped with self-contained breathing apparatus and turnout gear.

Exposure Standards and Guidelines

The reference dose (RfD) and acceptable daily intake (ADI) for fipronil are both $0.0002 \text{ mg kg}^{-1} \text{ day}^{-1}$. RfD and ADI values for fipronil-desulfinyl are about an order of magnitude lower, 0.00003 and 0.0002, respectively.

See also: Neurotoxicity; Pesticides.

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Relevant Websites

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- http://www.fao.org Food and Agricultural Organization (FAO). Fipronil data sheet (T*).

Fish Consumption Advisory

John L Hesse

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Introduction

In the United States, it was discovered in the early 1970s that fish tissue sometimes contains trace levels of environmental contaminants that may be harmful to humans if the fish are eaten too frequently. In response to this discovery, state, territorial, and tribal governments developed fish consumption advisories to protect the public from potentially harmful effects linked to eating sport-caught fish. Advisories are often targeted at the protection of women of childbearing age, the fetus, and young children since these population groups are most sensitive to the adverse effects of many of the contaminants. The goal of advisories is to create public awareness of the potential dangers as well as the benefits of fish consumption while recognizing the cultural importance of regular fish consumption for particular segments of the population.

Fish consumption advisories have historically been aimed at sport fish anglers and people who eat sportcaught fish. Consumers of fish sold commercially in markets or restaurants are protected by the US Food and Drug Administration (FDA) which has the authority to remove fish from the market that exceed FDA tolerance or action levels for chemical contaminants. However, in recent years, the FDA has also issued consumption advice to sensitive populations who may be consuming large amounts of certain commercially purchased fish (e.g., shark, swordfish, king mackerel, and tilefish). Many state, territorial, and tribal governments are now including some commercial fish species, such as these, in their consumption advisories for anglers.

Balancing the risks and benefits of fish consumption is becoming more and more complex as an everincreasing amount of research has shown that regular fish consumption can play a role in reducing cardiac disease, diabetes, arthritis, asthma, and certain forms of cancer. The benefits are, in large part, due to the high quality protein, omega-3 fatty acids, vitamins, and minerals found in fish. An additional benefit is that fish are very good sources of protein while containing much lower fat levels than many other protein sources in the diet; for example, red meats. Fish consumption advisories, by providing information on risk-reducing behaviors such as fishing less contaminated bodies of water, targeting fish species and sizes lowest in contaminants, and using fish preparation and cooking methods that reduce contaminant levels, can help consumers achieve the health benefits of fish while minimizing any potential health threats from contaminants.

Background and History of Advisories

The first fish consumption advisories were issued in the United States in the early 1970s in response to the discovery of mercury in fish in waters of southeast Michigan downstream of an industrial discharge of mercury. As more and more states tested fish for contaminants over the last 30 years, consumption advisories spread to nearly every state in the nation. In 2002, the US Environmental Protection Agency's National Listing of Fish and Wildlife Advisories included 2800 advisories in 48 states, the District of Columbia, and the US Territory of Samoa. The 2800 advisories in the national listing cover almost 33% of the nation's total lake acreage and 15.3% of the total river miles. Twenty-seven states and the District of Columbia have issued statewide advisories for lakes, streams, or coastal waters.

The US EPA has also been encouraging states to include information about safe sources of fish in their consumption advisories. In response, more and more states are informing the public about specific species of fish and fish from specific bodies of water that have been tested and have been shown to contain very low levels of contaminants. This information promotes the continued enjoyment of recreational fishing and the inclusion of fish as part of a healthy diet.

Bioaccumulative Pollutants

The US EPA's website identifies a total of 39 chemical contaminants that have triggered fish consumption advisories in the United States. Most advisories, however, have involved five primary contaminants: mercury, polychlorinated biphenyls (PCBs), chlordane, dioxins, and DDT. Chemicals of greatest concern are ones that accumulate in fish tissue at levels many times higher than those in the water, in some cases, more than 1 million times higher.

These chemicals are typically ones that are very persistent in the environment and remain biologically available in the sediments where they can be passed up the food chain from bottom-dwelling organisms to fish. As a result of biomagnification, the highest contaminant levels are found in top-predator fish species such as bass, walleye, pike, or muskellunge in freshwater environments and in sharks, swordfish, tuna, king mackerel, or bluefish in marine systems. This is

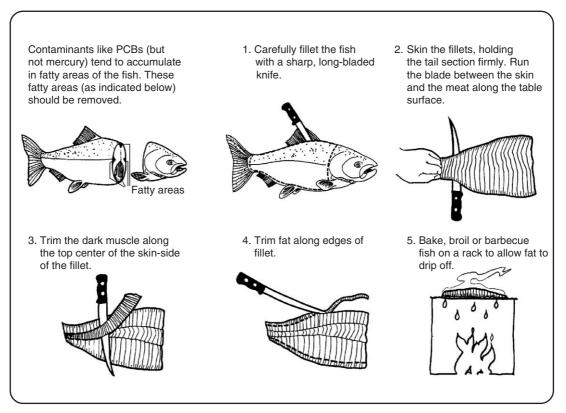


Figure 1 Recommended fish preparation method.

true particularly for mercury. Elemental mercury can be converted by microorganisms in the sediment and water column to its most toxic form, methylmercury, and is predominantly stored in this form in fish tissue.

Salmon, lake trout, and fatty, bottom-dwelling fish species such as carp and some catfish species tend to accumulate high levels of the lipophilic (fat-soluble) organic contaminants like PCBs, chlordane, DDT, and dioxins. Fish consumers can reduce their exposure to these lipophilic compounds by trimming the belly flaps, subcutaneous and dorsal fat, dark muscle, gills, eye, brain, and internal organs before cooking the fish. In addition, cooking the fish in ways that allow the fats to drip away further reduces the potential exposure to these contaminants. Figure 1 illustrates proper fish cleaning and preparation methods. Studies have shown that proper preparation and cooking can eliminate 50% or more of the organic contaminants. However, these methods do not significantly reduce mercury from the portion eaten since the mercury is not stored predominantly in fat.

Potential Health Effects of Eating Contaminated Fish

Surveys have clearly shown that sport anglers tend to eat more fish (two- to threefold higher on average) than the general US population. Consequently, human body burdens of mercury and persistent organic contaminants that are typically found in fish also tend to be higher in sport anglers than in the general population. Because the body burden level increases with the amount of fish eaten, subsistence anglers (e.g., low income) or populations who eat large amounts of fish for cultural reasons (e.g., native Americans and certain immigrant groups) usually have the highest exposures to contaminants and therefore may be at higher risk of adverse effects. The human fetus, infants, and children are often at greater risk because of their rapid growth and developing organ systems. Elderly people may also be at greater risk because their immune systems may already be compromised.

Epidemiological studies are now looking at effects of persistent contaminants on the immune system, the nervous system, prenatal and postnatal development, fertility, and the development of cancers. Methylmercury is known to be a neurotoxin and the developing fetus is considered to be the most sensitive life stage. The adverse developmental effects of methylmercury were first recognized in the 1960s through studies of a poisoning epidemic linked to fish consumption in Minamata, Japan, and investigation of an incident linked to mercury-contaminated grain in Iraq. In the Japanese poisoning incident, some children born to mothers who consumed heavily contaminated fish developed infantile cerebral palsy and other neurological deficits. Symptoms of mercury poisoning in adults include impairment of hearing and peripheral vision, numbness in extremities, uncoordinated movements, impaired speech, mental disturbances, and death in extreme cases.

High-level exposure to polychlorinated biphenyls can cause adverse health effects ranging from developmental effects in children to increased risk from cancer. Children born to exposed mothers have exhibited long-term adverse effects on cognitive development.

Sources of Contamination

Mercury and persistent organic pollutants in the environment come from a variety of sources. Mercury, for example, is a toxic metal that comes from both natural and manmade sources. Coal-fired power plants, municipal waste incinerators, medical waste incinerators, and cement kilns that burn hazardous waste or coal are currently among the major anthropogenic sources of mercury. Natural sources of atmospheric mercury include gases released from the Earth's crust by geysers, volcanic eruptions, and forest fires. PCBs are industrial chemicals used widely in the United States from 1929 until 1978 as coolants and lubricants and in electrical equipment. The manufacture of PCBs in the United States stopped in 1977, and use was restricted in 1979. Dioxins and furans are unwanted by-products of various industrial processes, including production of certain pesticides. DDT is an insecticide that was widely used in the United States from 1946 until 1972. DDT is still used in other countries and, by special permit, in the United States. Chlordane is a pesticide that was once used broadly in the United States but was banned in the 1980s. It is still used in some developing countries.

Content and Dissemination of Consumption Advisories

The core of a fish consumption health advisory is a set of fish consumption recommendations based on risk assessment and risk management considerations. There are three basic types of recommendations:

- Advisories for 'unlimited consumption' (no restrictions) are issued to inform the public that fish from specific water bodies have been tested for chemical contaminants and the results have shown that specific species of fish are safe to eat without a consumption limit.
- Advisories for 'no-consumption' are issued when contaminant levels in fish pose a potentially

serious health risk to the general population or sensitive subpopulations (such as children and pregnant women). These types of advisories recommend that members of the identified population or sub-populations not eat any amount of certain types of locally caught fish.

• Advisories for 'restricted-consumption' are issued when contaminant levels in fish may pose a health risk to the general population or sensitive subpopulations if too much fish is consumed over a specific time period. Examples of recommended time intervals between fish meals include: no more than one meal per week, one meal per month or six meals a year.

In addition to the specific recommendations in the advisories, as described above, risk communicators generally try to include supplemental information that supports the recommendations and provides alternative consumption options that also can be used to minimize the risk. Information on the potential health effects of the chemicals triggering the advisory is often provided. Similarly, the health benefits of fish consumption are also commonly included. Ideally, advisories will identify ways fish consumers can reduce any potential risks while still gaining the health benefits. These methods include fishing cleaner water bodies, eating smaller fish, avoiding certain fish species, and/or trimming and cooking the fish in ways that remove fatty tissues where some fish contaminants are concentrated.

In the past, the most common method of disseminating sport-caught fish consumption advisories has been in conjunction with fishing regulations issued to sport anglers. Although surveys of anglers have shown a general awareness of advisories in a majority of this population, the messages may not be reaching segments of the population at highest risk, such as women of childbearing age and young children, subsistence anglers, members of tribes and other ethnic groups for which fish has a strong cultural significance, and non-English speaking populations. More effective outreach programs to better educate at-risk populations are being developed cooperatively between regulators and communication partners at the local level who are familiar with and sensitive to the needs of the at-risk populations in specific locales. The US EPA has developed guidance for state, territorial, and tribal governments on the most effective risk communication methodologies.

See also: Chlordane; DDT (Dichlorodiphenyltrichloroethane); Dioxins; Food and Drug Administration, US; Mercury; Methylmercury; Polychlorinated Biphenyls (PCBs).

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Relevant Websites

- http://www.epa.gov Environmental Protection Agency website. For further information about the safety of locally caught fish and shellfish, visit the Environmental Protection Agency's Fish Advisory Website.
- http://www.cfsan.fda.gov For further information about the risks of mercury in commercial fish and shellfish, visit the US Food and Drug Administration's Food Safety Website.
- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fish Consumption Advisory.

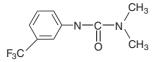
Flame Retardants See Polybrominated Diphenyl Ethers (PBDEs).

Fluometuron

Elmar Udarbe Zamora

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 2164-17-2
- SYNONYMS: *N*,*N*-Dimethyl-*N*'-[3-(trifluoromethyl)phenyl]urea; C-2059; Ciba-2059; Cotoran; Cotorex; Cottonex; Flo-Met; Higalcoton; Lanex; Pakhtaran
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Phenylurea herbicide
- Chemical Formula: C₁₀H₁₁F₃N₂O
- CHEMICAL STRUCTURE:



Uses

Fluometuron is a selective herbicide that acts on susceptible weeds and broadleaf grasses by inhibiting their photosynthesis. In the United States, fluometuron is only used for cotton and sugarcane production. It can be applied before or after planting for the control of weed and grass. Fluometuron is available in liquid, dry-flowable, and wettable powder formulations.

Exposure Routes and Pathways

The major routes of exposure are through the skin and inhalation. Protective clothing must be worn to prevent skin exposure. Animals can also be exposed by eating contaminated cotton leaves or cotton gin trash.

Toxicokinetics

Fluometuron is absorbed slowly through the oral route. Rats given fluometuron orally excreted the chemical unchanged in the feces and urine after 3 days. Fluometuron and its metabolites were detected in liver, kidneys, pituitary, adrenal gland, plasma, red blood cells, and spleen, with highest concentrations noted in red blood cells.

Mechanism of Toxicity

Little is known about the mechanism of fluometuron toxicity in mammals. Fluometuron is a weak inhibitor of cholinesterases.

Acute and Short-Term Toxicity (or Exposure)

Animal

Fluometuron is practically nontoxic to rats and rabbits through the oral and dermal routes. It takes a large dose to cause toxicity in rats and rabbits. There is moderate to low toxicity through inhalation (LD₅₀ of 2 mgl^{-1}). Guinea pigs appear to be more sensitive to fluometuron, however. It caused skin sensitization and cholinesterase inhibition after inhalation exposure $(0.6 \text{ mg l}^{-1} \text{ for } 2 \text{ h})$ in this species. Fluometuron caused slight eye and skin irritation in rabbits. Clinical signs associated with high dosages of fluometuron in rats include muscular weakness, tearing or watery eyes, extreme exhaustion, and collapse. Healthy sheep (6-9 months old) exhibited grinding of the teeth, ruminal tympany, mydriasis, difficulty in breathing, staggering, paresis of the hindlimbs and forelimbs, and recumbency after drenching with fluometuron.

Human

Signs of poisoning include nausea and vomiting. Fluometuron can irritate skin, and gastrointestinal and respiratory tracts.

Chronic Toxicity (or Exposure)

Animal

Conjunctivitis, skin sensitization, and congestion of major organs like the liver and kidneys as well as the spleen and adrenal glands can occur with repeated exposures. Abnormalities in the red blood cells also occur with repeated exposures. Pregnant rabbits treated orally from days 6 to 19 of gestation with 50, 500, or $1000 \text{ mg kg}^{-1} \text{ day}^{-1}$ fluometuron showed an increased incidence of resorptions. No carcinogenic response was noted in rats exposed by diet to 125 or 250 mg kg⁻¹ day⁻¹ fluometuron.

Human

Repeated exposure to fluometuron may cause conjunctivitis and skin sensitization. Fluometuron is not likely to lead to reproductive, teratogenic, mutagenic, or carcinogenic responses in humans.

In Vitro Toxicity Data

Fluometuron appears to have little genotoxic effect based on Ames assay, micronucleus test,

DNA damage, or rat DNA repair inhibition tests.

Clinical Management

If ingested, vomiting is induced or plenty of water or syrup of ipecac is given. Nothing should be given to an unconscious person. Eyes are flushed with plenty of tap water for 15 min. If inhaled, the victim should be moved to fresh air or respiration applied if possible. If exposure on skin, the exposed area should be washed with plenty of soap and water and medical attention provided as soon as possible.

Environmental Fate

Fluometuron adsorbs to soil particles. Half-lives for degradation in soil and water are estimated at 12– 17 days and 110–114 weeks, respectively. Breakdown in soil is mainly through photodegradation. Soil microbes also degrade fluometuron. In field studies in agricultural areas in California and Georgia, fluometuron residues were not detected in soil below 12 in. Roots more readily absorb fluometuron compared to leaves. Differences in the plant's ability to break down fluometuron should be considered in using this chemical in weed control.

Ecotoxicology

Fluometuron essentially is nontoxic to birds and bees. The LC_{50} is 30 mgl^{-1} in rainbow trout, 48 mgl^{-1} in bluegill sunfish, 170 mgl^{-1} in carp, and 55 mgl^{-1} in catfish. There is low potential for bioaccumulation.

Other Hazards

Fluometuron is stable under normal temperatures and pressures, but it may pose a slight fire hazard if exposed to excessive heat or flame. Containers may explode in the heat of a fire. It poses a fire and explosion hazard in the presence of strong oxidizers. It is incompatible with acids and bases. Thermal decomposition may release highly toxic fumes of fluorides and oxides of nitrogen and carbon. Water runoff during fire control may also give off toxic gases. In case of fire, water should not be used as it has the tendency to spread the flames. In case of spills, the area should be contained with absorbent material. The area should be cleaned with detergent and water.

Exposure Standards and Guidelines

No occupational exposure limits have been established for fluometuron by Occupational Safety and Health Administration, The National Institute for Occupational Safety and Health, or American Conference of Industrial Hygienists.

The National Fire Protection Association considers fluometuron as moderately hazardous (rating of 2). The reference dose of fluometuron is $13 \,\mu g \, kg^{-1} \, da y^{-1}$.

Fluoride

Greene Shepherd

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- REPRESENTATIVE CHEMICALS: Sodium fluoride; Hydrofluoric acid
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7681-49-4
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Halogen
- CHEMICAL FORMULA: F

Uses

Fluoride is used in a variety of industries, most notably as an additive in the majority of municipal water supplies in the United States and is used as a mineral supplement for children living in areas that do not have fluoridated water. It is used in hydrofluoric acid, fluoropolymers, and refrigerating agents.

Exposure Routes and Pathways

Ingestion, dermal exposure (most notably via hydrofluoric acid), and inhalation are possible routes of exposure. Fluorine gas (F_2) produces little toxicity unless it reacts with some other chemical to produce fluoride ion.

Toxicokinetics

Chronic ingestion of fluorides causes exaggerated buildup on teeth, bones, and ligaments. Exposure to skin, eyes, and mucous membranes has a corrosive effect.

Mechanism of Toxicity

Fluoride interferes with the metabolism of cells and enzymes. It is a cross-linking agent and rarely occurs in an elemental state in nature. It is a metabolic inhibitor, interfering with calcium metabolism and electron See also: Diuron.

Further Reading

US Environmental Protection Agency (1985) Chemical Information Fact Sheet Number 88: Fluometuron, pp. 9–12. Washington, DC: Office of Pesticides and Toxic Substances.

transport. Calcium is essential for maintaining cardiac membrane potentials and in regulating coagulation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Brief exposures (<60 min) to concentrations between 38 and 73 ppm in several animal species showed no effects. The LC_{50} for inhalation in animal models ranged between 150 and 200 ppm h⁻¹. Higher concentrations will produce lethality in a shorter duration of time. Chronic exposures at 5– 10 ppm produced irritation of the eyes, oropharynx mucosa, and respiratory tract.

Human

Chronic over-absorption can cause hardening of bones, calcification of ligaments, and buildup on teeth. Fluoride can cause irritation or corrosion to eves, skin, and nasal membranes. Large ingestion of fluoride salts or hydrofluoric acid may result in fatal arrhythmias due to profound hypocalcemia. Inhalation may be fatal. The American Conference of Governmental Industrial Hygienists threshold-limit value/time-weighted average for fluorine is 1 ppm. National Institute of Occupational Safety and Health reports that concentrations of 25 ppm are immediately dangerous to life and health. Inhalant abuse of fluoridated hydrocarbon refrigerants like Freon[®] has been associated with 'sudden sniffing death', which is thought to be a fatal arrhythmia caused by myocardial sensitization to catecholamines.

Chronic Toxicity (or Exposure)

Animal

Sheep fed fluoride 10 ppm in water over 7 years demonstrated decreased wool production. Flourosis, painful stiff gait, lameness, decreased milk production, and dental changes developed in cattle fed 40 ppm fluoride in their diet over 6 months to 1 year.

Human

Fluorosis is a chronic public health problem in many parts of the world. Exposures to fluoride in concentrations greater that 1 ppm result in bone deformities, spinal compressions, and restricted movements of joints. These symptoms are easily prevented by decreasing exposure to fluoride.

In Vitro Toxicity Data

Assessments of mutagenicity using the Ames Salmonella model have not demonstrated toxic effects at concentrations up to $500 \,\mu g$ per plate.

Clinical Management

Small ingestions of low concentration fluoride products (e.g., toothpaste) may generally be managed by dilution with milk. Dermal exposures should be

Fluorine

Robert Kapp

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- RELATED CHEMICALS: Fluorine, hydrogen fluoride and fluorides
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7782-41-4
- SYNONYMS: Bifluoriden (Dutch); Fluor; Fluor (Dutch, French, German, Polish); Fluorine-19; Fluoro (Italian); Fluorures acide (French); Fluoruri acidi (Italian); Saeure fluoride (German)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Halogen group
- Chemical Formula: F⁻

Uses

Fluorine is combined either directly or indirectly with other elements to form compounds such as hydrofluoric acid, fluoropolymers and is used in the synthesis of organic fluorine compounds such as fluorides as in the manufacture of Freon (i.e., dichlorodifluoromethane, CCl_2F_2), which is used as a refrigerant. Fluorine is used in the manufacture of uranium hexafluoride that is necessary for the separation of the isotopes of uranium in centrifuges in the production of nuclear weapons. Fluorine and its compounds are used in producing more than 100 magnesium-based gel. Affected eyes should be irrigated with running water or saline solution for 30–60 min. In cases where systemic toxicity is evident intravenous calcium may be utilized.

See also: Catecholamines; Fluorine; Hydrofluoric Acid.

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commercial fluorochemicals, including many wellknown high-temperature plastics. The presence of fluorine as a soluble fluoride in drinking water to the extent of 2 ppm may cause mottled enamel in teeth, when used by children acquiring permanent teeth; in smaller amounts; however, fluorides are added to water supplies to prevent dental cavities. Fluoride is found in a variety of dental products including toothpastes and mouth washes, or as a preventive measure against dental caries. Fluorine has been studied as a rocket propellant since it has an exceptionally high specific impulse value. Although certain fluorine-containing compounds have been identified as being involved in ozone depletion and global warming effects, legislative measures have been, or are being, put in place where necessary to reduce this impact. Fluorine-containing compounds have been said to have a great potential for clean synthesis (i.e., use in 'green chemistry'), and utilization of the high activity that fluorinated groups can impart molecules may help to reduce the quantities required of certain classes of chemicals that are used in the biosphere, including pesticides and pharmaceuticals. Fluorine will react with water or steam to produce heat, and toxic and corrosive fumes.

Background Information

Fluorine is a nonmetallic, pale yellow-green gaseous element with a pungent odor. It is the most electronegative and reactive of all the elements. Fluorine is an element that is widely distributed in the environment, but because of its high reactivity it is not found naturally in its elemental state. It is found in Cryolite, Fluorspar, and Fluorapatite. Fluorine combines with hydrogen to form hydrogen fluoride, which is a colorless gas. Hydrogen fluoride subsequently can dissolve in water to form hydrofluoric acid. Fluorine gas also combines with some metals such as sodium to form sodium fluoride and with calcium to form calcium fluoride. In 1529, Georigius Agricola described the use of fluorspar as a flux, and as early as 1670, Schwandhard found that glass was etched when exposed to fluorspar treated with acid. Fluorine is a naturally occurring compound that was first identified by Scheele. Many later investigators, including Davy, Gay-Lussac, Lavoisier, and Thenard, experimented with hydrofluoric acid, with some experiments ending in tragedy. Fluorine was first isolated by Moissan in 1886, when he noted the inclusion of fluorine in crystals of Fluorspar.

Exposure Routes and Pathways

Exposure to fluorides can be through contaminated air, food, drinking water, and soil. The exposure pathways for fluorine and fluoride are via dermal, inhalation, or ingestion pathways. Fluorides enter the environment naturally through the weathering of rocks and minerals, and by emissions from volcanoes. The greatest amount of the total volume of fluorides in the environment is released from natural sources, in particular volcanoes and oceans. However, the greatest concentrations are found near anthropogenic point sources. Fluorides are found in soil, air, water, and in most foods, from both natural and anthropogenic sources (though rarely as molecular fluorine). It is highly electronegative and therefore reacts vigorously with other compounds. Anhydrous hydrogen fluoride will react with moisture in the air and form hydrofluoric acid, which will gradually settle to the ground or be dispersed with the wind. Calcium fluoride is most common in alkaline soils, and fluoroaluminate complexes are most common in acidic soils. Thus, exposure to hydrofluoric acid would occur at a hazardous waste site only if someone came in contact with material leaking from a storage container or breathed contaminated air before it was dispersed. Once in a stable form, fluoride persists in the environment for a relatively long time unless transformed to another compound or decomposed by radiation.

The general population is exposed to fluoride through consumption of drinking water, foods, and dentifrices, primarily in the form of sodium fluoride and stannous fluoride. Populations exposed to relatively high concentrations of fluoride include workers in fluoride-processing industries and individuals residing near such industries. These individuals may be exposed to higher than back ground concentrations as a result of the levels of hydrogen fluoride and dusts from fluoride compounds in the air, and from foodstuffs produced within the vicinity that have collected excess fluoride from the environmental media. Populations near hazardous waste sites may be exposed to high levels of fluoride by similar routes, although no information has been obtained to support this.

Toxicokinetics

Fluoride, rather than fluorine, appears to be the agent that is toxicologically active in the body because fluorine is so reactive that it is not absorbed chemically unchanged. Existing data indicate that all common forms of inorganic fluoride are rapidly and quite extensively absorbed. The highest degree of absorption has been noted with aqueous solutions of sodium fluoride resulting in absorption within 30 min of oral exposure. Hydrofluoric acid has been shown to be absorbed across the skin. Rats exposed to fluorine via subchronic inhalation showed marked elevation in teeth and bones as well as in the bloodstream; however, there is no evidence of accumulation or retention of fluoride in soft tissues in humans. Chronic ingestion of fluorides can cause exaggerated buildup in teeth and bones. Cessation of exposure will decrease the fluoride levels in bone slowly; however, the rate of decay is undetermined in humans.

Mechanism of Toxicity

Hydroxyapatite is a mineral phase during bone formation. Apparently fluoride replaces the hydroxyl ion and/or perhaps the bicarbonate ion associated with hydroxyapatite. This results in the formation of hydroxyfluorapatite. The excess fluoride is excreted in the urine, feces, sweat, and saliva within 24 h. Skeletal sequestration and renal excretion appear to be two primary mechanisms of the body, which controls the toxic levels of fluoride. Fluoride appears to interfere with cell metabolism, and fluoride is a cross-linking agent. Fluoride ions carried in human blood exist in two forms, as an inorganic ion (F^{-}) and in combination with an organic molecule. The toxicological significance of the fluoride ion with the organic molecule is not known. The inorganic fluoride ion forms metal-fluoride-phosphate complexes that interfere with any enzymes that require a metal ion cofactor. The inorganic fluoride ion may interact directly with the enzyme. In addition, the fluoride ion is thought to be a general inhibitor of the energy production organpresent. Acute fluorine ingestion results in four specific categories of symptoms (A–D):

	A	В	С	D
Symptoms	Gastric symptoms: nausea, salivation, abdominal pain	Direct effects of fluoride	Inhibition of metabolism	Persistent abnormal serum fluoride levels
Etiology	NaF + HCl→HF	Glycolytic pathway, cholinesterase	Hyperkalemia, hypocalcemia	Mineral homeostasis, cellular damage

ization of the cell, specifically the oxidative phosphorylation necessary in ATP formation.

The acute toxic dose of fluoride ranges from 0.1 to 0.8 mg kg^{-1} of body weight.

Acute and Short-Term Toxicity (or Exposure)

Animal

Five species of laboratory animals were exposed to fluorine for brief periods (5, 30, or 60 min). The LC₅₀ values for these animals ranged from 150 ppm for mice (exposure time = 60 min) to 820 ppm for rabbits (exposure time = 5 min). Mice exposed to sublethal concentrations had pulmonary irritation and delayed development of focal necrosis in the liver and kidney. The animals showed no effects from 60 min exposures at levels from 38 ppm (dog) to 73 ppm (guinea pig), as judged by lack of irritation, dyspnea, and pulmonary congestion and hemorrhage. Repeated, short-term exposures (60 min exposures at weekly intervals for 4 weeks) at levels from 55 ppm (mice) to 75 ppm (rats) either showed no effects, or very slight effects grossly in the lung, liver, and kidney. Evidence for a mild degree of tolerance was found by elevated 60 min LC50 values in mice, and for decreased lung and kidney weights in the pre-exposed animals compared with previously unexposed controls. No cytogenetic changes occurred in the oocytes of mice given single or repeated treatments of sodium fluoride.

Human

Elemental fluorine and the fluoride ion are highly toxic. Low overdose ingestion produces local gastrointestinal upset, salivation, and a metallic taste that may last 48 h. Acute inhalation of fluorine can cause severe respiratory irritation, dyspnea, and death. High overdose ingestion, in addition to causing more severe local manifestations, may produce systemic symptoms of convulsions, coma, dysrhythmias, hypotension without compensatory tachycardia, acidosis, paresthesias, and coagulation disturbances. Hypocalcemia can develop very rapidly. Coagulopathies can develop as a result of hypocalcemia. Hyperkalemia may be

Chronic Toxicity (or Exposure)

Animal

High doses of fluorine gas and hydrogen fluoride in animal studies have resulted in testicular degeneration. The available animal and human data strongly suggest that the reproductive system is a target of fluoride toxicity at high exposure levels.

Feeding of sodium fluoride to mice at concentrations of up to 50 mg kg^{-1} diet for seven generations did not induce chromosomal aberrations or sister chromatid exchanges in the bone marrow. The (US) National Institute of Environmental Health Sciences, National Toxicology Program (NTP) oral carcinogenicity study on sodium fluoride concluded that there was "equivocal evidence that fluoride is a carcinogen in male rats, but not in female rats or mice of either gender." Another rat carcinogenicity study found no evidence of carcinogenicity of fluoride in rats.

Human

Chronic exposure to very small amounts of fluoride in the drinking water is recognized as being beneficial to the prevention of dental caries. Fluorination of the drinking water allows for the incorporation of fluoride into tooth enamel pre-eruptively, inhibition of demineralization, enhancement of remineralization, and inhibition of bacterial activity in dental plaque formation. Chronic exposure to excessive amounts of fluorine can result in mottled teeth (fluorosis), for example, a chronic fluoride ingestion of 1 ppm of fluoride in drinking water can cause mottling of the teeth, and an exposure of 1.7 ppm will produce mottling in 30-50% of patients. Chronic poisoning may cause osteosclerosis, calcification of ligaments and tendons, bony exostoses, and renal calculi. Chronic inhalation exposure to high levels of hydrogen fluoride and fluoride dusts, or chronic oral exposure to high levels of fluoride can cause skeletal malformations and severe joint pain, and an increased incidence of nonvertebral skeletal fractures. The existing data do not indicate that fluoride is a carcinogen in humans.

There have been reports of a decrease in fertility in women and decreased serum testosterone levels in men living in communities with high fluoride levels in municipal water.

In Vitro Toxicity Data

Sodium fluoride did not induce reverse mutations in *Salmonella typhimurium*, nor did it induce gene conversion in *Saccharomyces cerevisiae*. A fluoride level of $0.4-1.0 \text{ mgl}^{-1}$ inhibited DNA repair after irradiation of mouse spleen cells *in vitro*. Sodium fluoride was not mutagenic in cell cultures of human leukocytes at concentrations of 18 and 54 mgl⁻¹. Little or no effect was noted on chromosomes when mouse oocytes were exposed *in vitro* to a fluoride concentration of 200 mgl⁻¹ in media for up to 14 h.

Clinical Management

Skin or mucousal burns should be washed for 15-60 min under running tap water. Burns should be coated with a water-based magnesium dioxide ointment with ~20% glycerin and should contain no oil. Exposed eyes should be washed for 15 min with running tap water, and then irrigated with saline solution for 30 min. If ingested, soluble calcium should be administered. Gastric upset can be treated by ingesting milk or cream every 4 h.

Environmental Fate

Fluorine is not destroyed in the environment, but rather it combines with minerals to form salts, which remain in the soil. Hydrogen fluoride gas is absorbed into the atmosphere to form hydrofluoric acid, which eventually returns to earth in the form of precipitation. Fluorides form strong associations with sediment and soil particles, which eventually accumulate in plants and the bones and/or shells of animals.

In dilute solutions and at neutral pH, dissolved fluorides are usually present as the fluoride ion (F^-). As pH decreases, the proportion of F^- decreases, while hydrogen fluoride (HF^{2-}) and nondissociated hydrogen fluoride increase. Levels of nondissociated hydrogen fluoride also increase in concentrated solutions. In seawater, fluorides exist in equilibrium. Calcium carbonate precipitation dominates the removal of dissolved fluoride from seawater. The next most important removal mechanism is incorporation into calcium phosphates. Undissolved fluoride is generally removed by sedimentation. The residence time of fluoride in ocean sediments has been computed at 2–3 million years.

Several factors influence the level of fluorides in food. These include the locality in which the food is grown, the amount of fertilizer and pesticides applied, the type of processing the food receives, and whether fluoridated water is used in food preparation. Foods characteristically high in fluoride content are certain types of seafood $(1.9-28.5 \text{ mg kg}^{-1})$, especially those types in which the bones are consumed, bone products such as bone meal and gelatin, tea, and baby formula processed with fluoridated water.

Ecotoxicology

Considerable differences exist in plant sensitivity to atmospheric fluoride, but little or no injury will occur when the most sensitive species are exposed to about $0.2 \,\mu g \,m^{-3}$ air, and many species tolerate concentrations many times higher than this.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average, for fluorine is 1 ppm, and the short-term exposure limit, 15 min, is 2 ppm. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is $0.1 \text{ ppm} (0.2 \text{ mg m}^{-3})$. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day is $0.1 \text{ ppm} (0.2 \text{ mg m}^{-3})$, and the NIOSH Immediately Dangerous to Life or Health value is 25 ppm.

See also: Chlorine; Hydrofluoric Acid.

Further Reading

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Relevant Website

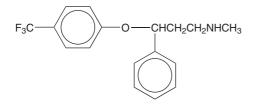
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fluorine.

Fluoxetine

Rebeca Gracia

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 54910-89-3
- SYNONYM: (±)-N-Methyl-3-phenyl-3-[(a,a,a-trifluoro-*p*-tolyl)-oxy]propylamine hydrochloride (Prozac)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Selective serotonin reuptake inhibitor; bicyclic antidepressant unrelated to tricyclic or tetracyclic compounds
- Chemical Formula: C₁₇H₁₈F₃NO
- CHEMICAL STRUCTURE:



Uses

Fluoxetine is used to treat depression, anorexia, bulemia, obesity, obsessive–compulsive disorder, premenstrual syndrome, panic attacks, narcolepsy, kleptomania, and diabetic neuropathy.

Exposure Routes and Pathways

Fluoxetine is available orally as capsules or liquid. Ingestion is the most common route of exposure.

Toxicokinetics

Fluoxetine is rapidly and completely absorbed orally, reaching a peak in 6-8 h. Food does not affect absorption. Fluoxetine is N-demethylated in the liver to an active metabolite, norfluoxetine, and many other minor inactive metabolites. Both fluoxetine and norfluoxetine are then conjugated prior to excretion. Protein binding is 94%. The volume of distribution is estimated to be 11-88.41kg⁻¹. Approximately 2.5% of the drug is renally excreted unchanged and 10% as the norfluoxetine metabolite. A total of 65% of radiolabeled fluoxetine is recovered in the urine after 35 days and 15% is recovered in the feces. The elimination half-life of fluoxetine is 48-72 h, averaging almost 70 h. The half-life of norfluoxetine is 7-9 days. The elimination half-lives for both are prolonged in patients with hepatic disease.

Mechanism of Toxicity

Fluoxetine has been found to cause selective central nervous system (CNS) neuronal uptake inhibition of serotonin. While fluoxetine may bind to adrenergic, muscarinic, and histaminic receptors, it has not been shown to have the profound effects on catecholamines that are common to tricyclic antidepressant overdose patients.

Acute and Short-Term Toxicity (or Exposure)

Animal

Six dogs were given intentional overdoses of oral fluoxetine during preclinical testing. Five of the six dogs had grand-mal seizures. All recovered with standard veterinary doses of intravenous diazepam. Chronic administration of fluoxetine has led to increased phospholipids in mice, rats, and dogs; this increase was reversed when the drug was discontinued.

Human

The therapeutic dose of fluoxetine ranges from 20 to $60 \,\mathrm{mg}\,\mathrm{day}^{-1}$. At therapeutic levels, the most commonly reported adverse effects are headache, nervousness, insomnia, drowsiness, tremor, nausea, anorexia, and diarrhea. Patients reported to have overdosed with fluoxetine have generally had a benign course with very little neurologic or cardiovascular toxicity. A decreased level of consciousness is the most common effect noted in overdose patients. Reported neurologic clinical symptoms include tremor, confusion, ataxia, insomnia, and coma. Seizures have been rarely reported following either therapeutic dosing or overdose. Cardiovascular symptoms seldom occur. The most common cardiovascular effects include mild tachycardia, bradycardia, and hypertension. No consistent EKG changes have been noted. Other symptoms reported are a flu-like syndrome, nausea, vomiting, and diarrhea. The estimated lethal dose is 1200-2000 mg. However, most fluoxetine-related fatalities occur in patients who have taken a concurrent tricyclic antidepressant overdose. It has been disputed that therapeutic dosing of fluoxetine has been associated with the development of mania, psychosis, and suicidal ideation. Fluoxetine use may be associated with serotonin syndrome following both therapeutic use and overdose, primarily in combination with other serotonergic agents. Recently, fluoxetine has been implicated in a discontinuation syndrome characterized by neurologic and gastrointestinal disturbances such as dizziness, nausea, lethargy, and headache. It is recommended to slowly taper dosage when stopping therapy to avoid untoward effects.

Chronic Toxicity (or Exposure)

Animal

Genetically obese mice were treated with fluoxetine to assess the ability of fluoxetine to produce weight loss. In this model, fluoxetine did produce initial weight loss but overtime had no positive impact on mouse weight.

Human

Fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) have been associated with increasing suicidal ideation in some populations of patients. Recent studies have led the British Department of Health to warn physicians against using paroxetine off label. Fluoxetine was specifically exempted from this recommendation. Long-term studies of patients with depression who were treated with fluoxetine have shown it to be fairly well tolerated. Primary adverse effects include nausea (23%), headache (21%), and insomnia (20%).

In Vitro Toxicity Data

In assays of mutagenicity and carcinogenicity, fluoxetine and its metabolite norfluoxetine have not demonstrated toxic effects in the Ames *Salmonella* assay, DNA repair assay in cultured rat hepatocytes, and mouse lymphoma assay or sister-chromatid exchange in Chinese hamster bone marrow cells.

Clinical Management

All basic and advanced life-support measures should be implemented. Gastric decontamination should be performed. Fluoxetine is readily adsorbed by activated charcoal and charcoal should be considered for substantial recent ingestions. Aggressive supportive care should be instituted. There is no specific antidote for fluoxetine overdose. Hemoperfusion and hemodialysis are ineffective.

See also: Charcoal; Diazepam.

Further Reading

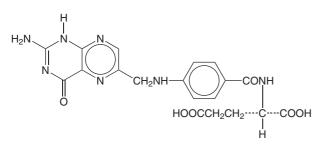
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Folic Acid

Diana Ku

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 59-30-3
- SYNONYMS: Folacin; Folate; Pteroylmonoglutamic acid; 4-(2-Amino-4-hydroxypteridin-6-yl)methyl-aminobenzoyl-L-glutamic acid
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Watersoluble vitamin
- CHEMICAL FORMULA: C₁₉H₁₉N₇O₆
- CHEMICAL STRUCTURE:



Uses

Folic acid is a nutritional supplement frequently used during periods of deficiency. Folic acid needs increase during chronic diseases, such as malabsorption liver disease, alcoholism, and anticonvulsant or oral contraceptive use. Folic acid supplementation during pregnancy is strongly recommended to prevent neural tube defects to the unborn child. The active form of folic acid, folinic acid, is used in the management of certain medical diseases (e.g., patients taking methotrexate, and 5-fluorouracil).

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, intramuscular, and subcutaneous. Dietary sources of folic acid are green leafy vegetables, some fruits, legumes, eggs, yeast, whole grain cereals, lean beef, veal, liver, and kidneys. Heat destroys folic acid in cooked foods.

Toxicokinetics

Folic acid is almost completely absorbed from the gastrointestinal tract, mostly in the upper duodenum,

Peak serum levels occur within 30–60 mm. Folic acid is converted in the liver to tetrahydrofolic acid in the presence of ascorbic acid by dihydrofolate reductase. Tetrahydrofolic acid and its derivatives are distributed into all body tissues with approximately half of it in the liver. It is excreted renally almost entirely as metabolite. Excessive amounts of folic acid (beyond the daily needs) are excreted unchanged in the urine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected.

Human

Toxicity is unlikely even after acute ingestions of 100 times the recommended daily allowance. Allergic reactions have been reported.

Chronic Toxicity (or Exposure)

Animal

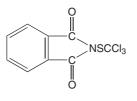
It would be unlikely for animals to be given chronic folic acid overdoses.

Folpet

Paul R Harp

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 133-07-3
- SYNONYMS: Acryptan; Faltan; Faltex; Folnit; Folpan; Folpel; Folpex; Ftalan; Fungitrol II; Intercide TMP; Phaltan; Phaltane; Spolacid; Thiophal; Vinicoll; ENT 26539; SHA 081601
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: N-[(Trichloromethyl)thio]phthalimide fungicide
- CHEMICAL STRUCTURE:



Human

Chronic large doses may interfere with sleep patterns and cause malaise, irritability, and gastrointestinal symptoms such as anorexia, nausea, bloating, flatulence, and bad taste. Seizure threshold may be lowered in epileptics and progression of neurologic injury in pernicious anemia has also been reported.

In Vitro Toxicity Data

Studies of human epithelial cells failed to demonstrate cytotoxic effects even at concentrations of $200 \,\mu g \, m l^{-1}$.

Clinical Management

Acute ingestions seldom require treatment. In cases of chronic excessive use, the patient should be instructed to discontinue the supplement. Any toxic symptoms should be treated symptomatically.

See also: Vitamin A; Vitamin D; Vitamin E.

Further Reading

Butterworth CE Jr. and Tamura T (1989) Folic acid safety and toxicity: A brief review. *American Journal of Clinical Nutrition* 50: 353–358.

Uses

Folpet is a broad-spectrum contact fungicide used on various fruits, vegetables, berries, flowers, and ornamentals. It is also used on seeds, plant beds, and structural surfaces and is added to some paints and plastics for antifungal purposes. Folpet is effective only for prevention of fungal growth, not for treatment of an existing infection.

Exposure Routes and Pathways

Folpet is available as a wettable powder or suspension concentrate. Eye, skin, or respiratory exposure may occur during production or application of folpet. Ingestion of contaminated food products is also a potential route of exposure.

Toxicokinetics

Folpet is readily absorbed following oral administration in rats. Metabolites of folpet in rats are tetrahydrophthalimide and phthalimide, which may be further metabolized to phthalic acid and ammonia. Absorption following dermal exposure is limited. Both folpet and certain metabolites are highly reactive with thiol groups, which results in rapid degradation and limits adverse effects to the contact area. Due to the reactive nature, a variety of enzymatic and nonenzymatic reactions are possible and elimination occurs rapidly.

Mechanism of Toxicity

Interaction with thiol groups initiates both the fungicidal activity in target organisms and the toxicological activity in nontarget organisms. An intermediate, thiophosgene, which readily reacts with thiols and other functional groups, is likely involved with the variety of reported mechanisms, which include inhibition of glyceraldehyde-3-phosphate dehydrogenase and O-demethylase activity in liver microsomes, uncoupling of oxidative phosphorylation, and activity as a hapten, stimulating the immune system to produce allergic responses against folpet and other structurally similar compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, folpet and the related fungicides captan and captafol have been shown to decrease cytochrome P450 activity and increase serum enzymes, suggesting a hepatotoxic effect. Reported LD_{50} values vary greatly depending on the route of exposure. Folpet is slightly toxic via oral and dermal routes with reported oral LD_{50} values >10000 mg kg⁻¹ in rats and dermal LD_{50} values >22600 mg kg⁻¹ in rabbits. In contrast, intraperitoneal LD_{50} values of 40 and 68 mg kg⁻¹ have been reported in rats. The large differences in LD_{50} values are most likely due to route-dependent differences in absorption.

Human

Local irritation can result following dermal, ocular, or respiratory contact with folpet. Ingestion of folpet may cause vomiting and diarrhea, leading to dehydration and electrolyte depletion. Exposure to folpet has been linked to contact dermatitis but incidence rates in humans appear low.

Chronic Toxicity (or Exposure)

Animals and Humans

Folpet has been classified by US Environmental Protection Agency (EPA) as a probable human carcinogen (B2) since the mid-1980s based on induction of neoplastic growth in the duodenum of several strains of mice and positive results in multiple *in vitro* mutagenicity assays. EPA has not yet reviewed folpet under their most recent guidelines for carcinogenic risk assessment, which place more emphasis on mechanism of action and evidence for thresholds. Reevaluation under the new guidelines will likely result in a decreased classification of carcinogenic risk to humans. Researchers have thoroughly examined the possible teratogenic effect of folpet due to its structural similarity to the known human teratogen thalidomide. All test results for teratogenicity were negative.

In Vitro Toxicity Data

As indicated above, folpet is mutagenic in a number of assays.

Clinical Management

For eye contact, the eyes should be flushed immediately with generous amounts of water. For dermal exposure, contaminated clothing should be removed and the skin should be washed thoroughly with soap and water. A physician should be contacted promptly if irritation does not subside. For cases of substantial ingestion of folpet within the last few hours and in the absence of significant vomiting, gastric lavage or a combination of activated charcoal and sorbitol may be indicated. Sorbitol should not be administered if diarrhea is present or if only a small amount of fungicide was ingested. In those cases, only activated charcoal should be administered. Acute exposure to folpet is not likely to result in toxicity; treatment, if necessary, is symptomatic and supportive.

Environmental Fate

Degradation is likely similar to that of captan, in which trithiocarbonate, thiophosgene, and phthalimide are produced. Folpet can produce phytotoxicity, in particular in dry conditions.

Ecotoxicology

Folpet is slightly toxic to birds including quail and ducks. The LD_{50} for bobwhite quail is >2510 mg kg⁻¹ and the dietary LD_{50} for the mallard duck is >5000 ppm. Folpet is highly toxic to fish including rainbow trout and bluegill sunfish. The LC_{50} (96 h) of Folpet in bluegill sunfish was 675 ppb and for rainbow trout was 185 ppb. Folpet is also highly toxic to aquatic invertebrates, the LC_{50} (48 h) for *Daphnia magna* was 0.60 ppm. Folpet is relatively nontoxic to honeybees.

Exposure Standards and Guidelines

The reference dose for folpet is $0.09 \text{ mg kg}^{-1} \text{ day}^{-1}$. The acceptable daily intake is $0.1 \text{ mg kg}^{-1} \text{ day}^{-1}$. See also: Captafol; Captan; Pesticides.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Food See Dietary Restriction; Dietary Supplements; Food Additives; Food Quality Protection Act, US; Food Safety and Toxicology; Food, Drug, and Cosmetic Act, US; Food and Agriculture Organization of the United Nations; Genetically Engineered Foods; Monosodium Glutamate.

Food Additives

James C Griffiths and Joseph F Borzelleca

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Food additives are substances or ingredients, which are incorporated into the final food product, either as a result of direct addition to achieve a desired effect (functionality or technical effect) or indirectly as a result of production or processing. Direct additives include antioxidants, leavening agents, texturizing agents, preservatives, colors, and flavors. The levels of these agents in food are usually low; with the levels of colors and flavors usually the lowest. Direct food additives may increase stability and prolong shelf life, improve the organoleptic qualities (appearance, texture or mouth-feel, aroma, and/or flavor) of food and increase market penetration. The number of additives used in processing foods has been increasing due to advances in food technology and consumer expectations. These additives are identified on the food label.

These increases in the use of food additives and/or their improper use may pose potential health hazards to the consumer or introduce an element of deception, disguising unpalatable/unhealthy food. It is appropriate and necessary that food additives be safe and suitable for their intended use. This is assured by governmental oversight and regulations that establish safety and conditions of use.

This section will focus primarily on the regulatory principles and practices in the United States, and these will be compared with the European and the UN's Codex Alimentarius Commission.

Definitions

Definitions of food additives (direct food additives, ingredients added to foods for a specific purpose)

vary among government agencies and organizations and include the following:

• US Food and Drug Administration (FDA): The term 'food additive' means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use), if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case as a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use; except that such term does not include: (1) a pesticide chemical in or on a raw agricultural commodity; or processed food; or (2) a pesticide chemical; or (3) a color additive; or (4) any substance used in accordance with a sanction or approval granted prior to the enactment of this paragraph¹ (footnote 2) pursuant to this Act, the Poultry Products Inspection Act (21 U.S.C. 451 and the following) or the Meat Inspection Act of March 4, 1907 (34 Stat 1260) as amended and extended (21 U.S.C. 71 and the following); (5) a new animal drug; or (6) an ingredient described in paragraph (ff) in, or intended for use in, a dietary supplement (US FFDCA §201 $(s)).^1$

¹US FFDCA §201(s) = United States Federal Food Drug and Cosmetic Act (1938 enaction, as amended), section 201 'Definitions'.

- European Economic Community (EEC): A food additive is any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods. 89/107/EEC.
- World Health Organization (WHO): Food additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities Codex Alimentarius, second edition (revised 1995), volume 1A (General Requirements), p. 11.

Principles

Several principles are common to the use of food additives including:

- A food additive must be safe and suitable, it must present no risk to the health of the consumer at the levels of intended use, it must not be used to deceive the consumer (wholesomeness and stability must not be compromised).
- A food additive must be used in accordance with the principles of current good manufacturing practice (cGMP) which dictates that the lowest possible amount necessary to accomplish the desired technical effect be used and that the preparation and handling of the food additive be to the same quality as food itself.
- A food additive may serve one or more technological functions (functionality) listed in **Table 1**. US Regulations may include broader generalizations and finer subcategories. For example, Section 172 of 21 CFR includes these broad categories:
 - \circ food preservatives,
 - coatings, films and related substances,
 - special dietary and nutritional additives,
 - o anticaking agents,

- flavoring agents and related substances,
- gums, chewing gum bases and related substances,
- other specific usage additives, and
- multipurpose additives.
 - Whereas some of the subcategories also listed in Section 172 of 21 CFR on a case-by-case basis include:
- antigushing agent,
- bleaching agent,
- cure accelerator agent,
- malting agent,
- pesticide surfactant agent,
- retard struvite (innocuous magnesium ammonium phosphate crystals) formation agent, and
 wetting agent.
- 0 0

Regulations – US

United States food law is codified in the Federal Food Drug Cosmetic Act (FFDCA) with amendments and further clarification in the Code of Federal Regulations, (CFR), Title 21. This deals with food and food additives.

Product categorization (e.g., food or drug) determines the nature and extent of testing. More rigorous and restrictive requirements, including premarket approval by the FDA, are applied to a drug than to a 'food'. There may be distinct advantages for classifying a product as a dietary supplement instead of a conventional food and this will be discussed in more detail in the section entitled 'Dietary Supplements Ingredients'. Inconsistent regulatory treatments of similar (or in some cases, identical) products can lead to a blurring of the lines between different 'intended use' FDA categories. Placement in a category depends in part on how the manufacturer positions the product for consumer use. A soluble fiber found in conventional breakfast cereals may be a food or a food additive; it also may be used as a dietary supplement and as an over-the-counter (OTC) drug, with concomitant allowable health benefit claims.

Premarket clearance (clearance prior to marketing) may be required by the FDA in some instances but not in others, The prevailing legal principle in the FFDCA is the prohibition to the use of 'any poisonous or deleterious substance which may render...[the food] injurious to health...'.

The following seven categories will be briefly described: (1) traditional foods used as food ingredients; (2) prior-sanctioned ingredients; (3) generally recognized as safe ingredients; (4) direct food additive ingredients; (5) indirect food additive ingredients; (6) dietary supplement ingredients; and (7) bioengineered food ingredients.

Table 1 Technological functions of a food additive

Technical effect	Notes	Example	<i>US</i> ^a	JECFA	EU
Acidity regulators	Buffer acid/base	Ammonia solution	U*	J	
Acids	pH change, tartness	Lactic acid		J	Е
Adjuvants		Polyvinylpyrrolidone		J	
Adsorbents	Remove moisture	Activated carbon	U**	J	
Anticaking agents	Prevent clumping	Aluminum silicate	U	J	E
Antifoaming agents	Prevent foaming	Polydimethylsiloxane		J	Е
Antioxidants	Prevent oxidation	Ascorbic acid	U	J	Е
Bulking agents	Add bulk	Ethyl cellulose		J	Е
Carrier solvents	Dissolve	Ethyl alcohol		J	
Clouding agents	Add opacity	Brominated vegetable oils		J	
Color retention agents	Preserve color	Cupric sulfate	U***	J	
Colors	Add color	Canthaxanthin	Ŭ	J	Е
Curing and pickling agents	Add unique color/flavor		Ŭ	0	-
Dough strengtheners	Modify starch, improve dough		U		E*
Emulsifiers	Modify surface tension	Lecithin	U, U****	J	E, E*
Enzyme preparations	Improve food processing	Avian pepsin	U, U	J	с, с Е
Extract solvents	Dissolve	Acetone	U	J	L
	Remove sediment	Polyvinylpolypyrrolidone	0	J	
Filtering aids Firming agents	Add firmness	Calcium sulfate	U	J	Е
0 0		Disodium 5'-guanylate	U	J	E
Flavor enhancers	Modify original flavor Add flavor	Citral	U	J	E
Flavoring agents			U	J	Е
Flour treatment agents	Improve milled flour	Stearyl tartrate	0		E
Foaming agents	Add foam	Methyl ethyl cellulose		J	
Formulation aids	Produce texture	A 111	U		
Freezing agents	Freeze	Nitrogen		J	
Fumigants	Control pests		U		_
Gelling agents	Gel	Sodium alginate		J	E
Glazing agents	Glaze, surface-treat	Beeswax	U****	J	E
Humectants	Add/retain moisture	Xylitol	U	J	Е
Lubricants/release agents	Prevent sticking, including molds		U	J*	
Miscellaneous		Helium; Gelatin; Carbon dioxide		J	
Nonnutritive sweeteners	Non caloric sweeteners	Sucralose	U		
Nutrient supplements	Add vitamins/minerals	Potassium gluconate	U	J	Е
Nutritive sweeteners	Caloric sweeteners	Sucrose	U		
Oxidizing and reducing agents	Improve stability		U		
Preservatives	Prevent microorganisms growth	Hydrogen peroxide	U*****	J	
Processing aids	Enhance food		U******		
Propellants	Discharge pressurized foods	Argon	U******	J	E
Raising agents	Enable dough to rise	Ammonium carbonate	U*******	J	E
Reduced-energy fat and oil replacement	Low-fat oil	Salatrim		J	
Sequestrants	Form soluble metal complexes	Stearyl citrate	U	J	Е
Stabilizers	Improve dispersions	Gellan gum	U	J	Е
Sweeteners ^b	Sweetening capacity	Aspartame		J	Е
Synergists	React with another ingredient	Sodium percarbonate	U	J	Е
Texturizers	Affect mouth feel, appearance		U		
Thickeners	Produce viscosity, body	Carob bean gum	U	J	
Yeast foods		Urea		J	

^aUS 21 CFR170.3(o).

^bUS splits sweeteners into nutritive and nonnutritive sweeteners. (see appropriate spaces in Table 1)

U^{*}, pH control agents; U^{**,} drying agents; U^{**,} coloring adjuncts; U^{***,} emulsifiers and emulsifier salts; U^{****,} surface-active and surfacefinishing agents; U^{*****,} antimicrobial agents; U^{******,} includes clarifying, clouding, filtering, etc.; U^{*******,} propellants, aerating agents and gases; U^{********,} leavening agents; J^{*}, release agents; E^{*}, modified starch; E^{**}, emulsifiers and emulsifier salts.

Traditional Foods Used as Food Ingredients

Prior Sanctioned Ingredients

Common or traditional foods that have a history of safe use (e.g., most vegetables, fruits, grains, meats, poultry) are recognized as safe for human consumption. They are an integral part of the final food that is consumed. When the key amendments were made to the FFDCA in 1958, it was recognized that some exceptions were needed to the food additive category, such as ingredients previously established as safe and already listed in FDA regulations. Some of the

prior-sanctioned ingredients include gum guiac, calcium propionate, linseed oil, and sodium nitrate. A complete listing appears in 21 CFR Section 181.

Generally Recognized as Safe Ingredients

Also in 1958, it was recognized that expertise other than that in the FDA could be utilized to determine safety of foods and food ingredients. Statutory language states a substance is generally recognized as safe (GRAS) and thus outside the scope of the food additive definition if it is "recognized, among experts qualified by scientific training and expertise to evaluate its safety, as having been adequately shown through scientific procedures...to be safe under conditions of its intended use ... " Over the years, the Agency has continued to modify this process. There are currently two approaches, the GRAS selfdetermination (usually managed by the interested company) and the GRAS Notification (managed by the FDA). This latter regulatory process allows a company to submit the details of their successful GRAS self-determination to the FDA, which will critically evaluate all aspects within a predetermined time period. The FDA will then issue a letter to the petitioner and post it publicly on the FDA's webpage (see section on Relevant Websites). The letter with the best outcome for the submitter may be interpreted as an affirmation of GRAS but, it is a 'no objection letter', that is, the FDA had no objections to the GRAS self-determination. For example, the public letter may read, 'Based on the information provided by Company X, as well as other information available to FDA, the Agency has no questions at this time regarding the conclusion of Company X that Ingredient Y is GRAS under the intended conditions of use. The Agency has not, however, made its own determination regarding the GRAS status of the subject use of Ingredient Y. As always, it is the continuing responsibility of Company X to ensure that food ingredients that they market are safe, and are otherwise in compliance with all applicable legal and regulatory requirements'. An alternative response from the FDA is an 'objection letter', which clearly identifies deficiencies in the submitted GRAS Notification.

Direct Food Additive Ingredients

If a candidate substance is to be used directly in/on food, and is not already covered under prior sanction or GRAS categorization, and is not bioengineered nor a dietary supplement, then it is most likely a *bona fide* food additive/ingredient and stringent premarket (i.e., FDA approval before marketing) approval is mandated by a petition process. If successful, official regulations will be published and codified in the 21 CFR and there are likely to be specific conditions of use including food categories in which it may be used and appropriate levels of use.

Indirect Food Additive Ingredients

If a substance is found in food but was not directly added to the food and if it does not have a technical effect in the food, it is considered an indirect food additive. This category includes food contact materials such as packaging. Originally these ingredients were subjected to similar, rigorous regulations comparable to direct food additive ingredients, but this has been significantly simplified into a premarket notification instead of premarket approval.

Dietary Supplement Ingredients

Dietary supplement ingredients were removed from the FDA's food additive regulations in 1994 with the passage of the Dietary Supplement Health and Education Act (DSHEA) creating a more favorable, less onerous process (FDA premarket notification) with the advantage that labels and labeling were permitted to provide 'statements of nutritional support' but, these materials are prohibited from being 'represented for the use as a conventional food'.

Bioengineered Food Ingredients

Although controversial, bioengineered foods, genetically modified organisms, and products of biotechnology are not required to undergo food additive scrutiny by the FDA as long as there are no significant changes in composition, nutrient value, allergenicity (beyond the conventional 'version') or, other safety concerns compared to the familiar, conventional, and traditional food. The bioengineered food ingredient should be substantially equivalent to the conventional/traditional food. FDA does expect a premarket consultation to review safety and any other potential concerns.

Regulations – Europe

European Union (EU) countries regulate food additives through a number of key directives that are approved by the members. The legislative processes are very complex and beyond the scope of this discussion. Suffice, the EU is 'run' by a number of institutions, including the European Commission (EC), the Council of Europe, and the European Parliament (EP). The EC initiates proposals for legislation, is guardian of treaties and ensures that EU legislation is applied correctly by member states, and manages EU policies and international trade

relations. The EC is further subdivided administratively into departments referred to as Directorate-Generals (DGs), one of which, the DG for Health and Consumer Protection is the most important in the area of food law. A number of advisory Scientific Committees, most critically, the Scientific Committee for Food (SCF) prepares scientific opinions and risk assessments. The three types of European legislation are (1) directives, (2) regulations, and (3) decisions. Directives express obligatory objectives but do allow the members flexibility in the translation of the directive into their national law. Regulations are inflexible, apply to all members, are binding and circumvent the member's national legislation. Decisions are binding but are more specifically addressed to discrete member states, companies, or individuals.

The key Directive, 89/107/EEC, provides the framework for general regulatory and safety aspects of food additives, including the aforementioned definition of food additive "...any substance not normally consumed as food ... addition of which ... for a technological purpose...becoming...a component of food". This definition does not include processing aids (similar to the US indirect food additive ingredients), plant health products, flavorings and substances used as nutrients. Additional directives have been promulgated that address several specific subcategories including among others, 'sweeteners', 'colors', and 'flavorings'. Authorized food additives, conditions of use, limitations, purity criteria, etc. are listed in the directive. The process can occur in stages, as there are provisions for temporary use, specific national (member countryspecific) marketing of a nonlisted additive as well as suspension or restriction of an authorized food additive in their country on grounds of suspected danger to health or erosion of 'traditional national foods', such as Greek feta cheese, German beer (Reinheitsgebot) and Spanish 'lomo embuchado'. Authorized additives are listed in one of the newer directives (94/35/EC (sweeteners); 94/36/EC (colors); 89/107/EEC (flavors); and 95/2/EC (amended 96/85/EC, 98/72/EC, 2001/5/ EC; for all other food additives)).

Regulations – Codex Alimentarius

The Codex Alimentarius (CA; latin for 'food law' or 'food code') Commission (CAC) is an intergovernmental subsidiary to the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) of the United Nations (UN) with the aim to formulate and implement internationally accepted food safety standards for the protection of consumer health and to ensure fair trade practices in the food industry. The principal result has been the publication of food commodity standards, hygiene and technical codes of practice, guidelines and other recommendations. The CAC professes to take into account the individual concerns of governments, nongovernmental organizations, industry, and the consumer. The CAC issues CA standards, which are requirements aimed at providing consumers with a 'sound, wholesome food product' free from adulteration, correctly labeled and presented, and CA codes of practice as advisories to member nations providing flexibility in translation and implementation.

The adoption of standards is a multistep process requiring the involvement of appropriate expert committees charged with preparing the 'proposed draft standard' which undergoes a series of circulations to governments and other interested international organizations for comment.

Safety – US

Recognizing that the establishment of safe food (and color) additives would be predicated upon appropriate and responsible safety assessments, the FDA issued 'Guidelines' in 1982 entitled 'Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food (termed the "Redbook")' and currently has a web-based dynamic version entitled 'Redbook 2000: Toxicological Principles for the Safety Assessment of Food Ingredients' (see section on Relevant Websites). Safety is defined by the Agency as 'a reasonable certainty that a substance is not harmful under the intended conditions of use'. The Redbook guidelines are an attempt to 'delineate the sensitivity and rigor of toxicological and other information needed to make safety determinations' for these additives. The guidelines outline a 'tiered' approach linking the level (and therefore financial and temporal costs) of effort to the chemical structure of the ingredient and to the potential exposure. Sample protocols are provided to ensure consistency in study design facilitating Agency and/or other expert evaluation. The food additive petition process requires the submission of appropriate data derived from animal and/or human safety studies such as described in the Redbook. The animal toxicity data must supply substantive information that addresses (1) the identification of any hazards inherent to the use of the substance; (2) the indication of a dose-response relationship for those identified hazards; and (3) the extrapolation of these data to establish a safe exposure for consumers (e.g., an acceptable daily intake).

Safety studies that are consistent with other internationally accepted guidelines (e.g., those of the Organization for Economic Cooperation and Development (OECD)) may be acceptable to the FDA.

Safety – Europe

The original and overriding Directive, 89/107/EEC, mandates that food additives can be approved only provided that "... they present no hazard to health of the consumer at the level of use proposed, so far as can be judged on the scientific evidence available ... " Further, to assess the possible harmful effects of a food additive "it must be subjected to appropriate toxicological testing and evaluation. The evaluation should also consider, for example, any cumulative, synergistic or potentiate effect of its use and the phenomenon of human intolerance to substances foreign to the body." And the EU did not consider this to be a once and done activity as the regulation further stipulates that "all food additives must be kept under continuous observation and must be reevaluated whenever necessary in the light of changing conditions of use and new scientific information."

Safety – Codex Alimentarius

The CAC has emphatically stated that all CA standards, codes of practice and other texts shall be firmly anchored in sound scientific analysis and evidence, involving a thorough review of all relevant information so that the issued standards ensure the objective, safe food. An instrumental expert committee, the Joint Expert Committee on Food Additives (JECFA), serves as a scientific advisory body to FAO, WHO, to the UN member country governments and to the Codex Alimentarius Commission. JECFA is charged with evaluating the safety of food additives (as well as contaminants, naturally occurring toxicants and the residues of veterinary drugs in food derived from animals used for human food). JECFA evaluations provide impartial advice and technically rigorous science-based risk assessments. There is another committee, the Joint Meeting on Pesticide Residues (JMPR), which critically evaluates data on pesticides and recommends ADIs and residue levels. It is also an advisory body to FAO, WHO, and to UN member countries.

Evaluation Steps

The evaluation of the safety of food ingredients can be resource-intensive depending on the chemical nature of the ingredient and the extent and conditions of exposure. An outline of the process follows:

- 1. Initial evaluation
 - 1.1. Clear definition of the issues
 - 1.2. Test material characterization
 - 1.3. Exposure assessments (assume 100% replacement)

- 1.4. Critical evaluation of the literature
- 1.5. Initial safety assessment
- 1.6. Issues to be addressed
 - 1.6.1. Safety testing must simulate human exposure conditions
 - 1.6.2. Absolute versus relative safety
 - 1.6.3. Animal versus human; animal and/or human; sensitive subsets of population
 - 1.6.4. History of use (US and other countries)
 - 1.6.5. Nature of material, extent of exposure (levels (dose), duration)
 - 1.6.6. Initial sensory evaluation ('sip and spit')
- 2. Primary evaluation
- 2.1. Acute toxicity test(s)
 - 2.1.1. Oral (dermal, ocular, inhalation)
- 2.2. Repeated dosing tests (7-30 days)
- 2.3. Initial genotoxicity tests (short term tests)
- 2.4. Initial sensory evaluation ('sip and spit')
- 2.5. Comparative (animal, human) ADMEK (absorption, distribution, metabolism, excretion, kinetics) (single-dose human study)
- 2.6. Special studies
- 3. Secondary evaluation
 - 3.1. Subchronic tests (<1/2 lifetime of the animal; usually 90 days)
 - 3.2. Repeated human dosing tests
 - 3.3. Reproductive/developmental toxicity tests
 - 3.4. Genotoxicity tests
 - 3.5. Special studies
- 4. Definitive evaluation
 - 4.1. Chronic toxicity (lifetime) tests (several species; *in utero* exposure)
 - 4.2. Long-term human dosing tests
 - 4.3. Special studies
 - 4.4. ADI (acceptable daily intake)
- 5. Risk assessment
- 6. Postmarketing surveillance (PMS)
 - 6.1. Active versus passive

See also: European Union and Its European Commission; Food and Agriculture Organization of the United Nations; Food and Drug Administration, US; Food, Drug, and Cosmetic Act, US; Organisation for Economic Cooperation and Development.

Further Reading

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Food Additives: Joint FAO/WHO Expert Committee See Joint FAO/WHO Expert Meetings (JECFA and JMPR).

Food Quality Protection Act, US

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In 1996, the US Congress unanimously passed landmark pesticide food safety legislation supported by the Administration and a broad coalition of environmental, public health, agricultural, and industry groups. President Clinton promptly signed the bill on August 3, 1996, and the Food Quality Protection Act (FQPA) of 1996 became law (P.L. 104-170, formerly known as H.R. 1627). The FQPA requires the US Environmental Protection Agency (EPA) to consider new factors when making pesticide regulatory decisions. Registrants, applicants, or petitioners for pesticide product registrations or reregistrations, or for tolerances or tolerance exemptions, whether pending or future, are advised to consider comprehensively the provisions contained in the FQPA, specifically the factors relevant to aggregate exposure assessment, children's exposure, and other issues raised by the new statutory standard.

EPA regulates pesticides under two major federal statutes. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), EPA registers pesticides for use in the United States and prescribes labeling and other regulatory requirements to prevent unreasonable adverse effects on health or the environment. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), EPA establishes tolerances (maximum legally permissible levels) for pesticide residues in food. Tolerances are enforced by the Department of Health and Human Services/Food and Drug Administration (HHS/FDA) for most foods, US Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) for meat, poultry, and some egg products, and the US Department of Agriculture/Office of Pest Management Policy.

For over two decades, there were efforts to update and resolve inconsistencies in the two major pesticide statutes, but consensus on necessary reforms remained elusive. The 1996 law represented a major breakthrough, amending both major pesticide laws to establish a more consistent, protective regulatory scheme, grounded in sound science. It mandates a single, health-based standard for all pesticides in all foods; provides special protections for infants and children; expedites approval of safer pesticides; creates incentives for the development and maintenance of effective crop protection tools for American farmers; and requires periodic re-evaluation of pesticide registrations and tolerances to ensure that the scientific data supporting pesticide registrations will remain up to date in the future.

Effective upon signature, the FQPA significantly amended the FIFRA and the FFDCA. Title IV of the FQPA amended the Federal Food, Drug and Cosmetic Act. The most important aspect of this title is the establishment of a single, health-based standard for setting pesticide residue tolerances. This eliminated the longstanding problems posed by different standards for pesticides in raw and processed foods. The provision removed the requirement of food additive tolerances for processed foods and instead regulates them all under the same tolerance provision. A tolerance (or exemption from tolerance) for a pesticide residue on a raw agricultural commodity (RAC) also applies to residues in a processed food derived from the RAC that are not higher than the RAC tolerance. If the levels in the processed food are higher, a separate tolerance must be set for that processed food. Residue levels in both the RAC and the processed food must be determined by EPA to be 'safe'.

The new safety standard, provided in section 408(b) (2) (A) (ii) of the FQPA, is a 'reasonable certainty of no harm' standard for aggregate exposure using dietary residues and all other reliable exposure information. When setting new or reassessing existing tolerances or tolerance exemptions under the new standard, EPA must now focus explicitly on exposures and risks to children and infants. EPA must explicitly determine that the tolerance, or exemption from tolerance, is safe for children; consider the need for an additional safety factor of up to tenfold to account for uncertainty in the data base relative to children unless there is evidence that a different factor should be used; and consider children's special sensitivities and often unique exposure patterns to pesticides.

In addition, when making a determination as to whether or not there is a reasonable certainty that a pesticide chemical will cause 'no harm', EPA must now consider other nonoccupational sources of pesticide exposure when performing risk assessments and setting tolerances. This includes dietary exposure from drinking water, nonoccupational exposure, exposure from like pesticides that share a common mechanism of toxicity as well as other exposure scenarios. When setting new or reassessing existing tolerances and tolerance exemptions, EPA must also evaluate the potential for endocrine disruption. The new law directs the Agency to use its authority to require specific tests and information on estrogenic effects for all pesticide chemical residues.

EPA began the task of implementing the requirements of the FQPA by explaining its goals and immediate plans in a letter sent in August 1996, to all current pesticide manufacturers, grower and other pesticide user groups, industry, environmental, consumer, and public interest groups. A second letter, containing more detailed information, was sent on September 1996, to all holders of pesticide registrations. In its September 1996 letter, the Agency stressed that work was continuing on many registration and reregistration activities and that interim decisions were being made. However, to ensure compliance with the new law's provisions to protect against pesticide uses that may pose unacceptable risks to children, additional time was needed to adequately review certain applications, especially food use applications.

Highlights of the FQPA

New Safety Standard for all Pesticide Residues in Food

- 'Reasonable certainty of no harm' from exposure to residues.
- Aggregate assessment of all nonoccupational sources of exposure, including drinking water, residential, and dietary exposure.
- Assessment of cumulative exposure to a pesticide and other substances with common mechanisms of toxicity.

Reduced Risk Pesticides

• Streamlined the registration process of reduced risk pesticides, including new active ingredients, new uses of existing active ingredients already found to be reduced risk, and amendments to all uses deemed to reduce risk.

• Adoption of integrated pest management techniques through research, education, and procurement and regulatory policies.

Tolerance Assessment and Reassessment

- Application of the new safety standard to all tolerances issued after August 3, 1996.
- Reassessment, within 10 years, of all tolerances issued prior to enactment of FQPA to ensure they meet the new safety standard.
- Establishment of tolerances for emergency exemptions issued under Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act.
- Authorization to charge fees for performance of tolerance functions.

Pesticide Reregistration and Periodic Registration Review

- Reauthorization and increase of maintenance fees to complete review of older pesticides first registered prior to November 1984.
- Authorization for a 15 year registration review program.

Right-to-Know

- Development of a simple, understandable consumer brochure on pesticide residues to be distributed to large, retail grocers for public display.
- Publication of an informative statement about the data relating to a tolerance.

Special Protections for Infants and Children

- Consideration of children's special sensitivity and exposure to pesticides.
- Use of an extra tenfold safety factor in addition to the traditional 100-fold safety factor, unless, on the basis of reliable data, a different factor is determined to be safe for children.
- Explicit determination that a tolerance is safe for children.

Endocrine Disruptors Screening and Testing Program

- Development and application of a screening and testing program for chemicals with the potential to disrupt the endocrine process.
- Progress report to Congress by August 2000.

Antimicrobial Pesticides

- Reform of the antimicrobial registration process to meet shortened review period goals while still ensuring efficacy and safety.
- Annual report to two Congressional Committees on progress in meeting the reform goals.

Minor Use Pesticides

- Incentives to maintain existing minor uses and to develop new ones.
- Establishment of minor use offices within EPA and the US Department of Agriculture.

See also: Delaney Clause; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Food, Drug, and Cosmetic Act, US; Pesticides; Risk Assessment, Human Health; Toxic Torts.

Further Reading

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Food Safety and Toxicology

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The Federal Food Safety Program resides in part under the authority of the US Food and Drug Administration. The specific statutory authority is the Federal Food, Drug, and Cosmetic Act (FFDCA). The areas of responsibility include the consideration of the safety or risk of food and color additives, both direct and indirect, and food-borne contaminants, both natural and anthropogenic. The FFDCA proscribes somewhat different standards of safety/risk for intentional food additives that undergo pre-market assessment of safety, specifically, versus those dietary constituents that are found in food as contaminants because they occur naturally or because they arise from anthropogenic sources.

The prototypical safety assessment for food-borne compounds is the acceptable daily intake (ADI) methodology, which was first documented in 1954, and has come to be employed throughout the world. This paradigm has also been codified in the consideration of food (e.g., aspartame) and color additives (e.g., Red Dye No. 2), and pesticides (e.g., atrazine). It is also routinely used in the consideration of incidental food-borne chemical contaminants (e.g., lead), particularly as a tool for screening out trivial incidents of exposure. This procedure specifies that an acceptable dose of a chemical may be calculated with the following equation:

ADI = NOEL/SF

where ADI is the acceptable daily intake, NOEL is the no-observed-effect level, and SF is a safety factor. In subsequent considerations an identical methodology has been implemented where new terms have been substituted for those originally described for the ADI safety assessment methodology. These terms are the reference dose (RfD), minimal risk level (MRL), and tolerable daily intake (TDI) for the ADI. In these methodologies, NOEL has been further defined to be the no-observed-adverse-effect level (NOAEL) and the SF is called an uncertainty factor (UF). These alternative terms have supplanted the ADI in considerations of food-borne contaminants, because unlike food additives, 'acceptance' is not an applicable term for chemical contaminants that do not have a premarket approval evaluation, and therefore these alternative terms were devised. Since contaminants are not deliberately added, and for some are fairly widespread in the environment, including the food chain, it is often much more difficult to achieve population exposures that are below an RfD/MRL/TDI.

As a general rule the NOEL/NOAEL is a dose from a controlled animal experiment where no adverse effect (i.e., an effect not considered harmful) is noted. The experiment does not establish that no effect can possibly occur at that dose under any conditions - it only denotes that none of the effects looked for in the experiment was observed. Since a statistical significance test is typically used to establish whether or not an effect occurred, the NOEL/ NOAEL will tend to diminish as the sensitivity of the measurement or the number of observations increases. Since the burden of proof is on science to show that an effect has occurred, greater uncertainty tends to raise the level of exposure that is deemed acceptable/tolerable. The selection of the effect that is considered adverse is a matter of societal values (i.e., localized, reversible, mild discomfort versus frank, irreversible systemic toxicity). That is, establishing that an effect has occurred is a separate consideration from how much one cares if it will occur or not.

In the original safety assessment paradigm a single safety factor (SF) of 100 was used to derive an ADI from a NOEL. The justification of the SF (also called an uncertainty factor (UF)) was based on scientific considerations as well as a judgment about how to manage the inherent uncertainty associated with the assessment. Scientific issues raised included the notion that humans may be more sensitive to chemicals than rodents used in a laboratory test, and that there may be substantial variability among individuals in a population. The necessity of managing uncertainty was acknowledged through the recognition that it is impossible to demonstrate that no adverse affect could not occur under any circumstances. As the ADI approach evolved, the single safety factor was replaced by two or more safety/uncertainty factors of 10, with each factor applied as a response to a particular scientific issue. For example, an NOEL/NOAEL from an animal experiment is normally divided by a factor of 10 as a matter of policy in response to the supposition that humans may be more sensitive than laboratory animals and by another factor of 10 to account for variability of response in the population of concern. SF/UFs dictate the impact of uncertainty of some quantity on the decision. Although the magnitude of the uncertainty is not precisely described, the general idea is that the greater the uncertainty, the larger the SF/UF needs to be. Thus uncertainty in SF/UFs and uncertainty in the derivation of the NOAEL push in opposite directions. As a matter of practice, the uncertainty underlying an SF/ UF application is not quantified, so that a factor of 10 is almost always employed. Even if the uncertainty were quantified, the magnitude of the uncertainty factor would still depend on some judgment about what degree of risk-adversity is appropriate.

One outcome of the dependence of the NOEL/ NOAEL on the statistical significance test is that it tends to penalize chemicals for which there is more or better data. To remedy this problem, the benchmark dose (BMD) concept was introduced as an alternative approach. The BMD depends on the specification of a low level effect that would typically be unobservable. The endpoint may be the specified percentage (5 or 10%) above background of a population for an endpoint deemed to be adverse. Since the endpoint is defined, determinations for different chemicals and different data sets tend to be more comparable.

Other efforts have endeavored to give SF/UFs a scientific basis by assembling a range of observations that are analogous to the occasions for SF/UF application. As indicated previously while the RfD/MRL/TDI methodology is essentially the same as for the ADI derivation, the endpoint has been recast as a threshold estimate. However, the ADI/MRL/RfD/TDI interpretation of the product of a NOAEL as a threshold presents some difficulties. First, since the number generated is partly a product of the management of the uncertainty, it does not make sense to say that threshold of safety itself is uncertain. Second, there is always some uncertainty about

whether or not there is a threshold, especially when considering subpopulations of individuals whom may already be ill. Perhaps more importantly, even if the NOAEL/UF procedure is considered as a rough estimate of a threshold dose, it only provides information about what may happen at that particular dose; there is no indication about the likelihood or frequency of an adverse effect at higher doses.

An important part of the safety assessment process lies in establishing the impact of scientific uncertainties on the eventual decision. This is typically done by making conservative estimates that deliberately err on the side of safety. Since scientists are more familiar with the scientific issues, the responsibility for this is often delegated to them. There are two potential difficulties with this. The first problem is that the technical person is entrusted with a decision that is partly a social/political one. This is particularly true if there is an interaction between degree of uncertainty and degree of harm. For example, a larger degree of uncertainty may be tolerated when the adverse effect is relatively trivial than when it is severe. However, if the technical person is not aware of all the competing dietary and nondietary risks that impinge on the decision, then they may not be in a position to judge the relative importance of a particular risk. The second problem is that a technical person may dictate the impact of the uncertainty for only part of the problem, without being aware of the other uncertainties involved. There is therefore no way to judge the 'appropriate' degree of conservativeness for the particular problem. Because the safety assessment process may have many steps, the decision process may be fragmented to a degree that no one can judge whether or not a decision is reasonable.

From an administrative standpoint, a great benefit of the safety assessment process is its relative simplicity. It deals with a broad spectrum of dietary public health issues that may be quite complex both socially and scientifically with a few short rules. For small problems, the benefit of either more carefully considering the scientific issues or encouraging widespread participation in the decision process may not justify the effort that must be expended to do so. The efforts to improve the scientific basis of safety assessment often result in the complexity that may result from a risk assessment without the benefit of clarity of purpose. There are occasions where the safety assessment process may prove to be too simple. The bigger the problem is economically and politically, the less comfortable the general public may be with delegating important social/political decisions to scientists. As a result, the safety assessment process is more useful for identifying small problems, than it is in dealing with large problems. For food

additives, the safety assessment process ensures that any risk from a compound that is intentionally added to food is trivial.

As with food additives, the safety assessment process for contaminants is helpful in identifying chemicals that pose a trivial risk. However, the safety assessment process may not be useful for those contaminants where the level of exposure in a population already exceeds what has been identified as the threshold of safety. The level specified by the safety assessment process may be unattainable in all circumstances, and control may best be directed at reducing exposure in general rather than attaining a particular level. Considering the best options available may require better information than the safety assessment process can deliver. In this case a formal risk assessment is required, where the goal is to provide an estimate of the probability of harm for a public health threat. A key difference between safety and quantitative risk assessment (QRA) is that while the former is a decision process in and of itself, a QRA is intended to provide information as part of a larger decision process - this view of risk assessment is illustrated in Figure 1.

Since QRA is an applied analysis, the goal is to describe what is known in response to a particular question. Where matters of degree of exposure and risk are involved, numbers provide more precise descriptions than words, particularly for exposures that exceed the threshold of safety. In QRA, numbers may be used to describe both empirical quantities and, for the purpose of describing uncertainty, degrees of knowledge. As these are two fundamentally different purposes, the benefits of using quantitative tools are somewhat different. For describing empirical quantities (e.g., body weight, heart rate), numbers are useful because they are more accurate, in that they provide a more precise statement of what is known.



Figure 1 A dietary public health decision paradigm.

On the other hand, when describing uncertainties, the primary advantage of quantitative methods is that they are more transparent, thereby allowing more people to participate in the decision process.

QRA can be depicted as a series of four steps, as illustrated in Figure 2. Note that this breakdown of the risk assessment process maintains the relationships to risk management and research that are depicted in Figure 1. The interactions with risk management occur at the beginning (formulating the question) and end (making the decision) of the risk assessment process. The interaction with research occurs primarily at the data generation and modeling steps. The design process involves assembling a chain of quantitative inferences that answer the public health question. The end result is the risk assessment model that explicitly states how a particular adverse effect is causally related to a particular dietary exposure to a contaminant.

Uncertainty

Public health risk assessments used in considerations of food safety are intended to make predictions, particularly quantitative, of plausible negative impacts. Obviously depending on the underlying scientific information, some predictions are more reliable than others, and the extent to which predictions are reliable should be taken into account in the public health decision process. In order to accomplish this, the characterization of risk must contain a statement about the degree of uncertainty.

In discussing uncertainty, it is important to distinguish efforts to quantify uncertainty from the related and oft confused endeavor of theorizing about the nature and extent of frequencies in a population or series. Although uncertainties may often be reasonably calculated or based on frequencies (i.e., statistical uncertainty), frequencies may be of interest on their own (e.g., population variability), while some uncertainties are not frequency based (e.g., model uncertainty). As depicted in Figure 3 the difference between probability and frequency is often a matter of context. The same distribution might be used to describe the frequency of an event when the question is about a population, or serve as the basis

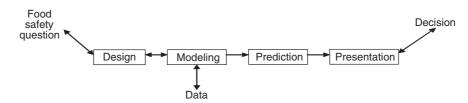


Figure 2 Four steps of quantitative risk assessment.

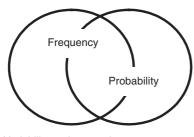


Figure 3 Variability and uncertainty.

for a quantitative probability statement when the question is about an individual. Consider two examples. First, a distribution describing the variability of a scientific instrument over time may be used to describe the uncertainty of individual measurement made by the instrument. Second, a distribution describing body weight could be used to predict the frequency of values expected in a population, or the uncertainty of the value in a particular individual for whom a measurement is unavailable.

Different results may be observed under conditions that are ostensibly the same. To keep track of this variation, we must maintain records or statistics. There are two general strategies that we may employ. First, we may simply store the results. That is, if we have a thousand observations, we can maintain access to all the individual values. The record may then be employed as an empirical distribution function, in which particular percentiles may be identified on demand. Second, we may use a mathematical model to summarize the distribution. There are two very different reasons for doing this. First, a statistical model may be used to provide a concise summary. The facility with which an analyst can store and retrieve data makes this motivation less compelling than it once was. Second, when a sparse data set is not considered representative of a large population, a model may also be used to infer or predict values that are not represented in the data set.

When mathematical models are used to draw inferences, the values of the model parameters may be a source of uncertainty. As the values of model parameters are not measured by direct observation (they are estimated as part of the model fitting process), the uncertainty of a parameter cannot be characterized by simply recording variability in a series of measurements. However, once the best model criterion has been established, the variability associated with a parameter can be linked to the variability in the data. If standard statistical assumptions are employed, the variability of and correlations among the parameters may be calculated directly.

Model uncertainty arises from the availability of multiple equations with different parameters with

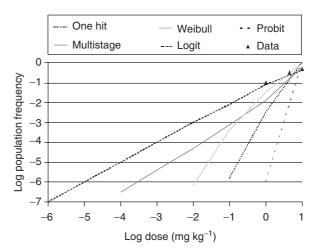


Figure 4 Model uncertainty in cancer risk assessment.

which an inference or prediction might be made. As a practical matter, model uncertainty is often not an important issue. In particular, so long as there are a number of observations to guide the model that come from similar circumstances as the problem that the analysis is concerned with, then any plausible model should yield very similar results. However, when models are used to extrapolate values, model uncertainty can be an important problem. Prime examples are the use of models to predict outcomes at low doses (e.g., Figures 3 and 4), and the use of distributions to estimate the frequency of occurrence of rare events.

Model uncertainties are generally represented by constructs that are commonly referred to as probability trees. The basic idea behind a probability tree is that the sum of the probabilities of all the models under consideration is one. Selecting and assigning probabilities to theories can be resolved by resorting to expert opinion. If a more formal process is necessary or desired – possibly to enable the evaluation to be carried out by a computer, an algorithm may be used. Since presumably the same considerations are involved, the same criteria that are used to select a 'best' model may also be used to weight them.

Frequency Distributions

Frequency distributions may be used to represent or draw inferences about the variation in a particular value among a population or series (i.e., a single set of values such as body weight). There are a number of ways of fitting or estimating the parameters for a frequency distribution. The popularity of the normal distribution may be at least partly attributed to the fact that estimates for the parameters (with a particular set of assumptions about the relationship between the model and the data) can be calculated directly. There are a large number of frequency models to choose from. Although there is some theoretical basis for many of them, the theory is at best only loosely applicable to describing variability in biological populations – the biology is almost certainly more complicated than the model. It is often a good idea to use several models, and to use a probability tree to represent model uncertainty.

Dose-Response Models

Dose-response models describe a cause-effect relationship. There are a wide range of mathematical models that have been used for this purpose. The complexity of a dose-response model can range from a simple one-parameter equation to complex multipharmacokinetic/pharmacodynamic compartment models. Many dose-response models, including most cancer risk assessment models, are population models that predict the frequency of a disease in a population. Such dose-response models typically employ one or more frequency distributions as part of the equation. Dose-response may also operate at an individual level and predict the severity of a health outcome as a function of dose. Particularly complex dose-response models may model both severity of outcome and population variability, and perhaps even recognize the influence of multiple causal factors.

Making Predictions

Once the overall risk assessment model is constructed, it may be used to make predictions. Running a model and collecting the results is often referred to as a simulation. If there are statistical components to the model, the model may be run repeatedly using different random numbers to select values from the statistical distributions each time. This process is known as Monte-Carlo simulation. In public health models, distributions can be used to describe variability in populations or the uncertainty in a value, parameter, or model. Since uncertainty is ever present, the presence of distributions in the model that are intended to describe variability usually results in a two-dimensional (2D) distributional model, where one dimension represents population variability and another represents uncertainty in the outcome. To use the Monte-Carlo method to assimilate the results of the model, a 2D simulation may be used. A program written to accomplish this task will look something like this:

- Begin Uncertainty Loop.
- Randomly Select New Values from Uncertainty Distributions.

- Begin Variability Loop.
- Randomly Select New Values from Variability Distributions.
- Calculate Output using Selected Values.
- Collect Output values into 2D Array.
- Repeat Variability Loop.
- Repeat Uncertainty Loop.

Because the total number of iterations is the product of the variability and uncertainty iterations, a 2D Monte-Carlo procedure is very calculation intensive. Even with longer calculation times, fewer iterations will generally be performed for each dimension than for a 1D simulation – with a concomitant decrease in the reliability of the estimates at the tails. The results of a 2D simulation will be a 2D array, rather than a 1D array. In order to reduce the number of values that need to be stored, it may be desirable to calculate summary statistics for each variability distribution as the simulation progresses.

QRA Output

Whether it is 1D or 2D, Monte-Carlo simulation produces many individual estimates - one per iteration. While these numbers are the end result of the risk assessment, no one can look at all these numbers and make sense of them. Therefore, they need to be sorted and tabulated and summarized. However, deciding how to do this involves considering how the results are going to be used and by whom. To use the classic example, reporting a distribution as a mean entails that the mean is what will be used in making a decision. It is important to inform the decision maker on the summary process so that they understand that a distribution lies behind the single number. The simplest way to do this is to simply display a list of percentiles along with the summary statistics. The results of a 2D simulation will necessarily be more complicated. If simulations are being run for a number of different scenarios (e.g., expected values with and without public health intervention), it is preferable to generate a table of summary statistics such as shown in Table 1.

The units reflect a scale constructed for the assessment, which ranges from 0 to 4. The mean and standard deviation for the uncertainty distribution are given for the mean, median, 95th percentile, and 99th percentile population values.

Although they may allow for quick comparisons, tables inherently compare one value at a time. Graphing or visualization is in some ways a better means of digesting the entire distribution. A 1D simulation will produce a frequency (when simulating variability) distribution or a likelihood distribution

Scenario	Average	Median	95th Percentile	99th Percentile
No tuna	0.447 ± 0.079	0.370±0.143	0.963 ± 0.026	1.362±0.117
2.0 ppm	0.447 ± 0.079	0.370 ± 0.143	0.963 ± 0.026	1.362 ± 0.117
1.0 ppm	0.447 ± 0.079	0.370±0.143	0.962 ± 0.026	1.362 ± 0.117
0.5 ppm	0.447 ± 0.079	0.370 ± 0.143	0.962 ± 0.026	1.362 ± 0.117
0.2 ppm	0.447 ± 0.079	0.369 ± 0.143	0.962 ± 0.027	1.361 ± 0.117
No limit	0.446 ± 0.079	0.368 ± 0.142	0.961 ± 0.026	1.359 ± 0.117

Table 1 Methylmercury exposure scenarios - consumption vs. level limits

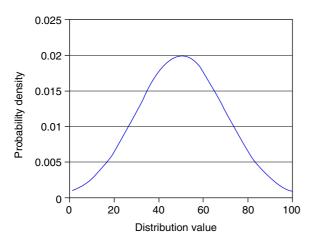


Figure 5 Frequency/likelihood curve: density vs. value.

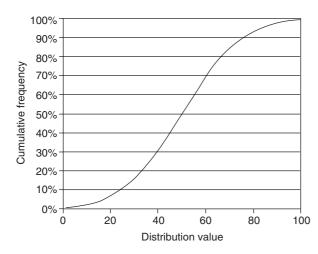


Figure 6 Frequency/likelihood curve: cumulative frequency/percentile vs. value.

(when representing uncertainty). There are two ways of plotting frequency or likelihood curves; the first is to plot density versus value (Figure 5), which emphasizes the values which are the most common or likely and the second is to plot cumulative percentiles versus value (Figure 6), which allows the percentile corresponding to a particular value to be read from the plot.

Two-dimensional results are more difficult to display. Two strategies for adding an extra dimension

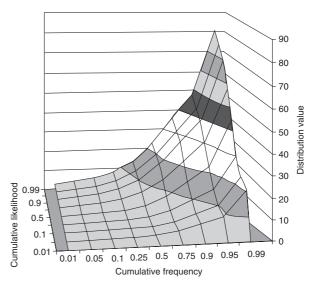


Figure 7 Three-dimensional representation of risk: severity (distribution value), frequency and uncertainty (cumulative like-lihood).

are illustrated below. The first uses 3D perspective to portray the third dimension (**Figure** 7). The second uses shading, where darker hues are used to represent either higher density or more central values (**Figure** 8). This is particularly useful for displaying uncertainty, as the less well-defined parts of a curve appear fuzzy and unclear.

Identifying Data Gaps and Planning Research

Putting a formal QRA together will inevitably raise areas of uncertainty that can be addressed through further research. Since it has identified the issues that are important for the decision, a risk assessment can be useful in planning the research that will have the most impact on future decisions. Research proposals are justified on the basis of an expectation that they will reduce uncertainty. There are three general purposes that additional research may be geared toward:

1. Measurement of values used directly in risk assessment (e.g., in empirical distribution functions).

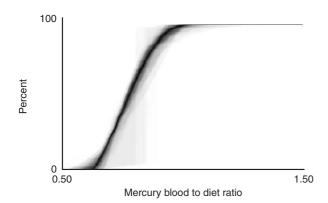


Figure 8 Variability in mercury blood to diet ratios.

- 2. Collection of data that will allow more accurate estimates of model parameters.
- 3. Collection of data that will allow discrimination among models.

Summary

Although contaminants are not intentionally added, the assessment of contaminants typically begins with the application of the same safety assessment process that is used for the evaluation of food additives. In most instances, such an assessment is sufficient in providing the assurance of safety of the potential exposure to many dietary contaminants. There are, however, other instances where the environmental contaminant is so ubiquitous, and therefore difficult to avoid, that a more informative analysis needs to be considered. The output of such an assessment is intended to describe the degree of harm expected in the population and the degree of uncertainty associated with the estimates. Specific areas of uncertainty can also be identified that will inform and suggest meaningful areas of research.

See also: Cumulative Risk Assessment; Monte Carlo Analysis; Safety Pharmacology; Uncertainty Analysis; Uncertainty Factors.

Further Reading

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- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Food Safety and Toxicology.
- http://www.cfsan.fda.gov US Food and Drug Administration, Toxicological Principles for the Safety Assessment of Food Ingredients.
- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Food Safety and Toxicology.
- http://foodsafe.msu.edul National Food Safety and Toxicology Center (at Michigan State University).

Food, Drug, and Cosmetic Act, US

Robert Kapp

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- AGENCY: US Food and Drug Administration (FDA)
- YEAR OF INITIAL ENACTMENT: 1938, followed by numerous amendments

Background Information

The first federal legislation dealing with oversight of food and drug distribution in the United States included the Pure Food and Drugs Act of 1906 (Public Law 59-384) and the Meat Inspection Act, both of which were precipitated, in part, by the Upton Sinclair Novel entitled the *Jungle* (Doubleday, Page & Company, New York, 1906). This book was an exposé of unsanitary conditions in meat packing plants in America. Dr. Harvey W. Wiley of the Bureau of Chemistry, whose laboratory experiments and persistent demands led to this initial legislation regulating the food and drug industries, championed the legislation. This early legislation prohibited interstate commerce in misbranded and adulterated foods, drinks, and drugs and permitted inspections of meat packing facilities. The disclosures of unsanitary meat packing plants' conditions, the use of poisonous dyes and preservatives in foods, and the 'cure-all' claims for ineffective and toxic medicines set the stage for this early food and drug legislation. The Sherley Amendment was passed in 1912 to prohibit labeling of medicines with false therapeutic claims intended to defraud the public. In 1913, the Gould Amendment was passed which required that food packages be "plainly and conspicuously marked on the outside of the package for weight, measure or numerical count." In 1914, the Supreme Court issued its first ruling on food additives (US vs. Lexington Mill and Elevator Company). The ruling found that the mere presence of a toxic ingredient was not sufficient to render the food illegal. The government now had to prove a relationship between the chemical additive and the harm it allegedly caused in humans. The Harrison Narcotic Act was passed that required increased record keeping for physicians and pharmacists who prescribed medications that exceeded the allowable limit of narcotics.

The Food, Drug and Insecticide Administration was renamed the Food and Drug Administration (FDA) in 1931. The FDA recommended a complete revision of the 1906 Food and Drugs Act in 1933 launching a 5 year legislative battle. Finally, Senator Royal S. Copeland of New York sponsored and engineered the passage of the Federal Food, Drug, and Cosmetic Act of 1938 (Public Law 75-717) after several sulfanilamide fatalities.

Overview of the Food, Drug, and Cosmetic Act (FDCA)

This was originally passed in 1938 and modified the 1906 Pure Food and Drugs Act to extended control to cosmetics and therapeutic devices; prohibited false advertising; required informative labeling; authorized definitions and standards for foods and drugs; required that new drugs could not be introduced into interstate commerce without documentation that the drug was safe before marketing – starting a new system of drug regulation; provided that safe tolerances be set for unavoidable poisonous substances; authorized the operation of plants under federal permit; and increased criminal penalties and authorized seizures where necessary.

Numerous amendments have been passed since 1938, including the following critical items:

- 1939 First Food Standard issued for canned tomatoes.
- 1941 Insulin Amendment required the FDA to test and certify purity and potency of insulin.

- 1945 Penicillin Amendment required FDA to test and certify safety and effectiveness of penicillin products.
- 1948 Miller Amendment affirmed that the FDCA applies to goods regulated by the agency that have been transported from one state to another and have reached the consumer.
- 1949 FDA published the 'Black Book' containing industry guidance entitled *Procedures for Appraisal of the Toxicity of Chemicals in Food.*
- 1951 Durham-Humphrey Amendment defined the types of drugs that could only be used with medical supervision and restricted their sale by prescription of a licensed physician.
- 1953 Factory Inspection Amendment required FDA to give manufacturers written reports of conditions noted during inspections.
- 1954 Miller Pesticide Amendment clarified procedures for setting safety limits for pesticides on raw agricultural commodities.
- 1958 Delaney Food Additives Amendment required manufacturers of new food additives to establish safety and prohibited the approval of any food additive shown to induce cancer in animals.
- 1958 Publication of the first list of Substances Generally Recognized as Safe (GRAS). This list contained about 200 substances.
- 1960 Color Additive Amendment required manufacturers to establish safety of color additives in foods, drugs, and cosmetics and prohibited the use of any color additive shown to induce cancer as per the Delaney Amendment noted above.
- 1962 Kefauver–Harris Drug Amendments passed to ensure drug efficacy and greater drug safety in response to the thalidomide birth defects disaster.
- 1965 Drug Abuse Control Amendments are enacted to deal with problems of abuse of depressants, stimulants, and hallucinogens.
- 1968 Animal Drug Amendments consolidates all regulations of new animal drugs under Section 512 making approvals more efficient.
- 1970 FDA requires the first patient package insert with oral contraceptives that provides patients with risk/benefit information.
- 1973 Consumer Product Safety Commission was created and took over administration of the Federal Hazardous Substances Labeling Act duties originally assigned to the FDA.
- 1976 Medical Device Amendments required manufacturers with the FDA and follow quality control procedures with some products needing premarket approval and others needing to meet performance standards before marketing.
- 1976 Vitamins and Minerals Amendments known as the Proxmire Amendments stopped FDA from

establishing standards limiting potency of vitamins and minerals in food supplements or regulate them as drugs based upon potency.

- 1982 FDA publishes the 'Redbook' entitled Toxicological Principles of the Safety Assessment of Direct Food Additives and Color Additives Used in Food. This document was the successor to the 1949 'Black Book'.
- 1984 Fines Enhancement Laws of 1984 and 1987 amended the code to increase penalties for federal expenses to a maximum of \$100 000 for each offense and \$250 000 if the violation is a felony or causes death.
- 1988 Food and Drug Administration Act established FDA as an agency of the Department of Health and Human Services with a Commissioner of Food and Drugs appointed by the President.
- 1995 FDA declares cigarettes to be 'drug delivery devices' and restricts the marketing and sales to reduce smoking by adolescents.
- 1996 Food Quality Protection Act amended the Food, Drug, and Cosmetic Act to eliminate the application of the 1958 Delaney amendment to pesticides.
- 1997 Food and Drug Administration Modernization Act mandated many of the reforms in agency practices since 1938 including accelerated reviews of devices, regulated advertising of unapproved drug and device uses, and regulated health claims for foods.

The FDCA as directed by the FDA has the authority to control the introduction of human and animal drugs, direct and indirect food additives, and the components of cosmetics. Both the safety and efficacy of any new drug must be clearly established before FDA approvals can be obtained to market the drug in the United States. Both animal and human clinical data must be submitted as part of the new drug application. There are no formal testing guidelines upon which industry can rely to perform adequate animal studies; however, there are informal guidance documents that indicate what types of studies should be conducted at various stages of the clinical investigations. The FDA subsequently issued the Good Laboratory Practice Regulations in 1976 that govern the conduct of animal studies.

Industry must also show that any chemical intended to be added to the food (i.e., as a preservative, coloring, or flavoring agent) or any material used in packaging (i.e., plastic wrapping or can coating) that could possibly leach into the food must be documented as safe for its intended use. These study results are submitted to the FDA as part of a food additive petition which the FDA reviews and, if the data sufficiently demonstrate the additive is safe, a regulation is published in the Federal Register that states that the direct and/or indirect additive is approved for a particular purpose. The FDA has published guidelines for the types of toxicity studies that must be conducted to support a food additive petition in the 'Redbook' (see above).

The FDA currently has no specific testing guidelines or requirements for cosmetic formulations for safety or efficacy prior to marketing. The FDCA states that the cosmetics must be free of 'poisonous and deleterious' substances.

See also: Delaney Clause; Food Additives; Food and Drug Administration, US; Generally Recognized as Safe (GRAS); Good Laboratory Practices (GLP); Investigative New Drug Application; Toxic Torts.

Relevant Websites

http://www4.law.cornell.edu – Federal Food, Drugs and Cosmetics Act (from the US Code). http://www.fda.gov – The US Food and Drug Administration.

Foreign Body Response

Shayne C Gad

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The foreign body reaction to biomaterials (such as implanted medical devices) results in foreign body giant cells and the components of granulation tissue. These consist of macrophages, fibroblasts, and capillaries in varying amounts, depending upon the form and topography of the implanted material. Relatively flat and smooth surfaces such as those found on breast prostheses invoke a foreign body reaction of a layer of macrophages one to two cells in thickness. Relatively rough surfaces such as those found on the outer surfaces of expanded poly(tetrafluoroethylene) vascular prostheses have a foreign body reaction of macrophages and foreign body giant cells at the surface. Fabric materials typically generate a surface response of macrophages and foreign body giant cells, with varying degrees of granulation tissue subjacent to the surface response.

Thus, the form and topography of the surface of the biomaterial determines the composition of the foreign body reaction. With biocompatible materials, the composition of the foreign body reaction in the implant site may be controlled by the surface properties of the biomaterial, the form of the implant, and the relationship between the surface area of the biomaterial and the volume. Implants such as fabrics or porous materials will have higher ratios of macrophages and foreign body giant cells in the implant site than smooth surface implants, which will cause fibrosis at the implant site.

The foreign body reaction consisting mainly of macrophages and/or foreign body giant cells may persist at the tissue implant interface for the lifetime of the implant. Generally, fibrosis (i.e., fibrous encapsulation) surrounds the biomaterial or implant with its interfacial foreign body reaction, isolating the implant and foreign body reaction from the local tissue environment. Early in the inflammatory and wound healing response, the macrophages are activated upon adherence to the material surface.

While is it generally considered that the chemical and physical properties of the biomaterial are responsible for macrophage activation, the subsequent events regarding the activity of macrophages at the surface are not clear. Tissue macrophages, derived from circulating blood monocytes, may coalesce to form multinucleated foreign body giant cells containing large numbers of nuclei on the surface of biomaterials. While these foreign body giant cells may persist for the lifetime of the implant, it is not known if they remain activated, releasing their lysosomal constituents, or become quiescent.

The end-stage healing response to biomaterials is generally fibrosis of fibrous encapsulation. However, there may be exceptions to this general statement (e.g., porous materials inoculated with parenchymal cells or porous materials implanted into bone). As previously stated, the tissue response to implants is in part dependent upon the extent of injury or defect created in the implantation procedure.

Repair of implant sites can involve two distinct processes: regeneration, which is the replacement of injured tissue by parenchymal cells of the same type, or replacement by connective tissue that constitutes the fibrous capsule. These processes are generally controlled by either (1) the proliferative capacity of the cells in the tissue or organ receiving the implant and the extent of injury as it relates to the destruction, or (2) persistence of the tissue framework of the implant site.

The regenerative capacity of cells allows them to be classified into three groups: labile, stable (or expanding), and permanent (or static) cells. Labile cells continue to proliferate throughout life; stable cells retain this capacity but do not normally replicate; and permanent cells cannot reproduce themselves after birth. Perfect repair with restitution of normal structure can theoretically only occur in tissues composed of permanent cells and may give rise to fibrosis and fibrous capsule formation with very little restitution of the normal tissue or organ structure. Tissues composed of permanent cells (e.g., nerve cells, skeletal muscle cells, and cardiac muscle cells) most commonly undergo an organization of the inflammatory exudates, leading to fibrosis. Tissues composed of the stable cells (e.g., parenchymal cells of the liver, kidney, and pancreas); mesenchymal cells (e.g., fibroblasts); and vascular endothelial and labile cells (e.g., epithelial cells and lymphoid and hematopoietic cells) may also follow this pathway to fibrosis or may undergo resolution of the inflammatory exudates, leading to restitution of the normal tissue structure.

The condition of the underlying framework or supporting stroma of the parenchymal cells following an injury plays an important role in the restoration of normal tissue structure. Retention of the framework may lead to restitution of the normal tissue structure while destruction of the framework most commonly leads to fibrosis. It is important to consider the species-dependent nature of the regenerative capacity of cells. For example, cells from the same organ or tissue but from different species may exhibit different regenerative capacities and/or connective tissue repair.

Following injury, cells may undergo adaptations of growth and differentiation. Important cellular adaptations are atrophy (decrease in cell size or function), hypertrophy (increase in cell size), hyperplasia (increase in cell number), and metaplasia (change in cell type). Other adaptations include a change by cells from producing one family of proteins to another (phenotypic change), or marked overproduction of protein. This may be the case in cells producing various types of collagens and extracellular matrix proteins in chronic inflammation and fibrosis. Causes of atrophy may include decreased workload (e.g., stress-shielding by implants) and diminished blood supply and inadequate nutrition (e.g., fibrous capsules surrounding implants).

Local and systematic factors may play a role in the wound healing response to biomaterials or implants. Local factors include the site (tissue or organ) of implantation, the adequacy of blood supply, and the potential for infection. Systematic factors may include nutrition, hematologic derangements, glucocortical steroids, and preexisting disease such as atherosclerosis, diabetes, and infection.

Finally, the implantation of biomaterials or medical devices may be best viewed at present from the perspective that the implant provides an impediment of hindrance to appropriate tissue or organ regeneration and healing. This reflects our current inability to control the sequence of events following injury in the implantation procedure, and restitution of normal tissue structures with function is rare. Current research is directed toward developing a better understanding of the modification of the inflammatory response, the stimuli providing for appropriate proliferation of permanent and stable cells, and the appropriate application of growth factors. This may provide keys to the control of inflammation, wound healing, and fibrous manipulation of biomaterials or implanted devices. For example, one area of recent research is understanding the functions of extracellular matrix (ECM) proteins as modulators of cell-matrix interactions. ECM proteins include thrombospondin (TSP)-1, TSP-2, SPARC, tenascin (TN)-C, and osteopontin, and they have been shown to participate in a number of processes related to tissue repair. Specifically, studies in knockout mice have indicated that a deficiency in one or more of these proteins can alter the course of wound healing,

Forensic Toxicology

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Introduction

Forensic toxicology is the branch of science that applies the principles and knowledge of toxicology to issues and problems in the field of law. To achieve this, techniques of analytical chemistry are combined with principles of toxicology to address issues related to the toxic effects of substances on humans that are germane to judicial proceedings. Analytical chemistry deals with the techniques and methods for determining the identity and relative amounts of unknown components in a sample of matter. Toxicology has been defined as the study of poisons. A frequently cited definition of a poison is one provided by the physician/alchemist, Paracelsus (1493-1541). He noted that "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy." For this reason, forensic toxicology involves the use of proper chemical or analytical techniques to identify and characterize any unknown substances in biological systems and examine the adverse effects of these substances on humans. Today, the practice of forensic toxicology encompasses three major subdivisions: postmortem and TSP-1, TSP-2, and SPARC have also been implicated in the foreign body response.

See also: Biocompatibility; Implant Studies.

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toxicology, forensic drug testing, and human performance toxicology. Of these three subdivisions, postmortem toxicology probably bears the closest relationship to the historical perception of forensic toxicology; one that is replete with vivid imagery of intricate homicidal poisonings. In spite of this expanded application of forensic toxicology, the basic responsibility of the forensic toxicologists still remains one of assisting the judicial system in deciding whether a particular substance could have a clinical or toxicological impact on the outcome of a legal matter. To this end, the forensic toxicologist must first establish the presence and exact identity of that chemical substance (prescription or illicit drug or poison) in an individual and establish a relationship between exposure to that chemical and the occurrence of an injurious effect or death.

Forensic drug testing arose primarily as a method of detecting drug use among individuals and as a way to curb or deter contemplated or further use. At the present time, drug use is a major medical and socioeconomic problem worldwide. In the United States, large amounts of financial and human resources are expended to combat the problem. This subdivision of forensic toxicology has wide application in areas such as workplace testing, testing of athletes, compliance with drug-related probation, and screening of new job applicants. Human performance toxicology is concerned with the relationship between the presence of a drug in an individual and changes in behavior or performance on assigned tasks. This subdivision of forensic toxicology forms the scientific cornerstone upon which law enforcement agencies build on to enforce laws dealing with driving under the influence (DUI) of drugs. Frequently, the forensic toxicologist is called upon to analyze biological specimens from individuals suspected of drug use and to connect the results to complex human activities such as driving. For some drugs, notably alcohol, pharmacological data exist on the principal target organs affected and the generally accepted performance impairing effects in humans. The forensic toxicologist can apply some of these generally accepted effects, vis-à-vis the analytical results obtained, to assist law enforcement agencies when human performance toxicology issues arise.

Poisoning is usually an act contrived of evil intentions. The poisoner seeking the death of another individual is often very discreet about the manner in which a poison is introduced into the body of a victim. Great precaution is often taken to conceal the steps or events leading to the completion of this activity. Therefore, establishing that the cause of death of an individual is due to poisoning, whether by accident, suicide, or homicide, is a difficult task requiring the application of a vast amount of knowledge. For the forensic toxicologist, this may involve knowledge of a number of factors and reliance on the expertise of other professionals. The forensic toxicologist must have an inquisitive mind, be familiar with a wide variety of chemicals and poisons, and be conversant with current knowledge regarding the 'drug culture', including the flow, distribution, and patterns of use of illicit drugs. In the normal course of their duties, forensic toxicologists work closely with other professionals. For example, when called upon to assist in establishing the cause of a sudden death, the forensic toxicologist would probably work with a medical examiner, a team of scientists, nurses, police officers, and other law enforcement personnel. The knowledge and information provided by each of these professionals can assist, either collectively or individually, the forensic toxicologist in resolving the mysteries surrounding the death.

Collection of Forensic Specimens

A typical forensic toxicological investigation to determine a cause of death or to determine the presence or absence of a drug or poison in an individual begins necessarily with the collection of an appropriate biological specimen. The biological specimens usually collected from living individuals are peripheral blood and urine. Blood is of particular usefulness to forensic toxicologists because the presence of a drug or poison in blood indicates that absorption has taken place. Additionally, good correlations exist between the blood levels of most drugs and poisons and their pharmacological and/or behavioral effects on humans. Urine drug levels, on the contrary, only indicate that a subject had past exposure to the drug in question without specifying the exact time of exposure or possible physiological effects. Nevertheless, the acquisition of a urine sample from an individual provides a noninvasive method of gaining valuable information regarding the presence or absence of a drug in the body of an individual. Additional advantages in the use of urine as a toxicological specimen include ease of testing, the presence of high concentrations of parent drug and/or metabolites and the relatively low cost of testing. In the United States, for example, the mandatory guidelines for workplace drug testing require the use of urine as the specimen of choice.

Occasionally, gastric contents, saliva, and hair samples may also be collected from living individuals. In one case, an arresting police officer saw a man suspected of possessing and distributing a white powder believed to be cocaine. However, before an arrest could be made the suspect stuffed the white powder and its plastic wrapper into his mouth and swallowed the crucial evidence. Upon finally arresting the man, part of the forensic investigation involved toxicological analysis of available body fluids. In this instance, acquisition of gastric or stomach contents from the suspect is indicated and despite the risks involved, a gastric lavage must be performed. The forensic toxicologist should request and analyze the gastric contents from this living individual as part of the battery of tests to be performed. By analyzing a sample of the stomach contents the toxicologist may be able to answer questions about the chemical identity of the white powder. Finally, a biological specimen that has received prominence in certain parts of the world in forensic toxicology testing is human breath. It is sampled to determine the bodily alcohol content of an individual and the result is compared with the legal definition of intoxication in driving-related offences. It may also be sampled for the presence or absence of inhalants, most of which are volatile organic solvents that are not easily detected in blood.

For deceased subjects, the specimens should be collected before embalming the body. Embalming may cause a dilution, destruction, or false indication of the presence of a drug or chemical in the decedent's body. The specimens collected from a decedent will include samples from a number of body fluids and organs since drugs and other chemicals distribute themselves in body fluids and organs with varying affinities. Whenever possible, a portion of the liver and whole kidneys should be obtained from deceased persons suspected of dying from a drug overdose or chemical toxicity. The liver is the organ primarily responsible for the detoxification of foreign substances such as drugs and chemicals in the body and tends to sequester most of them in high concentrations. The kidneys are the major organs responsible for the excretion of most drugs and poisons, particularly the heavy metals, and are also expected to contain high concentrations of these poisons.

Additionally, depending on the case history presented, the forensic toxicologist should request and analyze samples from specific tissues and organs collected from a decedent. For instance, the eye fluid or vitreous humor may be the preferred specimen to be analyzed for the presence of alcohol in the driver of a fatal accident whose blood or other bodily fluids become contaminated by stomach alcohol as a result of injuries received. The vitreous humor is preferred because it is anatomically well isolated from the stomach and is well protected from microbiological degradation. Certain toxicants, such as the organometallic compounds (methylmercury and trimethyltin), and drugs, such as the anticancer drug doxorubicin, have great affinities for the nervous system, and sampling of brain tissue may be indicated if these toxicants are suspected in a death investigation. For example, if called upon to investigate the cause of a sudden death of a cancer patient being treated with doxorubicin, the toxicologist should also analyze a sample of the brain tissue for the presence and concentration of the drug, in addition to analyzing the usual biological specimens, since the drug is selectively toxic to brain cells and tissues. If doxorubicin is found to be present in high concentrations in the brain, the forensic toxicologist may be able to ascribe the cause of death to doxorubicin toxicity rather than to the effects of cancer, with a high degree of certainty.

The specimens should be collected by qualified personnel and each container into which a specimen is placed must bear a label with the name of the subject, the type of specimen in the container, the date and time of the collection of the specimens, and the signature of the person collecting the specimen. Forms and labels are usually developed to facilitate inventory of the specimens collected and to document the activities at the collection site. Frequently, a police officer is at the scene of the collection of the specimen and that officer should also append his or her signature to the labels and form. The specimens collected should be properly packaged with the proper documentation and case history if available and transferred to a forensic laboratory for analysis. From a legal perspective, the specimens are part of the evidence that can be introduced in legal proceedings, as is any specimen analysis performed by a forensic toxicologist. For this reason, the processes involved in the transfer of the specimens from the collection site to the forensic laboratory must be carefully documented to establish a 'chain of custody'. The chain of custody ensures that only authorized personnel handle the specimens and thereby ensure their integrity.

Analysis of Forensic Specimens

Once in the laboratory, the types of toxicological analyses to be performed on the specimens will depend on several factors. In fact, the types of drugs or poisons to which any population of people are exposed will vary with the prevailing social, political, economic, and religious climate. Sometimes a specimen may arrive in a toxicology laboratory with a request for the analysis of a specific type of drug or poison. Other times, however, the type of analysis to be performed will be determined largely by the case history or other factors associated with the specimen. For example, the analysis performed on biological specimens taken from the driver of a vehicle involved in a traffic accident may involve first and foremost the determination of the presence or absence of alcohol and/or other commonly abused drugs such as marijuana or cocaine, primarily because of the overwhelming involvement of these drugs in traffic fatalities. However, the type of analysis to be performed on a decomposed body will involve searching for drugs and poisons other than alcohol. During decomposition, certain drugs initially present at death may be destroyed and others produced either by virtue of bacterial activity or by changes in the ambient environment.

To the untrained individual, determining both the presence and amount of an unknown drug or poison in an individual is a daunting task. However, systematic and well-standardized methods aimed at detecting the largest possible number of commonly encountered toxic substances have been developed over the years to assist the forensic toxicologist. Generally these methods have focused on the type of biological matrix being analyzed and the chemical class to which a drug or poison belongs. Thus, the method used in analyzing for a poison such as arsenic in hair will be different from that used in analyzing for alcohol in blood.

The type of procedure or instrument used for the detection of a particular drug or analyte will depend on the type of analyte or drug sought. Usually, however, the first line of tests performed includes a protocol of immunoassay screening tests designed to determine the presence or absence of a class or group of drugs. If a positive result is obtained with these tests, a second test using a different procedure based on physicochemical principles different from the first is performed to identify and confirm the particular drug. Some of the instruments that are currently used for the unequivocal identity of most drugs or chemicals are gas chromatography/mass spectrometry, atomic absorption spectrometry, and high-performance liquid chromatography.

A classification scheme that is commonly employed involves placing poisons in the following groups: corrosive agents, gases and volatile agents, metallic poisons, nonvolatile organic agents, and miscellaneous poisons. The corrosive agents include mineral acids and bases. This group of poisons also includes a number of household products formulated with caustic compounds. These poisons can be analyzed using basic chemical and clinical techniques which take advantage of physical properties such as solubility, acidity, or basicity, and observable color changes of the poisons. Gaseous and volatile poisons include several compounds such as acetone, acetaldehyde, carbon monoxide, cyanide, ethanol, methanol, and several other organic solvents. This class of poisons can generally be determined using gas-liquid chromatography techniques. Metallic poisons include arsenic, mercury, lead, and other heavy metals. The method of choice in analyzing for metallic poisons is atomic absorption spectrometry. The nonvolatile organic group contains by far the largest number of prescription and illicit drugs. Drugs such as the antipsychotic agents, antidepressants, amphetamines, central nervous system (CNS) stimulants, and hallucinogens belong to this group. Extraction techniques which take advantage of the acidic, basic, neutral, or amphoteric nature of these drugs are combined with appropriate instrumental methods to analyze for these compounds. Miscellaneous poisons will include agents such as plant and animal toxins and any other chemical substance whose detection from biological specimens will involve the application of some or all of the techniques described, including immunoassay techniques.

Regardless of the type of toxicological analysis eventually performed on a biological specimen, it is essential to follow specific and well-established scientific and good laboratory procedures. Needless to say, the quality of the analytical result is only as good as the quality of the overall process governing the analysis. A clean laboratory environment should be maintained and only chemicals of the highest grade and purity should be used for analysis. Instruments and equipment should be properly calibrated and maintained in proper working condition. Following the analysis, a written report detailing the outcome of the test must be prepared and submitted to the agency or party requesting the analysis. The results should be presented and interpreted in accordance with the definitions and framework established by the legal system of the particular country or state. Usually, this report will conclude any further involvement of the toxicologist in the issues surrounding the specimens or case. For example, a test result of '0.08g of ethanol per 100 ml of blood' that is reported in a case of a motorist suspected of operating his vehicle under the influence of alcohol may be all that is needed by the arresting agency to sustain a charge of 'operating under the influence of alcohol' against the motorist. This is because some jurisdictions adopt per se limits for alcohol in blood and an individual is presumed to be DUI at or above this level.

Interpreting Specimen Analysis

However, instances arise when the forensic toxicologist will have to provide detailed interpretation of the result of the analysis. The relevance and importance of the toxicological analysis to the overall forensic investigation resides in the correct interpretation of the test results. To this end, the forensic toxicologist must bring his or her knowledge of human anatomy, biochemistry, pathology, pharmacology, physiology, general toxicology, and concepts in other basic sciences to bear on the test results. Generally, the objectives of the forensic toxicologists are to answer the following questions. Are drugs or poisons present in the subject? When did exposure to the drug occur? How much did the subject take? Is the drug responsible for a specific type of behavior or prompt a particular type of behavior? In fatal cases, was the drug the cause of death?

Ethanol, the active ingredient in most alcoholic beverages, is perhaps the most widely studied drug in terms of its effects, disposition and fate in humans. It is so common in many societies that it is usually considered a social beverage and its classification as a drug comes as a surprise to some when it is implicated in legal issues. For this reason, forensic alcohol analysis is the most frequently performed analysis in many forensic laboratories. Similarly, it is often in the support of a blood or breath alcohol analysis that the forensic toxicologist comes face to face with the legal community. In the majority of cases involving alcohol and driving, the judiciary is concerned with establishing the following facts: (1) Whether the driver's alcohol result exceeded an established statutory alcohol level of intoxication. (2) Whether the measured alcohol level may have been responsible for the bad driving or accident. (3) Whether at the time of the incident the alcohol level may have been higher or lower than that legally established.

The disposition, fate, and intoxicating effects of alcohol have been extensively studied and well documented. A large body of scientific evidence exists on the correlation between the effects of alcohol on humans and the blood alcohol levels. Alcohol is a CNS depressant and is expected to adversely influence the correct execution of tasks, such as driving an automobile, requiring the proper functioning of the CNS when it is present. The forensic toxicologist may rely on these and other data and factors to provide an interpretation of the results presented; however, bearing in mind that different individuals may respond differently to different levels of alcohol. In some instances, the scientific expectation of the outcome may be different from that which is actually observed. In order to establish whether the alcohol level of a driver may be higher or lower at the time of driving than that measured when an arrest is made, the forensic toxicologist may rely on concepts of retrograde extrapolation. Retrograde extrapolation, or relating back, requires the use of basic principles of the pharmacokinetics of alcohol in humans to arrive at an estimate of the alcohol level of an individual at a time in the past, when knowledge of the alcohol level at the current time exists. The acceptability of this type of calculation has been legally challenged because of different scientific opinions on the subject. However, the forensic toxicologist, using proper assumptions, can still provide an estimated value of the alcohol level and use of the result then goes to the weight of the evidence presented.

The interpretation of postmortem toxicology results probably constitutes the greatest challenge to the forensic toxicologist. This is because of the many factors that affect drugs in postmortem cases. For example, many drugs are unstable in vivo and in vitro and a search for a particular drug during an investigation of a drug-related fatality using information from the case history presented may be futile. An important factor affecting the interpretation of postmortem drug concentration is the phenomenon of postmortem redistribution. Postmortem redistribution is a complex phenomenon that is believed to account for the observation that blood concentrations of a drug may be higher at autopsy than those immediately after death. For this and other reasons, the forensic toxicologist attempting to interpret postmortem toxicology results should do so after gaining a thorough understanding of every available aspect of the circumstances surrounding the case. Mere reliance on tables of therapeutic, toxic, and

lethal concentrations of a drug may result in misinterpretation.

Another complicating factor in the interpretation of postmortem toxicology results is the phenomenon known as postmortem production. This phenomenon is most applicable to blood alcohol levels after a fatality has occurred. Postmortem production can account for measurable blood ethanol levels after a fatality that may have no connection to prior exposure to alcohol. Postmortem ethanol production can result from a number of sources that include the existence of large numbers of appropriate microorganisms in improperly preserved bodies, or from bodies that suffered severe trauma at death. In any case, the forensic toxicologist attempting to offer an interpretation of these results should carefully consider these facts.

Should the presence of the metabolite or biotransformation product of a drug be detected in the body of an accused individual, the forensic toxicologist will have to rely on several factors such as age, weight, gender, and health status of the accused as well as relevant concepts in toxicology to aid in the interpretation of that result. A 45-year-old female was charged with operating her vehicle under the influence of drugs, causing the death of another individual. The accused reportedly failed to obey a traffic signal and drove her vehicle through a red light into an oncoming vehicle, killing its occupant. She fled the scene of the accident but was later apprehended. In her defense, she stated that she was being treated by her physician for depression and had consumed her medication after the accident but prior to her arrest. Toxicological analysis showed the presence of the prescribed drug in addition to two major metabolites of the parent drug. In this case, simply sending out a toxicology report without interpretation or a summary of what the results mean is unlikely to assist the court in adjudicating the matter fairly. Because there is an admission by the defendant in this case to the consumption of the drug, it is in answering the question of whether the presence of the drug may have been responsible for the defendant's behavior at the time of operating her vehicle that the toxicologist's expert knowledge can assist the court in an impartial ruling on this case. If the prescribed medication is a short- or longacting drug, the toxicologist may be able to use information on the relative half-life (time required to break down half of the original dose) of the medication and amount of metabolite detected upon analysis to ascertain the approximate time of drug intake. The toxicologist should be ready to provide this type of information to assist in the resolution of the matter.

The Forensic Toxicologist as Witness

Because he or she may be called as a witness, the forensic toxicologist must be aware of the constraints and demands imposed by the judicial system and ensure that the techniques and procedures used in the laboratory are based on a firm, well-established, and generally accepted scientific foundation as well as satisfying the criteria of admissibility established by the courts. Historically, in the United States, most courts deferred to the landmark ruling of Frye versus United States of America in 1923 as a criterion for judging whether a scientific principle or method is 'generally accepted' by those expected to be familiar with its use. Recently, the 'Frye test' has undergone a change in US federal courts in order to allow the introduction of valid scientific data or information gathered from rapidly advancing scientific techniques or novel tests into evidence. In 1993, the US Supreme Court held that the general acceptance test was too restrictive and incompatible with modern rules of evidence in the case involving Daubert v. Merrell Dow Pharmaceuticals, Inc. The Daubert ruling was later expanded to include expert testimonies from engineers, scientists, and other experts who are not scientists. Although the new rules were initially applied in federal courts, some state courts have adopted the general requirements of the Daubert ruling and expect expert witnesses to satisfy the vital elements of Daubert.

A forensic toxicologist may be subpoenaed as a witness to offer two distinct types of testimony pertaining to the results of an analysis. First, he or she may testify only to the results of the analysis. This type of testimony is known as objective testimony. Objective testimony usually involves furnishing the court with information such as the identification and description of the specimen analyzed, the manner in which the specimen was received in the laboratory, the location of the laboratory, a description of the methods used for analysis, and education and training which qualify the toxicologist to perform the tests used. The second type of testimony offered by the toxicologist is known as expert testimony. For this type of testimony, the toxicologist is presented as an expert witness who can offer interpretive opinions on his or her own results as well as those obtained by other scientists. To be accepted by the court as an expert witness, the forensic toxicologist must be qualified, usually in the presence of a jury. As an expert witness, the forensic toxicologist should be very well prepared in his or her area of expertise and be aware that every trip to court is an engagement in a potentially hostile arena. Opposing counsels will seek to present differing points of view on the same subject and attempt to reach different conclusions through

their own experts. All conclusions must be based on sound scientific knowledge and the information presented to the court with impartiality, integrity, and honesty. It is only by providing the court with scientific knowledge in this manner that the forensic toxicologist truly functions in his or her role as one who applies the principles and knowledge of toxicology to the resolution of problems in the field of law.

Summary

The role of the forensic toxicologist continues to be pivotal to society, particularly when it comes to the administration of justice. Because many drugs, chemicals, or poisons do not always produce characteristic and clinically observable tissue or organ damage to the medical examiner, the contribution by a forensic toxicologist is invaluable if a cause of death is probably due to a drug or poison. The findings from a forensic toxicological analysis can be combined with those from a medical examiner or forensic pathologist to establish the cause or causes of death and the information used in a judicial proceeding. It is a truism that the administration of justice has become a multidisciplinary mosaic of law, science, and modern technology. In this regard, because of its long tradition of evolving according to new legislation and advances in science, toxicology as a whole and forensic toxicology in particular, will continue to offer exceptional value to the truth-seeking goal of the judicial process. As long as society strives to ensure that justice is properly carried out for all and sundry, reliance on the activities of the forensic toxicologist in cases involving human exposure to chemicals and their possible role in causing injury or death will be expected to continue. A society in which the unfortunate reliance on drugs (prescription and illicit) has become a way of life for some is bound to have its share of sudden unexplained deaths, traffic accidents, and other serious outcomes of drug exposure and toxicity. The forensic toxicologist will continue to contribute to the overall knowledge gained about drugs as society continues to grapple with the identity and toxicity of new drugs, particularly 'designer drugs' and their analogs.

See also: Analytical Toxicology; Law and Toxicology; Toxicology, Education and Careers; Toxic Torts.

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Formaldehyde

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 50-00-0
- SYNONYMS: Formalin; Formic aldehyde; Formal; Methaldehyde; Methanal; Methyl aldehyde; Methylene oxide; Oxomethane; Oxymethylene
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aldehyde
- CHEMICAL FORMULA: CH₂O
- CHEMICAL STRUCTURE:

О || НСН

Uses

Formaldehyde is compound that is ubiquitous in the environment. It is a gaseous contaminant of emissions from power plants, manufacturing sites, and automobiles. It is also present in cigarette smoke, produced in wood fires and in photochemical smog. It is a normal metabolic intermediate found in all cells. It has several commercial, industrial, and medical uses. It is utilized in the production of cosmetics, paint pigments, and in the processes responsible for generating wrinkle-free, crease-resistant fabrics. It was used as a component of urea–formaldehyde insulating foam. In medical specialties, it may be used as a disinfectant, antiseptic, deodorant, and a tissue fixative. It is also an embalming agent.

Exposure Routes and Pathways

Occupational and residential exposures to formaldehyde are not uncommon. Due to a very high vapor pressure formaldehyde readily vaporizes and is a gas at room temperature. The most likely route of exposure is by inhalation. Exposure may also occur by ingestion or dermal absorption; however, permeability is low through the skin.

Toxicokinetics

Cells in the lungs rapidly and nearly completely absorb inhaled formaldehyde. At the site of contact it will quickly undergo conversion to formate. The resulting formate is either oxidized to carbon dioxide and exhaled, or excreted as formic acid in the urine. Higher doses may overwhelm the metabolic capabilities of the site of exposure and will result in formaldehyde remaining unchanged. In humans exposed to 1.9 ppm formaldehyde, blood levels of the compound remained stable for 40 min. Under these conditions it may then undergo reactions characteristic to the aldehyde carbonyl group. In addition to oxidation to acid (formic acid), it may be reduced to alcohol (methanol) or undergo conjugation with glutathione (S-acyl glutathione). The conjugation product forms rapidly and is the direct substrate for oxidation reactions that may follow. Unmetabolized formaldehyde may enter the one-carbon pool and subsequently be incorporated into purines, thymidines, and amino acids.

Mechanism of Toxicity

At high exposure levels the carbonyl group of formaldehyde can react with nucleophilic sites on amino acids and DNA. At the site of contact, the primary metabolic products will contribute to the toxicity of formaldehyde. The formation of formic acid generally will cause an acidosis, corrosion of the gastrointestinal tract, and other systematic effects. Formaldehyde also serves as an allergen due to its ability to combine with protein in the epidermis. This combination results in a hapten–protein complex that sensitizes T lymphocytes. Exposure can result in sensitization and contact dermatitis upon subsequent exposures.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} ranges from 0.2 to 0.8 g kg^{-1} , the subcutaneous LD_{50} is 0.42 g kg^{-1} , and the inhalation LC_{50} is 250 ppm per 4h and 815 ppm per 0.5 h.

Human

The toxicity of formaldehyde is related to its metabolic products and, as a result, individual variability in metabolism will determine toxic outcomes. Human ingestion of 118 ml of formaldehyde was fatal in some cases but not others. Systemic acidosis may appear upon ingestion along with corrosion and hemorrhaging of the digestive tract. Allergic sensitization may occur after exposure. This may lead to contact dermatitis after subsequent skin exposure, as well as asthmatic attack upon inhalation exposure. Inhalation may also result in irritation of the respiratory tract and pulmonary edema.

Chronic Toxicity (or Exposure)

Animal

Formaldehyde is an animal carcinogen and a mutagen. High levels (14 ppm) have been associated with nasal cancers in rats and mice.

Human

In humans, long-term, high exposures to formaldehyde are linked to lung cancer.

In Vitro Toxicity Data

Formaldehyde has been shown to significantly inhibit the viability and proliferation of mouse lymphocytes. IC_{50} values in a 3 h exposure ranged from 1.19×10^{-5} to 8.20×10^{-4} moll⁻¹. At 1–3 mmoll⁻ formaldehyde concentrations, dissociated rat thymocytes showed a dose-dependent decrease in cell viability. This may have been associated with an observed reduced cellular content of glutathione or an increased concentration of cellular calcium ion.

Clinical Management

There is no specific antidote for formaldehyde exposure. Contact with skin should be followed by a soap and water wash for a minimum of 15 min. For inhalation exposure, the victim should be moved to fresh air and, if not breathing, given artificial respiration. If breathing difficulties are apparent, oxygen may be administered. After ingestion, decontamination with milk or water should be followed with a bolus of charcoal (1 g kg^{-1}) and a mild saline cathartic. Dialysis may be started if severe acidosis or deteriorating vital signs are apparent. Electrolytes and blood methanol levels should be monitored.

Environmental Fate

Atmospheric formaldehyde is rapidly degraded by photolysis and photooxidation. It will undergo significant biodegradation in the soil or in water and shows no evidence of bioconcentration in several types of fish and shrimp.

Ecotoxicology

Formaldehyde is highly toxic to algae, protozoan, and other unicellular organisms. Fish show slight toxicity (guppies have $TLm = 50-200 \text{ mg l}^{-1}$).

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 0.75 ppm. The short-term exposure limit is 2 ppm.

See also: Pollution, Air Indoor; Respiratory Tract; Sensory Organs; Skin.

Further Reading

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- Heck H, Casanova M, and Starr TB (1990) Formaldehyde toxicity new understanding. *Critical Reviews in Toxicology* 20: 397–426.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Formaldehyde.

Formamide

Gerald L Kennedy

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 75-12-7
- SYNONYMS: Methanamide; Carbamaldehyde; Formimidic acid
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aliphatic amide
- CHEMICAL FORMULA: HCONH₂

Uses

Formamide is a good solvent for proteins and salts owing to its high dielectric constant. Its main applications are as a solvent in the chemical industry, as a softener for paper, as an intermediate for the manufacturing of formic acid and esters and hydrocyanic acid, and as a reaction medium.

Exposure Routes and Pathways

Occupational exposure to formamide may occur through inhalation and dermal contact with this compound at workplaces where formamide is produced or used. Formamide, a physiological product of *N*,*N*-dimethylformamide, was detected in the urine of synthetic leather factory workers. Formamide may be inhaled, swallowed, or absorbed through the skin. The chemical is moderately irritating to the skin and can produce from mild to severe irritation to the eye. In its usual application, inhalation is the most common route of exposure; although dermal contact is always possible.

Toxicokinetics

Formamide is reported to be a minor metabolite from demethylation of the solvent dimethyl formamide. The molecule is relatively difficult to metabolize with the amide group hydrolyzed to a slight extent by liver extracts at pH 7.4. The dog, cat, and rat excrete a large proportion of an oral dose of formamide unchanged in the urine.

Mechanism of Toxicity

The mechanism of toxicity of formamide is not known; the response profile is quite different from the better studied dimethyl derivative.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rodents ranges from 3.2 to 6 g kg⁻¹ with intravenous LD_{50} in rodents ranging from 5.1 in mice to 5.6 in rats. The dermal lethal dose in rabbits is 6 g kg⁻¹ and the inhalation lethal concentration for rats exposed for 6 h is 1500 ppm. Fetal malformations and fetotoxicity were induced when laboratory animals were treated by the oral, dermal, and injection routes during pregnancy.

Human

No reports could be found in the literature concerning the potential acute human health effects of formamide.

Chronic Toxicity (or Exposure)

Animal

Repeated oral administration to rats caused tissue changes at a number of sites including the gastrointestinal tract, spleen, testes, and blood. Multiple dermal or inhalation exposures induced blood effects, changes in organ weights, and testes damage in rats. Chromosome damage was reported in rats treated with formamide.

Human

No reports could be found in the literature concerning the potential human health effects of chronic exposure to formamide.

In Vitro Toxicity Data

No evidence of mutagenicity was seen in Ames bacterial tests.

Clinical Management

Exposed persons should be removed to fresh air and get medical attention as needed for any breathing difficulty. If swallowed, several glasses of water should be given to dilute the chemical and again medical attention is needed if large amounts are ingested. Formamide is moderately irritating to skin and mucous membranes. For skin contact, the exposed area should be washed with soap and water, and medical attention should be sought if irritation develops. For eye contact, the eyes should be flushed with water for at least 15 min by lifting the lower and upper eyelids occasionally and immediate medical attention should be obtained.

Environmental Fate

If released to air, formamide will exist solely as a vapor in the ambient atmosphere. Vapor-phase formamide will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals. The half-life for this reaction in air is estimated to be 8.0 days. If released to soil, formamide is expected to have very high mobility. Volatilization from moist soil surfaces is not expected to be an important fate process. If released into water, formamide is not expected to adsorb to suspended solids and sediment. Several biodegradation screening studies have observed significant biodegradation of formamide, which suggests that biodegradation may be important. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's law constant. An estimated bioconcentration factor of 3 suggests that the potential for bioconcentration in aquatic organisms is low. Hydrolysis is expected to be slow.

Exposure Standards and Guidelines

Occupational Safety and Health Administration (OSHA) standards: Vacated 1989 OSHA permissible exposure limit time-weighted average (TWA) 20 ppm (30 mg m^{-3}) ; short-term exposure limit 30 ppm (45 mg m^{-3}) is still enforced in some states. American Conference of Governmental Industrial Hygienists threshold limit values (TLVs): 8 h TWA 10 ppm, skin. Excursions in worker exposure levels may exceed three times the TLV-TWA for no more than a total of 30 min during a work day, and under no cir-

cumstances should they exceed five times the TLV-TWA, provided that the TLV-TWA is not exceeded.

Atmospheric Standards

There is a standard of performance for equipment leaks of formamide and other volatile organic compounds (VOCs) in the Synthetic Organic Chemical Manufacturing Industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOCs, considering costs, nonair quality health and environmental impact and energy requirements.

Food and Drug Administration Classification

Formamide is an indirect food additive for use only as a component of adhesives.

Further Reading

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- Kennedy GL Jr. (1986) Biological effects of acetamide, formamide, and their monomethyl and dimethyl derivatives. *Critical Reviews in Toxicology* 17: 129.
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- Thiersch JB (1962) Effects of acetamides and formamides on the rat litter *in vitro*. *Journal of Reproduction and Fertility* 4: 219.
- Warheit DB, Kinney LA, Carakostas MC, and Ross PE (1989) Inhalation toxicity study of formamide in rats. *Fundamental and Applied Toxicology* 13: 702.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Formamide.

Formic Acid

Heriberto Robles

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 64-18-6
- SYNONYMS: Aminic acid; Formylic acid; Methanoic acid; Myrmicyl
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organic acid
- CHEMICAL FORMULA: CH₂O₂

Uses

Formic acid is used as an additive to silage to improve its nutritional value. It is also used as an animal feed additive, food preservative, and in flavor enhancer formulations. In the manufacturing industry it is used as an acidulating agent in dyeing and finishing textiles, in leather tanning, wool dyeing, preparation of organic esters, pesticide manufacturing, electroplating, as an antiseptic in wine and beer brewing, and as a coagulating agent for rubber latex. In nature, formic acid is produced by bees, wasps, and ants.

Exposure Routes and Pathways

In industry, exposure to formic acid can occur through the oral, dermal, and inhalation routes. Formic acid can also be produced in the mouth and stomach from ingested formaldehyde. Formic acid can also be produced in the liver and other organs from the metabolism of methanol and formaldehyde. Stings by bees, wasps, and ants may result in the subcutaneous injection of formic acid.

Toxicokinetics

Formic acid can be readily absorbed from the digestive tract and the respiratory system. Systemic absorption produces acidosis, neuropathy, and visual and mental disturbances. Acidosis can also be produced when formic acid is produced by liver aldehyde dehydrogenase from formaldehyde. Formaldehyde in turn can also be produced metabolically by alcohol dehydrogenase from methanol. Formic acid is oxidized to carbon dioxide by the folate-dependent pathway. Some formic acid is excreted unchanged in the urine.

Mechanism of Toxicity

Exposure to formic acid may produce irritation and acid burns at the site of contact. Oral exposure may produce salivation, vomiting (may contain blood), diarrhea, gastritis, and pain. Dermal contact produces dermal irritation, dermatitis, and ulceration of membranes. Accidental splashes in the eyes may result in irritation, lacrimation, and pain. Inhalation of vapors, mists, or aerosols may result in increased nasal discharge, cough, throat discomfort, and pulmonary edema. Systemic absorption of large doses of formic acid may result in damage to the liver, kidneys, and eyes. Acute ingestion of high doses may result in shock, breathing difficulties, circulatory collapse, and death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Formic acid is slightly toxic by the inhalation route. The LC₅₀ for the rat and mouse has been estimated to be 15 g m^{-3} per 15 min and 6.2 g m^{-3} per 15 min, respectively. Rats consuming a diet containing 0.5–1.0% formic acid for 6 weeks experienced a reduced organ and total body weight compared with controls. The same response was noted when rats were given formic acid in their drinking water at a concentration of 0.5–1.0%. The oral LD₅₀ for mice and rats has been reported to be 1076 and 1830 mg kg⁻¹, respectively.

Human

The main target organs for formic acid poisonings are respiratory and gastrointestinal systems as well as the skin, eyes, liver, and kidneys. Direct contact with formic acid may result in severe tissue damage (burns), ulceration, and permanent scarring. Systemic absorption can result in severe acidosis. Signs and symptoms of overexposure include eye irritation, lacrimation, throat irritation, coughing, severe osmolar gap, hypotension, renal failure, apnea, ocular damage, circulatory collapse, and death.

Chronic Toxicity (or Exposure)

Human

It has been reported that chronic intake may result in albuminuria and hematuria.

Clinical Management

If ingested, the formic acid should be diluted with milk or water in alert patients. Careful gastric aspiration with a nasogastric tube may be attempted to limit systemic absorption. The goal of the clinical management is to correct the acidosis. Acidosis may be treated with sodium bicarbonate or by hemodialysis. Immediate hemodialysis may remove formic acid from systemic circulation. Acid–base balance, electrolytes, and kidney function should be monitored closely.

Environmental Fate

Formic acid is found in nature as it is produced by plants, insects, and bacteria. However, it is also used in industry for the manufacture of numerous consumer products. Therefore, the chemical may be released to the environment as a waste product or from unintentional, accidental releases. If released to soil it is expected to biodegrade and has a short half-life. If released to water, it is expected to biodegrade and hence not likely to bioaccumulate in aquatic organisms. If released to air, it is expected to react with hydroxyl radicals contained in water vapor.

Exposure Standards and Guidelines

Food

Formic acid is a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant in accordance with the following conditions: (1) the quantity added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritive, or other technical effect in food, and (2) when intended for use in or on food it is of appropriate food grade and is prepared and handled as a food ingredient.

Formic acid may be safely used as a preservative in hay crop silage in an amount not to exceed 2.25% of the silage on a dry weight basis or 0.45% when direct-cut. The top foot of silage stored should not contain formic acid and silage should not be fed to livestock within 4 weeks of treatment.

Occupational Safety and Health Administration Standards

Permissible exposure limit: Table Z-1 8 h timeweighted average (TWA): 5 ppm (9 mg m⁻³). Threshold limit values: 8 h TWA: 5 ppm; 15 min short term exposure limit: 10 ppm. National Institute for Occupational Safety and Health recommendations: recommended exposure limit: 10 h TWA: 5 ppm (9 mg m^{-3}) .

Further Reading

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- Klaassen CD (ed.) (2001) Casarett & Doull's Toxicology: The Basic Science of Poisons, 6th edn. New York: McGraw-Hill.
- Rossoff IS (2002) *Encyclopedia of Clinical Toxicology*. Boca Raton, FL: The Parthenon Publishing Group.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Formic Acid.

Foxglove

Fermin Barrueto Jr.

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• SYNONYMS: Digitalis purpurea, Digitalis obsura, Digitalis lanata, Digitalis ferruginea – Scrophulariaceae family; Digitalis; Fairy bells; Fairycap; Fairy glove; Fairy thimbles; Rabbit flower; Lady's thimbles; Lion's mouth; Throatwort; Witch's thimbles; Folks glove; Willow-leaves foxglove

Uses

Foxglove contains approximately a dozen different cardioactive steroids, the most prominent being digitoxin. An extract of foxglove has been used medicinally for decades. The pharmaceutical drugs digoxin and digitoxin are contained within foxglove and are used to treat atrial fibrillation with a rapid ventricular response and for congestive heart failure.

Background Information

Foxglove is an erect biennial herb with simple toothed leaves and a central stalk of pink, purple, yellow, or white tubular, bell-shaped pendent flowers ~ 3 in. long and numerous in number. This herb grows up to 4 ft high. Foxglove is a cultivated plant in the western United States and Hawaii; it is native to Britain and Europe. It is found in open land, roadsides, waste areas, and is commonly grown in gardens.

Exposure Routes and Pathways

The most common route of exposure is ingestion of any part of the plant or of any material or drug derived from the plant.

Toxicokinetics

Limited useful data are available for plants containing cardiac glycosides; however, some reference will be made to digitoxin, which is a major constituent of *Digitalis purpurea*.

Digitoxin is readily and completely absorbed (90–100%) from the gastrointestinal tract and the aglycones or genins of the plant-derived cardioactive steroids are even more rapidly absorbed. Peak absorption occurs between 4 and 12 h postingestion, but in overdose, peak amounts and absorption can be delayed. Plasma protein binding is extensive (97%) and there is a relatively small apparent volume of distribution (0.61 kg^{-1}) . The elimination half-life is very long and variable with this mix of cardioactive steroids, ~ 100 h or 4–6 days. It is slowly eliminated, with 60–80% of the dose appearing as metabolites in the urine.

Mechanism of Toxicity

Foxglove contains approximately a dozen cardioactive steroids, most prominently digitoxin. There are also other physiologically active chemical constituents including digoxin, digitonin, digitalin, antirhinic acid, digitalos, and digitoflavone. These toxins are cardiotonic or cardioactive steroids. They consist of a steroid backbone with a five-membered lactone ring attached to it, forming an aglycone or genin. Most plant-derived cardioactive steroids have a five-membered lactone, except red squill; whereas almost all animal-derived steroids have a six-membered lactone. The addition of sugar residues to the aglycone at C3 then creates a cardiac glycoside. The aglycones are derivatives of cyclopentenophenanthrene, and the sugars are unusual methylpentoses. These cardioactive steroids influence the heart in two ways: stronger cardiac contractions due to increased intracellular calcium from inhibiting Na^+, K^+ ATPase and slower contractions through vagal stimulation, prolonging diastole. These cardioactive steroids inhibit the Na⁺,K⁺ ATPase pump mechanism, which disturbs the sodium gradient increasing intracellular sodium. There is a corresponding increase in extracellular potassium and intracellular calcium. This leads to electrical conduction impairment and reduction of the normal resting membrane. With a decreased ability of the myocardial cells to act as pacemakers, the myocardium becomes sensitized. This leads to premature ventricular contractions, ventricular dysrhythmias, and virtually any dysrhythmia except for a supraventricular tachycardia with a rapid ventricular response.

Acute and Short-Term Toxicity (or Exposure)

Human

Foxglove plant poisoning is fairly uncommon but has occurred from unintentionally making a tea out of the plant's leaves and from eating the plant. Some references state that as little as two or three leaves can produce serious toxicity, although no direct observations have been found to support these statements. Foxglove toxicity resembles digoxin or digitoxin toxicity. Gastrointestinal symptoms develop within several hours and include mouth and throat pain, nausea, vomiting, cramping, abdominal pain, and diarrhea. This is followed by central nervous system changes (e.g., severe headache, drowsiness, vision disturbances, confusion, hallucinations, tremors, and convulsions), hyperkalemia, dysrhythmias, and heart block. Yellow haloes are classic symptoms of chronic digitalis leaf poisoning but usually are not seen with pharmaceutical grade digoxin. Severe overdose results in hyperkalemia, myocardial sensitization, and dysrhythmias. There is often marked difficulty in managing these cases due to the sensitized myocardium. Placing an intravenous pacemaker has been associated with an increase in mortality due to the mechanical stimulation of the sensitized myocardium. The myocardium can lose its ability to respond to electrical pacing. Foxglove tea poisoning has been associated with ventricular tachycardia, junctional rhythms, and atrial fibrillation with high-grade atrioventricular block requiring 6 days to revert to normal. One report described a patient suffering from confusion and visual disturbances lasting 5 days and EKG changes for 10 days. Elevated digitoxin and digoxin levels, hyperkalemia, and the electrocardiogram will help confirm toxicity (in the absence of simultaneous consumption of digitalis preparations).

Therapeutic digitoxin levels range from 18 to 22 ng ml^{-1} (23–28.18 nmoll⁻¹). Toxicity in most patients is above 25 ng ml^{-1} (32 nmoll⁻¹). For digoxin, therapeutic serum concentrations range between 1.5 and 2.5 ng ml⁻¹. The serum concentration cannot be used to guide management or determine level of exposure as it is merely a cross-reaction of the mix of cardioactive steroids with the assay and not an accurate quantization. Qualitatively, if there is any level, besides undetectable, it indicates the presence of cardioactive steroid. In combination with the electrocardiogram, hyperkalemia, and gastrointestinal symptoms, these findings are enough to determine toxicity and need for treatment with digoxin-specific Fab.

Chronic Toxicity (or Exposure)

Animal

Signs and symptoms of toxicity in animals and livestock would be similar to those in humans. Livestock symptoms include diarrhea, bloody stools, anorexia, weakness, urge to urinate, and dysrhythmias. Treatment should consist of symptomatic and supportive care.

Human

Chronic toxicity develops following the use of herbal products or teas that contain foxglove. The development of toxicity is unpredictable since the digitalis glycoside content of these products is not standardized. This is now extremely rare.

In Vitro Toxicity Data

Studies of digoxin in an *Escherichia coli* model of genotoxicity were inconclusive.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Activated charcoal should be considered for all patients that present within 1 h of exposure. Treatment with digitoxin- or digoxin-specific Fab should be considered in those with severe symptomatology who fail to respond to conventional therapy, with ECG evidence of digoxin/digitoxin toxicity, or potassium greater than $5.0 \,\mathrm{mEq} \,\mathrm{l}^{-1}$. Digoxin-specific Fab should be administered over any antidysrhythmic as this is a polyvalent antibody that will bind digoxin, digitoxin, and other cardioactive steroids. There is marked variability in response to ingestion of cardiac-glycosidecontaining plant parts (e.g., leaves and stems) depending on various factors (e.g., the season, age of plant, and humidity). Therefore, all patients with a history of ingestion should have decontamination with activated charcoal, a baseline ECG, and electrolyte monitoring and replacement (if necessary) and should be observed for 4-6 h. Patients presenting with any sign of toxicity

should be admitted to a monitored setting for a least a 24 h observation.

See also: Charcoal; Digitalis Glycosides; Red Squill.

Further Reading

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Fragrances and Perfumes

Anne Marie Api and Pertti J Hakkinen

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Introduction

Fragrances, like color and music, can play a major part in feelings about what is experienced in life. Scent is present in flowers, rain, fresh air, the sea, and many other natural sources. Scents can also been created by those skilled in the art of perfumery. Perfumers can create scents that mimic those that occur naturally. The use of fragrances dates back at least 10 000 years to the Egyptians, who used scented oils to clean and soften the skin, and to mask body odor. Today, fragrances enhance the quality of life, and researchers claim that the use of fragrance can have a strong psychological impact, boosting moods, keeping people alert, providing a feeling of calmness, and may even enhance the learning process. Repeated exposure to a scent can trigger a conditioned response, for example, how ones feels when smelling a favorite food.

The terms fragrances and perfumes can generally be used interchangeably as the mixtures of chemicals artfully assembled by a perfumer. Fragrances and perfumes are used in a wide variety of consumer products, ranging from the 'fine fragrances' applied directly to the skin, to perfumes used in creams, lotions, detergents, and many other personal and household products. In addition to enhancing the use of many products, fragrances can be used to neutralize the unpleasant odors associated with many cleaning agents.

The Safe Use of Fragrance Ingredients

Fragrances today are much different than those of olden days. A single fragrance or perfume might contain several hundred or more natural and synthetic materials. The natural ingredients come from materials found in roots, bark, flowers, and from other parts of plants from many regions of the world. They are obtained through physical processes such as distillation or extraction. The synthetic ingredients used in fragrances and perfumes are manufactured through chemical processes. In the past, some important natural ingredients were from animal sources such as whales and the civet cat; however, synthetic replacements made of individual chemicals or mixtures of natural plant and/or synthetic chemicals providing the same or nearly the same smell as the animal-derived chemicals are available to perfumers as replacements. More and more synthetic ingredients are being used today due to conservation and availability issues and time and cost efficiencies.

Because of the widespread use of a large variety of consumer products ranging from perfumes to

cosmetics to skin products and other personal and household hygiene products as well as air fresheners, scented oils, and candles, it is important to examine the dermal effects, systemic toxicity, and environmental consequences of the use and exposure to fragrance materials. It can be argued that the natural and synthetic chemicals used in fragrances and perfumes are among the most studied chemicals used in consumer products. The D-limonene entry in this book provides an example of a fragrance and perfume chemical that has undergone extensive toxicology testing, exposure assessment, and risk assessment. This was done to understand the reasons behind D-limonene's skin sensitization potential (oxidation of D-limonene is necessary for its sensitizing potential). In addition, it was done to understand the human relevance of carcinogenicity observed in male rats (the tumorigenic activity of D-limonene has been concluded to be nonrelevant to humans because of the role that α 2u-globulin plays in the nephrotoxicity and carcinogenicity in male rats).

The fragrance industry has maintained a strict system of safety assurance for more than 30 years. Originally designed to be self-regulatory, it is based primarily on a scientific assessment of potential hazards and exposure to fragrance materials by the scientific staff of the Research Institute for Fragrance Materials, Inc. (RIFM). RIFM, an independent nonprofit institute, was founded for the purpose of obtaining and evaluating safety data on fragrance ingredients. RIFM maintains a comprehensive scientific program that covers human health methodology, environmental methodology, respiratory safety, fragrance allergy, group health and environmental testing, and use level testing.

All of RIFM's test results are evaluated by the RIFM Expert Panel (REXPAN), an independent, international group of scientists. The REXPAN includes experts in dermatology, pharmacokinetics, toxicokinetics, toxicology, pathology, environmental science, and other experts. The experts have no commercial ties to the fragrance industry. Outside experts and RIFM scientists provide consultation as needed. The evaluations by the REXPAN are based on existing data or, where insufficient data exist, on testing performed by RIFM itself. REXPAN's findings and conclusions are published in peer-reviewed journals. RIFM also maintains the most extensive technical database of human health effects, environmental fate, and product regulations on fragrance ingredients available worldwide.

The overall RIFM process results in well-documented conclusions that are provided to the International Fragrance Association (IFRA) as the basis for consideration of a new or existing Fragrance Material Standard, and to industry for appropriate product risk assessment and risk management. The IFRA standards regarding use restrictions (e.g., the maximum allowable level of a particular chemical or related class of chemicals in a type of consumer product) are carefully reviewed by the IFRA Scientific Committee. IFRA then disseminates the information worldwide to its national and regional associations for subsequent distribution to member companies. These determinations reflect industry's stewardship and are interpreted to have the same legal value as other sources of law, such as legislation.

The IFRA Code of Practice and Standards is comprehensive and applies to the manufacture and handling of all fragrance materials for all types of application. It formulates the basic principles, which are the standards of good operating practice by the fragrance industry. Compliance is encouraged throughout the supply chain by a system of notifications and potential enforcement action. When IFRA is informed about a suspected infringement to the code, its staff investigates the facts and contacts the parties in question, as needed. In the few cases that have arisen in the past, a satisfactory resolution has been achieved. The application of IFRA's Code of Practice and Standards does not dispense individual manufacturers from the obligation to comply with all national or international regulations relevant to their operations. IFRA also analyzes and reviews pending regulation applicable to fragrances, as well as legislative trends in related areas such as cosmetics, intellectual property, chemicals, and occupational health and safety.

Both RIFM and IFRA develop and maintain open communication and cooperation with national and international government bodies, concerned members of the medical and scientific community, the industry customers using fragrances, and other stakeholders.

See also: International Fragrance Association (IFRA); Limonene; Research Institute for Fragrance Materials (RIFM).

Further Reading

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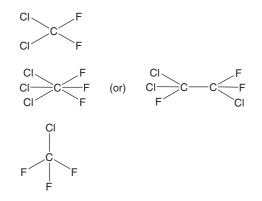
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Freons

Kathryn A Wurzel

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- Preferred Name: Chlorofluorocarbons (CFCs)
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: The CAS registry number is dependent on the specific freon compound
- SYNONYMS: Halons; Halocarbons; Freon 12 (CAS 75-71-8); Freon 13 (CAS 75-72-9); Freon 22 (CAS 75-45-6); Freon 113 (CAS 76-13-1); Freon 114 (CAS 76-14-2); CFC-12; CFC-13; CFC-22; CFC-113; CFC-114
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Halogenated solvent
- CHEMICAL FORMULAS: Freon 12, CCl₂F₂; Freon 113, C₂Cl₃F₃; Freon 22, CHClF₃
- CHEMICAL STRUCTURE:



Uses

Freons are commonly used as refrigerants and propellants in many types of aerosols. They are also used as selective solvents for degreasing.

Exposure Routes and Pathways

Inhalation (pulmonary route) is the main source of toxic exposures to chlorofluorocarbons. Dermal exposure may also occur. Ingestion would be intentional.

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Toxicokinetics

Chlorofluorocarbons have low dermal absorption characteristics. Absorption by the lungs is slow (based on data collected from animal studies). The main factor affecting distribution of chlorofluorocarbons in an individual is the amount of body fat. Chlorofluorocarbons are concentrated in the body fat and are slowly released into the blood at concentrations that do not present a risk of cardiac sensitization.

Loss of CFC-113 from tissues is rapid during the postexposure period with virtually 100% clearance within 24 h of exposure. Freons are eliminated entirely by the respiratory tract. Chlorofluorocarbon compounds partition preferentially into lipidrich tissues and are poorly metabolized. Significant accumulation occurs in brain, liver, and lung tissues compared to blood levels.

Mechanism of Toxicity

The exact mechanism of central nervous system (CNS) depression has not been determined, but the most plausible hypothesis is change in membrane fluidity that alters neural transmission. No significant histological damage has been noted in the brains of animals exposed to lethal concentrations.

Chronic skin irritation occurs as a result of defatting of the skin. Ventricular fibrillation is due to the direct sensitization of the myocardium to endogenous catecholamines.

Acute and Short-Term Toxicity (or Exposure)

Animal

The main effects observed in animals following exposure to chlorofluorocarbons are CNS depression, respiratory tract irritation, rapid breathing, lung congestion, and microscopic liver changes. Cardiac dysrhythmias and mild chemical conjunctivitis have also been noted. Chlorofluorocarbons are skin irritants and slight eye irritants, but are not skin sensitizers in animals.

Chlorofluorocarbons are more acutely toxic to rabbits than to mice via the oral route of exposure. Dogs have demonstrated vomiting, lethargy, nervousness, and tremors following inhalation exposure to chlorofluorocarbons. High-concentration exposures to dogs, monkeys, and rats resulted in cardiac arrhythmias.

Human

Eye and skin irritations have been observed following exposure to chlorofluorocarbons. No corneal opacity has been noted as a result of exposure to chlorofluorocarbons but frostbite of the eyelids may be severe.

Chlorofluorocarbons are very toxic when inhaled at high concentrations and/or for extended periods of time. Lower concentrations or brief periods of exposure result in transient eye, nose, and throat irritations. Temporary CNS depression, dizziness, headache, confusion, and incoordination are associated with exposure to high concentrations (≥ 2500 ppm in air). Gross overexposure may lead to abnormal liver function, refractory ventricular dysrhythmias, and sudden death. Intentional sniffing of aerosols has resulted in sudden death. There is significant individual variability in response to chlorofluorocarbons.

Chlorofluorocarbon compounds are cardiac sensitizing agents. Pulmonary edema, bronchial constriction, and lung irritation may also occur following inhalation exposure to high chlorofluorocarbon concentrations.

Chronic Toxicity (or Exposure)

Animal

A 2 year inhalation exposure to rats did not result in any hepatotoxic effects. Animal testing indicates no carcinogenic, mutagenic, embryotoxic, or reproductive effects. Generally, no changes in offspring were noted when doses of chlorofluorocarbons were below those associated with maternal toxicity (both oral and inhalation exposures).

Human

Chlorofluorocarbons are acutely toxic but do not appear to induce chronic toxicity.

Clinical Management

Oral exposures to liquid chlorofluorocarbons are rare but have resulted in severe frostbite to the upper respiratory system and gastrointestinal tract. Necrosis and perforation of the stomach have been reported. Thus, emesis, activated charcoal, and gastric lavage are not recommended.

Following inhalation overexposure, a calm environment with no physical exertion is imperative to avoid an endogenous adrenaline surge. Exogenous adrenergic drugs should not be used to prevent induction of sensitized myocardial dysrhythmias. Diphenylhydantoin and countershock may be effective for ventricular dysrhythmias.

Exposure of the eyes to liquid chlorofluorocarbons or significant air concentrations should be treated by irrigating the eyes with tepid water for at least 15 min. Cryogenic dermal injuries should be treated by water bath rewarming until vasodilatory flush has returned.

See also: Catecholamines.

Relevant Websites

http://www.inchem.org – Fully Halogenated Chlorofluorocarbons; Environmental health Criteria 113 from the International Programme on Chemical Safety.

Fuel Oils

Richard D Phillips

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- REPRESENTATIVE CHEMICALS: Fuel oils can be grouped into three categories: kerosene, gas oils, and heavy fuel oils.
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS¹: CAS 64747-41-9 (Kerosene); 68335-30-5 (Diesel fuel); 68476-30-2 (No. 2 Heating oil)

¹This is a sampling of CAS numbers for products and refinery streams considered to be in the fuel oil category. For a more complete list, refer to the CONCAWE reports given in the 'Relevant Websites' section.

• SYNONYMS: Jet fuel; Middle distillate; Light cycle oil; Gas oils; Marine diesel fuel; Bunker fuel are a sample of synonyms. See 'Relevant Websites' section for more information.

Uses

Kerosenes are used in blending aviation fuels and can be tailored to meet very strict specifications. Kerosenes are also used as domestic and industrial heating fuels. Kerosenes may be used in a wide range of products including insecticides, solvents and, mould releasing agents. Gas oils are used primarily as fuels in diesel engines and for both industrial and domestic heating. Heavy fuel oils are used in medium to large industrial plants, marine applications, and power stations in combustion equipment such as boilers and furnaces.

Exposure Routes and Pathways

Fuel oils may enter the respiratory system as a vapor or an aerosol. However, the heavier the oil, the lower the vapor pressure and the less likely that one would be exposed to vapor. Exposure to aerosol would be a concern in certain spray applications of kerosene type production. If fuel oils contact skin, this could be a pathway for exposure. Finally, drinking contaminated water or food could result in ingestion of fuel oils.

Toxicokinetics

Fuel oils may be absorbed through the respiratory tract, the gastrointestinal tract and percutaneously. The higher the molecular weight of the hydrocarbons in the oils, the less likely that absorption will occur. Metabolism via oxidation is also likely to occur since the components of fuel oils are hydrocarbons. The degree of metabolism would, of course, be dependent on the nature of the hydrocarbon (i.e., aliphatic, aromatic, etc.), the molecular weight and any other associated molecule (e.g., sulfur, nitrogen). Excretion from the body would also be dependent on the above and could occur via exhalation, in the urine or feces. These molecules are not likely to accumulate in the body.

Mechanism of Toxicity

A primary risk from ingestion of lighter gas oils, such as kerosene, is aspiration during vomiting, which can result in pneumonitis. Like most hydrocarbons, significant exposure may result in central nervous system (CNS) depression. However, fuel oils may have high amounts (10–20%) of three- to seven-ring aromatics which can be carcinogenic.

Acute and Short-Term Toxicity (or Exposure)

Gas oils have a low order of toxicity following acute oral, dermal, or inhalation exposure. Signs observed following high doses are indicative of CNS depression. Skin irritation may result from repeated or prolonged contact with the skin. This, of course, varies depending on the molecular weight (i.e., lower, more irritating), percentage of aromatics and substituted hydrocarbons. Potential for eye irritation varies from slight to mild. Gas oils are typically not skin sensitizers. Ingestion of large quantities of fuel oils can cause vomiting, diarrhea, gastrointestinal disorders, difficulty breathing, and even convulsions, coma, and death.

Heavy fuel oils may contain significant concentrations of hydrogen sulfide (H_2S) , which may accumulate in the headspaces of storage tanks. Hydrogen sulfide is neurotoxic.

Chronic Toxicity (or Exposure)

Animal

Heavy fuel oils may have significant amounts of aromatics produced from cracked petroleum stocks. If so, these products may be carcinogenic and have produced tumors in mice. Gas oils and kerosenes have also produced tumors in mice but generally under conditions of severe skin irritation. If skin contact and particularly skin irritation is minimized, the tumor response does not occur.

A long-term inhalation study was conducted with jet fuel vapor (JP-4). Rats and mice were exposed to 0, 1, or 5 mg l^{-1} for 6 h per day, 5 days per week for 12 months. At exposure termination, 10% of the rats were sacrificed, and the remainder held for an additional 12 month observation period. There were no toxicologically significant signs observed during the exposures. Body weights for male rats were reduced.

The only consistent pathological change was evidence of a mild progressive kidney effect only in the male rats. This is believed to be a rat specific phenomena and not applicable to humans.

Human

Very little is known about the long-term effects of low level exposure to fuel oils. For example, it is not known whether chronic exposure can cause cancer, birth defects, or reproductive impairment in humans.

In Vitro Toxicity Data

A number of *in vitro* genotoxicity studies have been conducted on fuel oils. Typically, kerosenes are negative in these studies. Gas oils range from inactive to weakly active in a number of *in vitro* assays.

Clinical Management

If symptoms arise from inhalation of fuel oil vapor or aerosol, the individual should be removed to fresh air as quickly as possible. If ingestion occurred, the airway should be protected. Vomiting should not be induced.

Where significant skin contact has occurred, the affected areas should be washed thoroughly with water, using soap if available. Contaminated clothing should be removed as soon as possible.

Eyes should be flushed gently with water for up to 10 min.

Environmental Fate

Most hydrocarbon constituents of kerosene will evaporate and be photodegraded in the atmosphere. This will be true for the lower molecular weight components of other fuel oils as well. The higher molecular weight component will persist in the aqueous environment for longer periods and will biodegrade slowly.

Ecotoxicology

The ecotoxicology of fuel oils is complicated by the complexity of the constituents and by the variety of methodologies used in testing. Sublethal effects have been observed in fish in response to kerosene. For example, lesions in tissues – gill, pseudobranch, kidney, and nasal mucosa.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value for kerosene is 200 mg m^{-3} , and for diesel, it is 100 mg m^{-3} . Oil mist from heavier fuel oils would be covered by the oil mist standard, which is currently 5.0 mg m^{-3} but has a notice of intended change to 0.2 mg m^{-3} .

See also: Diesel Fuel; Jet Fuels; Kerosene; Otto Fuel II.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fuel Oils.

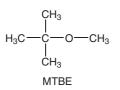
http://www.concawe.be – Conservation of Clean Air and Water in Europe (CONCAWE, 1995, 1996, 1998) Product Dossier nos. 94/106 Kerosene/Jet Fuels, 95/107 Gas Oils, 98/109 Heavy Fuel Oils.

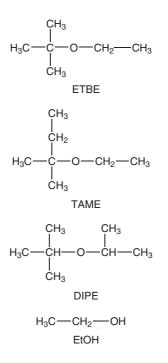
Fuel Oxygenates

Ann de Peyster

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Methyl *t*-butyl ether (MTBE) (CAS 1634-04-4); Ethyl *t*-butyl ether (ETBE) (CAS 637-92-3); *t*-Amyl methyl ether (TAME) (CAS 994-05-8); Diisopropyl ether (DIPE) (CAS 108-20-3); Ethanol (EtOH) (CAS 64-17-5)
- Chemical Formulas: MTBE $(C_5H_{12}O)$; ETBE $(C_6H_{14}O)$; TAME $(C_7H_{16}O)$; DIPE $(C_6H_{14}O)$; EtOH (C_2H_6O)
- CHEMICAL STRUCTURES:





Uses

Oxygenates have been used in gasoline mainly to reduce emissions of certain air pollutants such as benzene and carbon monoxide. Small quantities may be used as laboratory reagents and as pharmaceutical agents. For example, methyl *t*-butyl ether (MTBE) is a versatile organic solvent that has many applications, including clinical use in dissolving gallstones.

Exposure Routes and Pathways

Exposure to MTBE and other fuel oxygenates can occur at any point in their manufacture, distribution, and use. Opportunities for exposure closely parallel other organic hydrocarbons in gasoline. Exposure can occur by inhalation as oxygenates evaporate from fuel during refueling. Fuel oxygenates are highly water-soluble and contamination of drinking water can occur from spills or leaks.

Toxicokinetics

The toxicokinetics of MTBE have been studied in animal models, primarily rodents. The information available to date on the biological fate of ETBE and TAME indicates that their kinetics are expected to be similar to those of MTBE. This has been confirmed experimentally in rodents, in *in vitro* systems using liver microsome homogenates, and also in studies with human volunteers inhaling these fuel oxygenates while at rest or during light exercise.

Rodent and human studies have shown that MTBE is rapidly absorbed following inhalation exposure. In addition, rodent studies have shown rapid distribution of MTBE after oral and intraperitoneal exposure. Dermal absorption occurs more slowly. Evidence supports metabolic transformation of MTBE by P450 enzymes to the parent alcohol, *t*-butyl alcohol (TBA), and formaldehyde in rodents and humans. Further oxidative metabolism of TBA seems to be slow, and glucuronidation is a major competing pathway. Formaldehyde metabolism to formate is very rapid. The toxicokinetic parameters of MTBE and TBA depend on the dose and route of administration although they appear to be linear following inhalation exposures up to 50 ppm.

Inhaled ETBE and TAME are also eliminated by exhalation or through the urine. Major metabolites of ETBE are TBA and acetaldehyde. TAME breakdown in the body is slightly more complex than that of MTBE or ETBE, producing *t*-amyl alcohol as an initial intermediate of metabolism. Compared to MTBE, ETBE uptake in male human volunteers exposed by inhalation is lower and elimination through the respiratory tract is

slightly higher. Like MTBE, ETBE toxicokinetics are linear following exposures up to 50 ppm vapor.

The metabolites of MTBE, ETBE and TAME in humans are qualitatively similar to those observed in rodent studies, and there are no gender differences observed in humans. The half-lives of MTBE, ETBE, and TAME in rodents are less than 1 h, whereas halflives estimated in human studies suggest a more complex picture with multiple half-lives in blood and urine extending to 28 h for ETBE.

DIPE is rapidly absorbed into the blood from the lungs or GI tract. Elimination through lungs is the most probable route of excretion. Information on DIPE is currently limited.

Ethanol is readily absorbed following inhalation or oral exposure. In the bloodstream, ethanol is rapidly distributed into total body water. Ethanol is removed from the blood primarily by metabolism in the liver. Ethanol is metabolized to acetaldehyde and later to acetic acid by two major pathways: acetaldehyde (ADH) and the ethanol-oxidizing system in the endoplasmic reticulum.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat LD_{50} for MTBE is ~ 3.9 g kg⁻¹ body weight and the LC_{50} is between 18 000 and 40 000 ppm. These are extremely high doses compared to levels occurring in the environment. Death is preceded by ocular and mucous membrane irritation, ataxia, and central nervous system depression.

Systemic effects were observed at concentrations of 3000 ppm for MTBE, 4000 ppm for ETBE, and 0.5 g kg^{-1} for TAME when administered to rats for a period of 4 weeks. These effects included increased weights of livers, kidneys, and adrenal glands, and signs of ataxia and hypoactivity. Neurotoxic effects, primarily in the form of activity modification, were observed at 800 ppm MTBE. For ETBE, minor effects were seen only in rats exposed to 4000 ppm for 28 days (the highest concentration tested).

MTBE caused an increase in protein accumulation and cell proliferation in the kidneys of male F344 rats after inhalation exposure of 3000 or 8000 ppm; however, this increase was not accompanied by an increase in the level of α 2u-globulin. MTBE administered by gavage also caused an increase in hyaline droplet formation in the kidneys of male Sprague– Dawley rats at doses of 0.44 and 1.75 g kg⁻¹. Similar effects were observed in males after a 14 day exposure to 1.4 g kg⁻¹ MTBE. The male kidney appears to be the primary target of MTBE. No histopathologic changes were noted in the kidneys after exposure to ETBE or TAME, but no detailed analyses of protein changes were conducted.

In rats, MTBE did not seem to be a strong sensory irritant, in terms of inhibiting respiratory function. Such inhibition was noted after exposure to 8000 ppm, but not at lower concentrations. On the basis of this information and the Alarie model, a level for sensory irritation in humans was estimated to be 140 ppm MTBE. Consistent with the study in rats showing that MTBE alone is a weak respiratory irritant, the controlled human exposure studies failed to document significant sensory irritation – either subjective or objective – from MTBE alone.

A 28 day subchronic toxicity study of TAME administered by oral gavage was conducted in Sprague–Dawley rats. Dose levels were 0, 125, 500, or 1000 mg kg^{-1} body weight. Mean body weights were lower in male rats given 1000 mg kg^{-1} , and dose-related increases in adrenal gland and kidney weight were measured in males. There were no treatment-related histopathologic changes.

A 4 week inhalation study was conducted with ETBE in Sprague–Dawley rats. Exposures were at 500, 2000, or 4000 ppm ETBE vapor for 6 h day⁻¹, 5 days week⁻¹. There was a statistically significant increase in white blood cells at 2000 and 4000 ppm. In addition, kidney weights were increased in male rats at 4000 ppm; however, there was no histologic evidence of kidney damage.

In teratology studies of MTBE, maternal effects were observed in rats exposed to 3000 or 8000 ppm (but not 400 ppm), and in mice and rabbits exposed to 4000 or 8000 ppm MTBE (but not 1000 ppm). Pregnant rats exposed to 4000 or 8000 ppm MTBE showed a reduced number of viable fetal implantations. Small but statistically significant decreases were observed in the viability of offspring from pregnant rats exposed to 1300 or 3400 ppm MTBE when compared with control animals.

Rat pups exposed *in utero* to 8000 ppm MTBE and, to a lesser extent, those exposed to 3000 ppm, had statistically significant decreases in body weight during lactation compared with control pups and pups exposed *in utero* to 400 ppm. Effects on the central nervous system consisting of hypoactivity, ataxia, and loss of startle reflex were seen in adult rats exposed *in utero* to 3000 or 8000 ppm MTBE. No malformations were reported in the fetuses examined in the three studies in rats described above. Increased frequencies of skeletal malformations were found in fetuses from mice exposed to 4000 or 8000 ppm MTBE. When administered to mice at lower concentrations (1000 ppm), MTBE was neither teratogenic nor toxic to the mother or fetuses.

ETBE has been studied in subchronic inhalation experiments using Fischer 344 rats and CD-1 mice exposed to 0, 500, 1750, or 5000 ppm for 13-14 weeks. Although transient ataxia (uncoordinated gait) was a common observation in rats at the higher concentrations, no lasting neurotoxicity was reported. Few major changes in standard clinical pathology were noted in rats or mice, although liver weight increases and centrilobular hepatocyte hypertrophy suggested that ETBE is mitogenic. Like MTBE, ETBE produced evidence of a2u-globulin droplet accumulation in the male rat kidneys. An unexpected finding was degenerative changes in testicular seminiferous tubules in male rats (but not male mice) exposed to the 1750 and 5000 ppm concentrations. This had not been reported in similar studies with MTBE.

Inhalation effects of TAME were evaluated in a two-generation reproductive toxicity study in CD rats exposed to 0, 25, 1500, or 3000 ppm, and in developmental toxicity studies using timed pregnant CD (Sprague–Dawley) rats and CD-1 mice exposed to 0, 250, 1500, or 3500 ppm. In the reproductive toxicity study, exposure of male rats to high concentrations (1500 or 3000 ppm) 5 days a week for 10 weeks resulted in adult systemic toxicity at 1500 and 3000 ppm, some adult reproductive toxicity at 3000 ppm, and offspring toxicity at 1500 and 3000 ppm. Interested readers should consult 'Further Reading' below for details of experimental findings, which included increased percentages of abnormally shaped sperm in the F₀ generation, and other effects suggestive of hormonal imbalance (delayed preputial separation and vaginal opening, shorter anogenital distance) variously in the offspring. In the developmental toxicity studies, rats were exposed for 6 h a day on gestational days 6–19 and mice were exposed on gestational days 6-16. In rats, the NOAEL (noobserved-adverse-effect level) for maternal toxicity was 250 and 1500 ppm was the NOAEL for developmental toxicity. More severe developmental toxicity was observed in mice, in which NOAELs for maternal and developmental toxicity were both 250 ppm.

Diisopropyl ether (DIPE) has also been evaluated in Sprague–Dawley rats in subchronic inhalation studies with doses administered up to 7100 ppm, $6 h day^{-1}$ for 90 days. Male and female rats manifested evidence of liver and kidney hypertrophy but few other significant clinical signs. In a standard developmental toxicity evaluation also using Sprague–Dawley rats, the 6745 ppm inhaled dose administered during gestation days 6–17 produced an increase in rudimentary ribs in the offspring. This effect was considered of uncertain significance. The overall conclusion of these studies was that DIPE has a low order of toxicity.

Human

Ethers are odorous compounds that can be detected in air at very low concentrations. The odor detection thresholds are 53 ppb for MTBE, 13 ppb for ETBE, and 27 ppb for TAME. Adding MTBE at a concentration of 15% by volume dramatically lowered subjects' odor detection thresholds for gasoline by 54–80%, depending on the type of gasoline. For ETBE, the effect was even more dramatic, producing an 89% reduction in gasoline odor threshold.

Overall, the available data suggest that most people do not experience unusual symptoms or significant acute medical consequences when inhaling MTBE in fuel. Because some have reported acute symptoms under some circumstances, different individual sensitivity to MTBE has been suggested.

The consequences of acute ingestion of ethanol are overwhelmingly the result of its action on the central nervous system. They range from the easily recognized signs of intoxication, such as slurred speech and ataxia, to subtle impairment of performance detectable only by neurobehavioral testing. Sensitivity varies enormously among individuals even when body weight is accounted for. On the basis of the available literature, it can be projected that blood levels as low as 10 mg% ($100 \text{ mg}1^{-1}$) may induce performance deficits in some people under some conditions. Functions such as vigilance and attention seem to be those most affected at low levels.

Chronic Toxicity (or Exposure)

Animal

Results of chronic MTBE exposure studies are the most widely available of all studies on the ether-like fuel oxygenates (MTBE, ETBE, TAME, DIPE). Evidence from animal bioassays demonstrates that long-term, high-level exposures to MTBE by either ingestion or inhalation cause cancer in rodents. Inhalation exposure to MTBE produced an increased incidence of renal and testicular tumors in male rats and liver tumors in mice. Oral administration of MTBE produced an increased incidence of lymphomas and leukemias in female rats and testicular tumors in male rats. Chronic exposure to ethanol also produces cancers (e.g., esophageal) in laboratory animals.

Human

There are no adequate epidemiologic studies of chronic exposure to MTBE or to any of these other ether-like fuel oxygenates that are not confounded by other exposures. Extrapolating effects of chronic animal exposure study findings to humans is questionable for a number of reasons. First, the increased tumor incidences were observed at very high exposure levels of MTBE that were toxic and unlikely to be experienced by human populations. Second, each of the animal bioassays had some notable technical limitations. Third, some of the tumors are of questionable relevance to humans because they may be a species-specific phenomenon involving cytotoxic responses to the high-dose exposure regimen. How and why these tumors arose in animal studies (mode of action) is still not completely understood.

It should be noted that evidence derived largely from animal and cell-based studies indicates that both MTBE and ETBE are oxidatively demethylated to produce *t*-butyl alcohol (TBA). MTBE is also metabolized to formaldehyde. Both TBA and formaldehyde are potentially carcinogenic. In all the studies with rodents, MTBE and TBA increased tumor incidence only at very high oral or inhalation exposures, levels that would not be encountered by humans for prolonged periods of time.

Chronic ethanol exposure by ingestion of alcoholic beverages produces widespread toxicity, ranging from liver and nervous system damage to reproductive impairment and birth defects. People are not likely to be exposed orally to ethanol alone when used as a fuel additive, and systemic effects of chronic exposure to ethanol by respiratory and dermal uptake would be negligible.

In Vitro Toxicity Data

MTBE has been tested for genotoxicity with generally negative results. MTBE was neither toxic nor mutagenic in studies using the Salmonella mutation (Ames) assay, nor did exposing primary rat hepatocytes to MTBE in culture cause unscheduled DNA synthesis, an indicator of DNA damage. As part of the inhalation carcinogenicity bioassay study, bone marrow cells from male and female rats were analyzed for chromosomal aberrations. No MTBE-related chromosomal damage or increase in micronuclei was detected in these cells. Oral administration of MTBE (1, 10, 100, or 1000 mg kg^{-1}) to male and female CD-1 mice for 3 weeks did not produce mutations at the *hprt* locus of lymphocytes. In addition, MTBE did not induce sex-linked recessive lethal mutations in the fruit fly (Drosophila melanogaster) when administered at 0.03, 0.15, or 0.30% in food. The only report of MTBE-induced genotoxicity is an abstract indicating that it was mutagenic in an S9-activated mouse lymphoma assay, a response that has been attributed to formaldehyde production.

ETBE was negative in mutagenicity assays in bacteria and Chinese hamster ovary cells, in a bone marrow micronucleus test in mice, and in an *in vitro* chromosome aberration assay.

TAME was reported to be nonmutagenic in five standard *Salmonella* strains, either with or without activation, TAME was negative in the micronucleus assay.

DIPE has been tested for genotoxic activity in bacterial mutation assays, a yeast assay for mitotic gene conversion, and in tests using rat liver and Chinese hamster ovary cells with structural chromosome damaging the end point. Negative responses were observed in the bacterial and yeast assays.

Ethanol is negative in mutagenicity assays in bacteria, mouse sperm, cell transformation in hamster and rat embryo cells, and chromosome aberrations *in vitro*. It produces dominant lethal effects in rats and increases sister chromosome exchange *in vitro* in human and nonhuman lymphocytes.

Clinical Management

Individuals overcome by exposure to fuel oxygenates should be moved to fresh air and administered 100% humidified supplemental oxygen. Skin should be thoroughly washed with water. Following ingestion, the potential risk of aspiration outweighs the benefit of inducing vomiting. Following contamination of the eyes, they should be irrigated with copious amounts of water for at least 15 min.

Environmental Fate

The high vapor pressure of MTBE and other ethers leads to partitioning to the atmosphere when released to surface water or soil surfaces. When introduced into subsurface soils or to groundwater, MTBE may be fairly persistent since volatilization is reduced or prevented. The potential for bioconcentration appears to be very minor.

Although MTBE has a reasonably high water solubility, it shows little tendency to degrade from hydrolysis and very little tendency to adsorb to suspended particulates, soils, or sediments. In groundwater, MTBE can be fairly persistent since it shows limited susceptibility to either anaerobic or aerobic biodegradation.

MTBE is not expected to persist in the atmosphere because of its fairly rapid reactions with hydroxyl radicals. Based on what is known about the similar behavior of MTBE and other ether fuel oxygenates, the environmental fate of others is expected to be similar to that of MTBE. Ethanol volatilizes, photodegrades and biodegrades, and leaches into groundwater. It does not adsorb to sediments or bioaccumlate in fish.

Ecotoxicology

Depending on the time of exposure and endpoint measured, MTBE is acutely toxic to various aquatic organisms at concentrations of 44 to $> 1000 \text{ mg} \text{ l}^{-1}$ in invertebrates, and 388 to $> 3000 \text{ mg} \text{ l}^{-1}$ in vertebrates. In microalgae, decrease in growth was observed at 2400 and $480 \text{ mg} \text{ l}^{-1}$ within 5 days. MTBE does not appear to bioconcentrate in fish and is rapidly excreted or metabolized. The LC₅₀ in *Pi*-*mephales promelas* (fathead minnow) is 91.7 mg l⁻¹ in a 96 h flow-through bioassay. Ecotoxicological effects of other ether fuel oxygenates have not been well studied but are expected to be similar to the effects of MTBE.

Exposure Standards and Guidelines

- MTBE 50 ppm is the 8 h time-weighted average (TWA) American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV); 20–40 µg1⁻¹ is the US Environmental Protection Agency drinking water guideline for MTBE
- ETBE 5 ppm is the 8 h TWA ACGIH TLV
- DIPE 500 ppm Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) time-weighted average concentration (TWAC)
- EtOH 1000 ppm is the OSHA PEL/TWAC

MTBE is currently regarded as an animal carcinogen, but whether these effects seen in laboratory animals have relevance to humans is unknown.

See also: Diesel Fuel; Fuel Oils; Gasoline; Jet Fuels.

Further Reading

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butyl ether on Fischer-344 rats and CD-1 mice. *Toxicological Sciences* 51: 108–118.

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Furan

Heriberto Robles

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 110-00-9
- SYNONYMS: Axole; Divinylene oxide; 1,4-Epoxy-1,3-butadiene; Furfuran; Oxacyclopentadiene; Oxole; Tetrole
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Five-membered aromatic heterocyclics
- Chemical Formula: C₄H₄O

Uses

Furan is a solvent used in the organic synthesis of pyrrole, tetrahydrofuran, and thiophene. It is also used as a solvent for resins and in the production of lacquers, agricultural chemicals, and stabilizers.

Background Information

Furan occurs naturally in oils distilled from rosincontaining pinewood. In addition, many natural foods contain the furan ring structure and substituted furans may be formed through cooking of simple carbohydrates. Furan is also found in tobacco smoke as well as in wood smoke and gas emissions from gasoline and diesel engines. Furan has also been detected in industrial effluents and can be emitted to the air from petroleum refineries and coal mining and gasification plants.

Exposure Routes and Pathways

The most significant route of exposure for furan is via inhalation. However, oral and dermal exposure may also occur in industrial settings.

Relevant Websites

- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fuel Oxygenates.
- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Fuel Oxygenates.

Toxicokinetics

Animal studies suggest that furan is readily absorbed via all routes of exposure. Furan is oxidized by the cytochrome P450 enzymes in the liver and other tissues and may form epoxide intermediates; however, the precise intermediates have not yet been identified. Intermediates of furan may include enedials and dialdehydes, which are metabolized to CO_2 and eliminated through the lungs. Examination of urinary output following furan administration in animals revealed a complex mixture of mercapturate metabolites of furan.

Mechanism of Toxicity

Furan can cause eye, skin, and mucous membrane irritation, a burning sensation, and, in severe cases, corrosion. If inhaled, furan may produce pulmonary edema and bronchiolar necrosis. When absorbed, furan can cause central nervous system (CNS) depression to the point of narcosis and tonic seizures.

Furan is metabolized by the cytochrome P450 enzymes in the liver and other tissues. The furan ring undergoes oxidative cleavage and forms highly reactive furan radical cations or epoxides, which react directly with cellular nucleophiles. These reactive metabolites may react directly with DNA or with cellular proteins to produce disruption of cellular functions and cell death. Chronic cell death and regeneration produced by chronic furan exposure may be a significant factor in the carcinogenicity potential of the chemical. In addition, there is some evidence to suggest that the reactive metabolites of furan may induce mutations in cellular genes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Furan has been shown to be highly toxic by inhalation to laboratory animals. Reported symptoms of inhalation overexposure include increased respiratory rate, drop in blood pressure, convulsions, anesthesia, and death from paralysis of the medulla and asphyxia. Furan also has an irritating and corrosive effect on mucous membranes and the digestive tract.

The inhalation LC_{50} in mice was reported as 120 mg m^{-3} (acute pulmonary edema). The oral LD_{Lo} (lowest published lethal dose) in dogs has been reported as 234 and 140 mg kg⁻¹ (convulsions or effect on seizure threshold). The inhalation TC_{Lo} (lowest published toxic concentration) in rats has been reported as 200 mg m⁻³ per 4 h, 500 mg m⁻³, and 5 mg m⁻³ per 4 h (CNS disturbance).

Human

Inhalation of furan vapors may produce CNS-depressant effects including headache, nausea, dizziness, drowsiness, and confusion. Acute exposure to high concentrations may produce gastrointestinal congestion, liver and kidney damage, low blood pressure, unconsciousness, and/or death from respiratory arrest. Direct contact with vapors or liquid will irritate or burn the skin and eyes. Oral ingestion may be associated with CNS-depressant effects similar to those following inhalation exposure.

Chronic Toxicity (or Exposure)

Animal

Furan has been shown to be cytotoxic in animal models and may cause cell damage in tissues and organs which show cytochrome P450 oxidase activity. Furan was evaluated for carcinogenicity in rats and mice in a 2 year study by the National Toxicology Program. The primary toxic effects of furan were observed in the liver of treated animals. Cholangiocarcinogma of the liver was observed in all dosed rats and was detected as early as 9 months into the study. The incidence of combined liver carcinomas and adenomas in male rats showed a significant dose-related increase. A significant increase in liver adenomas was observed in treated females. The incidence of combined liver adenomas and carcinomas was also increased for all treated groups of mice. On the basis of animal studies, the International Agency for Research on Cancer has classified furan as being possibly carcinogenic to humans.

Several types of nonneoplastic liver lesions were observed in treated mice and rats. Toxic hepatitis of dose-related severity was noted in all dosed rats, in male mice at doses greater than or equal to 8 mg kg^{-1} , and in female mice at doses greater than

or equal to 15 mg kg^{-1} . Doses of 2 and 4 mg kg^{-1} day⁻¹ did not produce hepatitis.

Repeated exposure to furan vapors at various concentrations resulted in histopathological liver changes and structural or functional changes in the trachea or bronchi.

Human

Very little information is available concerning the chronic toxicity of exposure to furan. Industrial use of furan is confined to closed systems due to the volatility of the compound; therefore, the potential for direct exposure to furan is limited. The public exposure to commercial furan is minimal.

In Vitro Toxicity Data

Mutagenicity studies conducted using the Salmonella/microsome incubation assays gave negative results for furan. The same results were observed when Salmonella typhimurium strains were incubated in the presence or absence of rat and hamster liver S-9 fractions.

In vitro treatment of rat or mouse hepatocytes did not induce unscheduled DNA synthesis. However, furan treatment induced gene mutation in mouse lymphoma cells in the absence of metabolic activation. Furthermore, furan was shown to induce sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells both with and without metabolic activation. In a similar study, furan was shown to induce chromosomal aberrations only at comparatively high doses and in the presence of a live activation system.

Clinical Management

If inhalation exposure occurs, the source of conination should be removed or the victim should be moved to fresh air. Artificial respiration should be administered or, if the heart has stopped, cardiopulmonary resuscitation should be administered if necessary. If dermal contact has occurred, contaminated clothing should be removed and the affected area washed with water and soap for at least 5 min or until the chemical is removed. Contaminated eyes should be flushed with lukewarm, gently flowing water for 5 min or until the chemical is removed. If ingestion occurs, vomiting should not be induced. Water should be given to dilute the compound. If vomiting occurs naturally, have the victim lean forward to reduce the risk of aspiration. Aspiration of the compound into the lungs may produce chemical pneumonitis, requiring antibiotic treatment and administration of oxygen and expiratory pressure.

Environmental Fate

Furan may be released to the environment as a waste industrial product or from unintentional, accidental releases. If released to soil it is expected to volatilize. If released to water, furan is not expected to adsorb to suspended particles and sediment and is likely to volatilize to ambient air. Sulfate-reducing bacteria can degrade furan. However, under non-sulfate-reducing conditions, biodegradation in soil and water is expected to be slow. In the air, furan will exist as a vapor and will be subject to degradation by reacting with hydroxyl radicals.

Ecotoxicology

Feeding or abdominal injection of furan to *Drosophila melanogaster* flies did not induce sex-linked recessive lethal mutations.

TC₅₀ for fathead minnow (*Pimephales promelas*) in a flow-through bioassay was reported as follows: 29–31 days, 61 mg l^{-1} per 96 h, at 23.2 °C at a water pH of 8.0 and hardness of 44.5 mg l⁻¹.

Other Hazards

Furan is highly flammable. It can ignite in the presence of flames, heat, and sparks.

Exposure Standards and Guidelines

Given the toxicological properties of furan, the American Industrial Hygiene Association recommends that worker exposure to furan should be minimized to the maximum extent possible.

See also: Tobacco Smoke.

Further Reading

Hamadeh HK, Jayadev S, Gaillard ET, *et al.* (2004) Integration of clinical and gene expression endpoints to explore furan-mediated hepatotoxicity. *Mutation Research* 549(1–2): 169–183.

Relevant Websites

- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Furan.
- http://ehp.niehs.nih.gov Furan (Substance Profile from the National Toxicology Program's Tenth Report on Carcinogens, 2002).

Furfural

Richard A Parent

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 98-01-1
- SYNONYMS: 2-Furancarboxyaldehyde; 2-Furanaldehyde; 2-Formylfuran 2-furfural; Fural; α-Furaldehyde
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Five-membered heterocyclic aldehyde

Uses

Furfural is an insecticide, fungicide, and germicide. It has multiple industrial uses, including production of durite, solvent refining of petroleum oils, acceleration of vulcanization, and a component of rubber cements. It is used in the extractive distillation of butadiene. Furfuraldehyde is a synthesis source for furfuryl alcohol, tetrahydrofurfural alcohol, furan, tetrahydrofuran, poly(oxtetramethylene)glycol, and a variety of synthetic resins. It is also used as a constituent of rubber cements, as a synthetic flavoring agent, and as a solvent for synthetic and natural resins.

Exposure Routes and Pathways

Human toxicity can occur as a result of exposure to furfural by ingestion, inhalation, dermally, or via direct eye contact.

Toxicokinetics

Furfural is rapidly absorbed from the gut and lungs and is also absorbed percutaneously following dermal exposure. Metabolism is rapid. The aldehyde group is oxidized to form furanaryllic acid, the majority of which is conjugated with glycine to produce furoylglycine and furanacryloylglycine. A small portion of the acid is decarboxylated to form CO_2 or condensed with acetic acid.

Furfural has been shown to be distributed throughout the tissues and organs in rats. At 72 h following oral administration, the concentration of labeled furfural and its metabolites was highest in the liver and kidneys and lowest in the brain.

The major route of elimination is via the kidneys as the glycine conjugates, with more than 60% excreted as furoylglycine within the first 24 h after oral administration. Only 3–7% is excreted in the feces and less than 1% is excreted in expired air. The half-life of an oral dose is \sim 2–2.5 h.

Mechanism of Toxicity

Little is known about the mechanism of action in humans, but some information is available from animal studies. Inhalation exposure in rats is associated with pulmonary irritation, parenchymal injury, and the regenerative proliferation of type II pneumocytes. The activity of acid and alkaline phosphatases and glutamic-pyruvate transaminase is increased, whereas that of arginase and succinate dehydrogenase is decreased. The concentration of lactic acid in the lungs is increased. The activity of glutathione-Stransferase is also increased concurrently with a decrease in the concentration of glutathione. After single oral doses given to rats and mice, the effects on the liver are transient and involve scattered eosinophilic globular formation and increased mitotic figures without zonal or massive necrosis. Repeated oral administration results in cirrhotic changes including bridging necrosis and hydropic degeneration of hepatocytes in the parenchyma. Daily intraperitoneal administration to rats caused a time-reversible decrease of respiratory enzyme activity. This results in a degeneration of the processes of reverse resorption in the nephron and may be the cause of the observed functional insufficiency of the kidneys.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal exposures to furfural have demonstrated toxicity via the inhalation and oral routes of administration but little is available relative to its dermal toxicity. Furfural, after ingestion, has been reported to produce central nervous system depression with brain lesions in animals and hepatic cirrhosis in rats. Rabbits exposed by inhalation exhibited hepatic and renal lesions. Inhalation of 260 ppm was fatal to rats but caused no deaths in mice or rabbits. Dogs exposed to 130 ppm furfural in air for 6 h day⁻¹ for 4 weeks suffered liver damage but at 63 ppm no such effects were observed.

Ingested furfural has produced liver cirrhosis in rats. Rabbits exposed to vapors for several hours a day manifested hepatic and renal lesions and modifications in blood picture. Administration of a single lethal dose produced a pronounced inhibitory effect on the medullary vegetative centers and brain nuclei with signs of congestion in liver, kidneys, and brain, with degenerative lesions in liver and kidneys.

The inhalation exposure of cats to very high levels of furfural (2800 ppm) for 30 min resulted in death due to pulmonary edema. The oral LD₅₀ in rats is between 50 and 100 mg kg⁻¹ and the intraperitoneal injection LD₅₀ is between 20 and 50 mg kg^{-1} . The symptoms appear to be weakness, ataxia, and unconsciousness. Inhalation exposures (220 mg m^{-3}) 5 h day⁻¹, six times a week for 12 weeks) caused changes in the hypothalamic-hypophyseal-adrenal system of rats which affected bodily adaptation processes. Decreases in brain noradrenaline levels and adrenal gland adrenaline content occurred. Brain acetylcholinesterase concentration increased and urinary 17-hydroxycorticosteroid and 17-ketosteroid excretion decreased. Subchronic inhalation of furfural by Syrian golden hamsters resulted in atrophy and hyperplasia of olfactory epithelium in the nasal cavity, and cats exposed at higher levels demonstrated pulmonary edema.

Human

Furfural can cause skin sensitization and has been shown to cause irritant dermatitis which may become eczematous. It can be absorbed through the skin or by inhalation and it is an irritant to the eyes, skin, and respiratory system. No throat or eye irritation was noted in humans exposed to 10 ppm for 8h or 20ppm for 4h. No data are available relative to reproductive or developmental effects in humans exposed to furfural. When air concentrations reach from 2 to 14 ppm, headaches, itching of the throat, and red/weeping eyes occurred in exposed humans. If exposures are severe, respiratory tract irritation can progress to acute respiratory distress syndrome, which may be delayed in its onset by up to 72 h. The National Institute for Occupational Safety and Health has indicated that 100 ppm in air is a concentration immediately dangerous to life or health.

Chronic Toxicity (or Exposure)

Animal

A 2 year gavage study in F344 rats and B6C3F1 mice by the National Toxicology Program (NTP) indicated some evidence for carcinogenicity. Two male rats showed rare cholangiocarcinomas and two other animals showed bile duct dysplasia with fibrosis. In addition, there was clear evidence of carcinogenic activity in this mouse strain in that there were increased incidences of hepatocellular adenomas and hepatocellular carcinomas in male mice. Hepatocellular adenomas were also increased in female mice. Also, renal cortical adenomas and carcinomas in male mice and squamous cell papillomas of the forestomach in female mice may also have been related to furfural administration.

Human

There is inadequate evidence of carcinogenicity in humans and, consequently, it is characterized by the International Agency for Research on Cancer as a group 3 carcinogen (not classifiable as to its carcinogenicity to humans) whereas the American Conference of Governmental Industrial Hygienists (ACGIH) classifies it as an A3 carcinogen with confirmed evidence in animals but unknown relevance to man.

Workers chronically exposed to the vapor have reported complaints of headaches, fatigue, itching of the throat, lacrimation, loss of sense of taste, numbness of the tongue, and tremor. Occupational exposure is rare due to its low vapor pressure.

In Vitro Toxicity Data

Furfural is definitely genotoxic to mammalian cells. It has been reported to be mutagenic in Salmonella strains TA100 but not TA98 in the Ames test. Chromatid breaks and chromatid exchanges were increased in cultured Chinese hamster ovary cells and was likewise positive for induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* using an NTP protocol. In an assay involving monitoring sister chromatid exchanges in cultured human lymphocytes, both a positive response and a dose–response relationship was reported.

Clinical Management

Gastric lavage may be appropriate if performed soon after furfural ingestion (within 1 h). Administration of activated charcoal should be considered soon after ingestion. For inhalation exposures, the patient should be moved to clean air. Cough, difficulty breathing, bronchitis, and pneumonitis should be checked for. Oxygen should be administered and ventilation assisted as required. Bronchospasm should be treated with inhaled β -2 agonist and oral or parenteral corticosteroids. Severe irritation of the respiratory tract can progress to pulmonary edema with a delayed onset. For direct eye contact, eyes should be irrigated profusely with water at room temperature for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, a physician should be seen immediately. For dermal exposures, contaminated clothing should be removed and the exposed site washed with mild soap. Development of dermatitis or hypersensitivity should be monitored. The affected person should be treated, as appropriate, with corticosteroids or antihistamines.

Environmental Fate

Furfural is a naturally occurring product as well as being a synthetic one. When released into the environment, it volatilizes readily. In the air, furfural is degraded by reaction with hydroxy radicals produced photolytically. If released into the soil, it is highly mobile and has a great potential to leach into groundwater. Volatilization from the soil is expected to be slow. Although data is limited, furfural is expected to biodegrade readily in the soil under both aerobic and anaerobic conditions. Furfural will not bioconcentrate to any great extent in fish or wildlife.

Other Hazards

The flammability of furfural is comparable to kerosene. Its explosive limit potential ranges from 19.3% to 2.1% in air.

Exposure Standards and Guidelines

- Furfural is considered by the Food and Drug Administration to be an indirect food additive related to its use as a component of adhesives (21 CFR 175.105(4/1/88)).
- Occupational Safety and Health Administration permissible exposure limit, 8 h time-weighted average (TWA): 5 ppm with skin designation.
- ACGIH threshold limit value, 8 h TWA: 2 ppm with skin designation.
- ACGIH excursion limit recommendation: 6 ppm for maximum of 30 min.

ACGIH has listed furfural as a confirmed animal carcinogen with unknown relevance to humans (A3).

Miscellaneous

Furfural is a liquid with a pungent almond-like odor. It is found in food items as a natural product. It is soluble in water to the extent of $86 \text{ g} \text{ l}^{-1}$ at room temperature and the log of its octanol/water partition coefficient is 0.41 indicating that it is more soluble in water than in lipophilic solvents. It has a caramel-like taste and boils at 162°C. As a liquid, its density is ~1.16 at room temperature but its vapor density is ~3.3, causing it to settle in low places during an environmental release. Its odor threshold is somewhere between 0.024 and 20 mg m^{-3} .

See also: Pesticides; Sister Chromatid Exchanges.

Further Reading

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Substances: Furfural. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Standards Development and Technology Transfer, Technical Information Branch.

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- http://toxnet.nlm.nih.gov GENE-TOX Database.
- http://www.osha-slc.gov Occupational Safety and Health Guideline for Furfural, OSHA, US Department of Labor, online.

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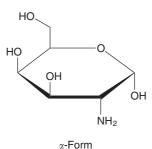
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Galactosamine

Udayan M Apte and Harihara M Mehendale

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 1772-03-8
- SYNONYMS: 2-Amino-2-deoxy-D-galactose; D-Chondrosamine hydrochloride
- CHEMICAL FORMULA: C₆H₁₃NO₅
- CHEMICAL STRUCTURE:



Exposure Routes and Pathways

Oral, inhalation, and ocular are possible routes of exposure.

Mechanism of Toxicity

Galactosamine is a model hepatotoxicant, induces hepatitis characterized by neutrophilic infiltration, and kills the animal by fulminant hepatic failure. Galactosamine induces liver injury by interfering with the uridine pool in the cell, which is essential for RNA and protein synthesis. Galactosamine is metabolized via the Leloir pathway of galactoase metabolism, which leads to generation of uridine derivatives of galactosamine. The two enzymes of the Leloir pathway, galactokinase and uridine diphosphate (UDP)-galactose uridyltranserase, convert galactosamine into galactosamine-1-phosphate and UDP-galactosamine, respectively, due to their low substrate specificity. UDP-galactosamine blocks the final enzyme in Leloir pathway, the UDP-galactose-4' epimerase resulting in accumulation of UDP-galactosamine in the cells. This results in the depletion of uridine triphosphate (UTP), UDP, uridine monophosphate (UMP), and the sugar derivative of uridine such as UDP-glucose and UDP-galactose essential for RNA and protein synthesis. Orotate, a precursor of the hexosamine biosynthesis pathway, has been used as an antidote to galactosamine toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} in mice is 2660 mg kg⁻¹ (intraperitoneal exposure). Galactosamine, an amino derivative of sugar galactose, has been used as a model hepatotoxicant since the first reports of hepatotoxicity induced by galactosamine in late 1970s by Keppler and associates. Galactosamine-induced hepatitis has been a model of choice to study various aspects of liver disease including mechanisms of toxicant-induced apoptosis and necrosis, liver tissue repair, neutrophil infiltration and transmigration, and role of endotoxin or lipopolysaccharide (LPS) in initiating liver injury.

Galactosamine induces depletion of UTP, a form of high-energy molecule needed for conducting highenergy requiring procedures, selectively without interfering with other nucleotides such as ATP, CTP, or GTP. Galactosamine treatment results in depletion of important uridine derivatives such as UDP-glucose and UDP-galactose, which play a critical role in glycogen synthesis and RNA production in the cell. Thus, the main mechanism behind galactosamineinduced hepatotoxicity is depletion of cellular uridine uridyl derivatives.

In the last decade, extensive evidence has gathered suggesting involvement of Kupffer cell-mediated inflammatory reactions in Gln-induced liver injury. It is known that Gln leads to activation of Kupffer cells resulting in secretion of proinflammatory cytokines such as tumor necrosis factor (TNF)- α . Increased levels of TNF- α leads to neutrophilic infiltration and cytotoxicity in the liver. Depletion of both Kupffer cells by glycine and gadolinium chloride and of neutrophils by antineutrophil antibodies protect from galactosamine-induced liver damage. The role of LPS has also been implicated in galactosamine-induced hepatitis. Galactosamine administration has been shown to increase LPS levels in portal circulation, which has been implicated in the neutrophilic infiltration following galactosamine treatment. In recent years, galactosamine in combination with LPS has been extensively used to study inflammatory reactions and neutrophil-mediated liver injury.

Galactosamine and galactosamine + LPS-induced hepatitis are highly reproducible and well-studied models of experimental hepatitis in rodents. A dose of 400–1000 mg kg⁻¹ of Gln alone or a dose of 300– 700 mg kg⁻¹ of Gln in combination with 0.1 mg kg⁻¹ of LPS has been successfully employed to induce experimental hepatitis in rodents. In 1950s and 1960s, the galactosamine + LPS-induced hepatitis was considered to represent the experimental model for viral hepatitis but investigations since then have revealed that it is pathologically different from viral hepatitis. In addition to the experimental hepatitis models, galactosamine (300 mg kg⁻¹) has been used in medium-term cancer bioassays as a promoting agent instead of partial hepatectomy.

Galactosamine is one of the hepatotoxicants known to have age-dependent hepatotoxicity (carbon tetrachloride-chlordecone combination, chloroform, and thioacetamide are other examples). It has been reported that neonatal (5 days old) and old rats (24 months old) are less susceptible to galactosamine-induced liver damage as compared to adult rats (5 months old) due to increased liver tissue repair. It is also known that primary hepatocytes isolated from old rats exhibited higher basal levels of UTP, UDP, and UMP in the liver, which may play a role in the protection observed by the old rats. Although the major organ affected by galactosamine is liver, reports of low to moderate kidney and lung injuries are also available in the literature.

Human

No data are available on human toxicity of galactosamine.

Chronic Toxicity (or Exposure)

Animal

Galactosamine has been classified as 'equivocal tumorigenic agent' by Registry of Toxic Effects of Chemical Substances (RTECS) and is a known mutagen in liver cells at high doses. In one study, chronic exposure to galactosamine produced liver tumors after 77 weeks of exposure. Three to four months of exposure to galactosamine induces chronic progressive hepatitis, while 8–10 months of exposure leads to hepatoma and cholangeofibrosis.

Human

No data are available on chronic human exposure to galactosamine.

See also: Liver.

Further Reading

Decker K and Keppler D (1974) Galactosamine hepatitis: Key role of the nucleotide deficiency period in the pathogenesis of cell injury and cell death. *Reviews of Physiology, Biochemistry and Pharmacology* 71: 78–106.

Gallium

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7440-55-3
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Metals
- CHEMICAL FORMULA: GA³⁺

Uses

Gallium is the 32nd most abundant element and constitutes 0.0005% of the Earth's crust. It is found

most commonly in association with zinc, germanium, and aluminum and is found primarily in the mineral germanite. Gallium(III) is the primary oxidation state for gallium compounds; its chemistry resembles that of aluminum(III). Gallium and gallium compounds have numerous uses in optoelectronics (e.g., LEDs), telecommunication, aerospace, and many commercial and household items, for example, alloys, computers, and DVDs. In addition, gallium is used in special high-temperature thermometers, in place of mercury, and in arc lamps. Medically, gallium alloys are used in dental prostheses, radioactive gallium has been used to locate bone lesions, and nonradioactive gallium has been used as an antitumor agent. Gallium has been used experimentally as an adjunct to *cis*-platinum cancer chemotherapy. It has also been used to treat hypercalcemia and inhibit bone resorption. Gallium maltolate is under development as a treatment for Paget's disease.

Exposure Routes and Pathways

The most common route of intended exposure to gallium is parenteral injection. Occupational exposure to gallium compounds may occur through inhalation of dust (e.g., gallium arsenide) and dermal contact with these compounds. In semiconductor and solar cell production, indoor gallium arsenide emission losses are relatively high. Because of increased use of gallium compounds in new and developing technologies, exposure to gallium compounds is expected to increase in the future. The many uses of gallium may result in the release to the environment through various waste streams. Gallium is present in parts per million (ppm) concentrations in coal, which may be released into the atmosphere. If released to air, gallium compounds are expected to exist solely in the particulate phase in the ambient atmosphere.

Toxicokinetics

Gallium is not readily absorbed orally, but when administered parenterally it is easily taken up by various tissues. Once absorbed, gallium concentrates in the bone (where it appears to be quite stable). Gallium also concentrates in the liver, kidneys, and spleen but is soon released and excreted in the urine.

There is no information on the effects of gallium on enzymes, either as an agonist or as an antagonist.

Mechanism of Toxicity

Gallium can interfere with the structural integrity of transferrin, the ircin-binding protein that transports iron in the serum. Gallium is believed to bind in the protein methionine. In microorganisms like *Escherichia coli*, gallium suppresses the synthesis of low-molecular weight polypeptides. It also concentrates on the surface of the cell envelope.

Acute and Short-Term Toxicity (or Exposure)

Human

When used as a diagnostic tool, gallium has produced dermatitis in some patients. Gallium can also lead to gastrointestinal distress. There are not any reported cases of gallium toxicity from occupational exposure.

Chronic Toxicity (or Exposure)

Animal

Gallium is a mutagen; in different species of animals, gallium was responsible for renal damage, blindness, and paralysis. Gallium concentrates in tumors in experimental animals.

Human

Reported bone marrow depression may result from radioactivity and not from gallium itself. Gallium nitrate has significant activity as a single agent in the treatment of advanced bladder cancer, especially since it causes minimal myelosuppression, and has activity in patients who did not respond to other treatments. Gallium nitrate is also active in combination regimens for advanced bladder cancer. Evaluation of gallium nitrate in combination with newer agents such as the taxanes or gemcitabine may also be warranted given its activity, different mechanism of action, and nonoverlapping toxicity profile.

Clinical Management

Experimentally, deferoxamine mesylate has been effective in treating gallium toxicity. Ethylenediaminetetraacetic acid and *meso-2*,3-dimercaptosuccinic acid have not been effective.

Environmental Fate

Gallium compounds cannot be oxidized and atmospheric transformations would not be expected to occur during transport. Particulate-phase gallium will be removed from the atmosphere by wet and dry depositions. Gallium compounds are expected to exist as ions in the environment and therefore volatilization from water surfaces is not expected to be an important fate process.

See also: Food Additives; Food and Drug Administration, US; Food, Drug, and Cosmetic Act US

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Gap Junctional Intercellular Communication in Epigenetic Toxicity

James E Trosko and Randall J Ruch

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Introduction: "How Does a Physical, Chemical, or Biological Agent Induce 'Toxic' Endpoints in Human Beings?"

The words, toxic, toxicity, toxicology, toxins and toxicants, have been defined many different ways. From the perspective of this short review, we have interpreted the science of toxicology as a multi-disciplinary approach to understand how physical agents (radiation, solid particles), natural (toxins) and synthetic chemicals (toxicants) disrupt critical molecular, biochemical, cellular, physiological, behavioral, and ecological processes that are needed to maintain the normal 'health' of a biological system. Those agents and conditions that irreversibly disrupt the normal health of a biological system would be referred to as 'toxic' agents, and the means by which they bring about toxic effects as their 'toxicity' or 'toxic mechanisms'.

In the case of complex biological systems, such as human beings, health depends on an intricate networking at the molecular (DNA or genes), bio-(proteins-enzymes; lipids-membranes; chemical carbohydrates; ions, etc.), cellular (neurons, hepatocytes; red blood cells), tissues/organ (liver, brain, skin), organ system (skeletal; respiratory, nervous), physiological (endocrine, immunological), and subconscious and conscious levels. All of these interact with each other and with physical, chemical and environmental factors (dietary, social, and cultural). A toxic agent, by interacting with a complex biological entity, such as a human being, can disrupt that 'cybernetic, hierarchical system' by interacting at any level in such a manner as to cause either immediate (acute) or long-term (chronic) toxicity. Depending on both the genetic background (genetic predisposition or genetic resistance) and the stage of development of the human being (at conception; embryogenesis, fetal, neonatal, sexual maturation, maturation or geriatric stage), the biological or health consequences of the toxic effects will be very different, even though the underlying toxic mechanism could be identical.

Of course, the situation is more complex than just knowing the genetic and developmental status of the individual exposed to the 'toxic' agent. It is also necessary to know how, at the cell level in tissues and organs of an individual, the toxic agent alters the normal behavior of a cell's repertoire, including its ability to divide, differentiate, die by programmed cell death, adaptively respond if it is highly differentiated, or senesce. At this level, cellular exposure to a toxic agent could lead to (1) no discernable effect; (2) mutation (irreversible alteration of the genetic information); (3) death of the cell by either 'necrosis' or programmed cell death (apoptosis), or (4) altered expression of the genetic information. The terms, mutagenicity or genotoxicity, refer to those toxic processes that can lead to either changes at the individual gene level within a chromosome or changes in chromosome morphology (deletions, chromosome exchanges) or chromosome number (polypoidy, aneuploidy) by either damaging the DNA, altering the replication of DNA, or altering the stability of chromosomes.

The terms, necrosis and apoptosis, refer to those toxic process that lead to the death of cells (cytotoxicity). Death of cells can come via many means – by toxic agents that damage DNA (mutagens) or by toxic agents that damage critical functions or structures in cells that have nothing to do with DNA (enzyme inhibitors; membrane disruptors) or by toxic agents that alter expression of genes that are designed to lead to cell 'suicide' or programmed cell death. Some toxic agents can induce apoptosis at nonnecrotic doses but induce necrosis at higher doses. In other words, since most agents at a high enough concentration can lead to cell death via necrosis, there seems to be a relationship between apoptosis and necrosis based on the concentration of the toxic agent. Again, it is not as simple as just the dose of the toxic agent; the cell type within the tissue can determine the cytotoxic consequence of the exposure. All tissues contain a few pluri-potent stem cells (cells capable of giving rise to many cell types of the tissue/organ), many progenitor cells (cells derived from the pluri-potent stem cell but which start to expand the specialized cell type within the tissue/organ), and terminally differentiated cells (highly specialized, non-dividing cells derived from the progenitor cells). These three classes of cells usually have differential sensitivity to cytotoxic agents, as is seen with the stem cells of the small and large intestine.

The term, 'epigenetic toxicology', was coined to refer to processes that alter gene expression after exposure to toxins or toxicants but do not cause mutagenicity or cytotoxicity. These epigenetic toxicants, by altering gene expression, can disrupt the repertoire of a cell's behavior. Cells have a choice of: (1) staying as is; (2) dividing; (3) differentiating; (4) apoptosing; (5) adaptively responding if terminally differentiated; or (6) senescing. Cells normally make the choice to divide, differentiate, apoptose, adaptively respond or senesce after endogenous (hormones, growth factors) agents trigger intracellular signals within target cells. At the same time these agents alter one of the critical biological structures/ functions of most normal cells within tissues/organs, namely that of gap junctional intercellular communication (GJIC).

By either increasing or decreasing this fundamental biological process, gene expression will be altered so the resting cell can alter its state to proliferate, differentiate, apoptose, adaptively respond, or senesce. This can be an adaptive response such as growth, maturation, or wound healing. On the contrary, it can be a maladaptive response if the modulation of GJIC occurs at inappropriate times or for inappropriate durations. To understand the role of GJIC in the field of toxicology, as this is a rather new concept of toxicity, we review the basic biology of gap junctions in the following sections.

Gap Junctional Intercellular Communication: Regulator of Cellular Homeostasis

Humans are composed of ~ 100 trillion cells, each of which starts out genetically identical but end up unique and organized in subgroups of similar cells (tissues) that perform the functions necessary for the maintenance of the whole organism. The fertilized egg from which each human grows contains a unique set of $\sim 30\ 000$ genes inherited from the parents that provides the foundation on which development and functioning rest. As this egg develops into the complex human organism and this organism matures, cells must respond and adapt to the environment. This requires cells to proliferate, differentiate, repair damage, die, or change in other ways. These response processes must be highly organized for organisms to grow and function properly and for this to happen, the cells that make up that organism must be able to communicate with one another rapidly and effectively. Considering the importance of these processes, it is not too surprising that there is more than one type of cell-cell communication and that the interactions among these types have become increasingly sophisticated over evolutionary time.

There are three different types of cell-cell communication: extracellular, intracellular, and GJIC. Extracellular communication occurs when cells release ions and molecules (growth factors, neurotransmitters, hormones, etc.) into the surrounding medium that are sensed by contiguous cells or into the blood where they are carried throughout the body and sensed by more distant cells. Usually receptors on the cell membranes of cells serve as sensors for these extracellular chemical signals although communication may also occur without a receptor. In such cases, the communication factor can move through the cell membrane into the cytoplasm. The receipt of these molecular signals by a receptor cell activates a cascade of signals inside this cytoplasm causing the cell to modify its activity (adapt), change its function (differentiate), divide, or die. The functioning of this cascade of signals within cells is known as intracellular communication.

Overlying both extracellular and intracellular communication is a third form of cell-cell communication, known as GJIC. This type of communication enables cells to exchange molecular and ionic signals directly through passageways, known as gap junctions, that connect the cytoplasms of contiguous cells. This exchange allows each cell to synchronize its response with that of other adjacent cells.

Figure 1 illustrates the relationships between the three forms of cell–cell communication in a tissue containing a stem cell, progenitor cells coupled by gap junction channels, and a terminally differentiated cell. The stem cell communicates with the progenitor cell through the extracellular substrate triggering intracellular signals inside the latter cell. As the progenitor cell is in surface contact with the stem cell, communication can also occur through cell adhesion molecules. All progenitor cells also communicate intracellular signals to their sister progenitor cells via gap junction intercellular channels. These intracellular signals stimulate the progenitor

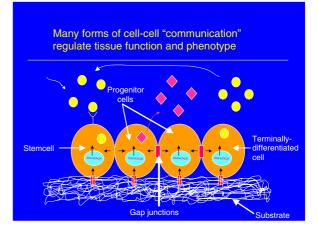


Figure 1 Illustration of how three different kinds of cells within a tissue – the stem cell, progenitor cells coupled by gap junction channels and the terminally differentiated cell – communicate with each other via signals from the substrate, cell-adhesion molecules, soluble secreted factors, and gap junctions.

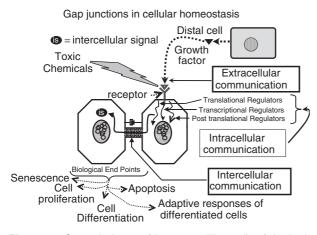


Figure 2 General picture of how 100 trillion cells of the body communicate with each other to maintain homeostatic control of cell functions. (Reproduced from Yamaski H (1990) Gap junction intercellular communication in carcinogenesis. *Carcinogenesis* 11: 1051–1058, with permission from Oxford University Press.)

cells to secrete extracellular communication factors that can enter the bloodstream and communicate with distant cells that have receptors for those specific factors; for example, hormones and growth factors. The progenitor cell can also communicate with its terminally differentiated daughter cell via gap junctions. Lastly, the terminally differentiated cell, by receiving signals through both the extracellular substrate and by the GJIC signal, is stimulated to secrete a signal that is sent back to the original stem cell.

Figure 2 provides a general picture of the interplay among the three types of cell-cell communication. The three cells represent gap junctionally coupled cells in a tissue communicating with a cell in a distant tissue via an extracellular-secreted factor; for example, hormones and growth factor. The extracellular signaling molecules then trigger intracellular signals in the target cell. These intracellular signals can subsequently be transferred to the neighboring cell via gap junctions. Depending on the nature of the extracellular stimulus, the intracellular communications can lead to either an increase or decrease in the cell's ability to transfer the signals via gap junctions to a contiguous cell. These increases and decreases in GJIC lead the cell to proliferate, senesce, terminally differentiate or die of a programmed cell death (apoptosis).

The Structure of Gap Junctions

The cells in a human body are organized into tissues; in turn, groups of tissues make up organs. Cells are attached to each other and to the human skeleton by

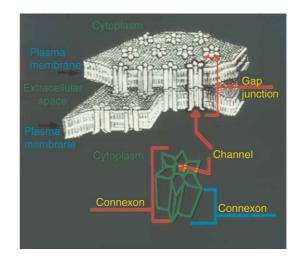


Figure 3 Components of a gap junction linking the cytoplasm of contiguous cells. (Reproduced from Yamaski H (1990) Gap junction intercellular communication in carcinogenesis. *Carcinogenesis* 11: 1051–1058, with permission from Oxford University Press.)

several kinds of cell-cell junctions. These junctions not only hold cells together, but also form barriers to other cells and molecules and, in some cases, act as sensors that detect signals from other parts of the organism. Gap junctions are unique types of cell-cell junctions because they have pores or channels that connect the cytoplasms of contiguous cells; other junctions do not have such channels.

As can be seen in Figure 3, gap junctions are made up of 'hemi-channels' or connexons consisting of six proteins called connexins that are coded by an evolutionarily conserved family of genes. When two hemi-channels or connexins unite across the extracellular space between two neighboring cells, they form a complete channel that allows ions and small molecules to move directly from one cell to the other without having to enter the extracellular space. The connexins on opposite cells are attracted to each other in a poorly understood way and connect or 'dock' tightly together. In most gap junctions, several complete channels cluster in one small region of the cell membrane resulting in a gap junction 'plaque'.

Figure 4 is an electron micrograph depicting a portion of a coupled pair of cells in which a cross-section of a 'plaque' or island of hundreds of coupled connexons aggregate to form a 'gap junction'. Each cell can have varying numbers and sizes of these gap junction plaques, depending on the physiological state of the tissue.

Gap junction channels are very small – approximately 1.5-2 nm in diameter. As illustrated in Figure 3, two connexons and 12 connexins make up one complete gap junctional channel. The human genome contains genes for ~ 20 different kinds of

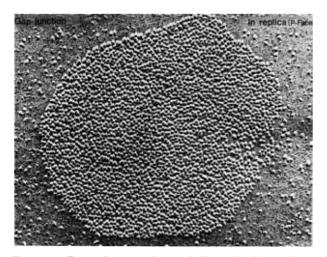


Figure 4 Freeze-fracture micrograph illustrating the gap junction plaque in the membrane between two cells.

connexins so there are many potential combinations of connexins that can make up gap junction channels.

Gap Junctional Intercellular Communication

Once two connexons are docked properly, the complete channel will open and ions and molecules can freely move between contiguous cells. However, gap junction channels allow only very small molecules to pass through. Amino acids, water, simple sugars, and most intracellular signal molecules freely move through gap junction channels but larger molecules, such as proteins and fats, cannot.

This cell-cell movement of ions and molecules through gap junction channels is known as GJIC. It is critical to the health of cells and the entire organism. GIIC helps balance and maintain cellular levels of critical ions and nutrients, helps supply neighboring cells with raw materials needed for synthesis of macromolecules, and helps coordinate cellular functioning. Within most tissues, several gap junction plaques, each composed of hundreds to thousands of channels, connect the cells. In cells that are well connected by gap junctions, ions and molecules move rapidly between cells so that within tissues, groups of cells respond more like a functional unit than individual cells. The GJIC provides for the rapid, direct flow of molecular and ionic information between cells that serves to synchronize the functions of multicellular tissues.

GJIC has been implicated in all of the five basic fates that cells may have: remaining quiescent, proliferating, differentiating, committing programmed cell death (apoptosis), and, in already differentiated

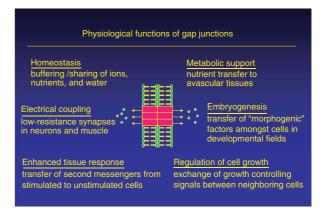


Figure 5 Cellular/tissue functions linked to gap junction function.

cells, adaptively responding. Each of these fates is critical for the development and maintenance of organismal structure and function. Both proliferation and differentiation are needed during growth and repair. Terminally differentiated cells must be able to adapt to changing conditions; for example, insulin cells responding to blood sugar levels. Cells in solid tissues must undergo apoptosis to facilitate tissue modeling or replacement of old cells with new, healthier ones. Lastly, some cells are destined to senesce and become nonproliferating, low activity, end-stage cells. One example of the role of GJIC in determining the fate of cells is the importance of gap junctional communication between sperm cells and Sertoli cells. This communication triggers the differentiation process of the spermatocyte into a mature sperm.

The situation in an organism is even more dynamic than might be suggested by the above summary. Cells uncouple and recouple with each other continuously as part of normal cellular functions. When cells are stimulated to divide by an extracellular growth factor, gap junction channels close. For proliferation to occur, gap junctions must be deactivated so that molecular signals that normally keep the stimulated cell inactive are blocked. Once the cell divides, and there is no longer any growth stimulus, the daughter cells form active gap junctions with contiguous cells.

Figure 5 illustrates a number of the physiological functions that are dependent on GJIC, functions that reflect its role during embryogenesis, development of the fetus and neonate, sexual and adolescent maturation and adult functioning of both electrically coupled tissues; for example, heart, and nonelectrically coupled tissues; for example, liver.

The importance of GJIC in the performance of these physiological functions is a reflection of the impact it has on individual cells. GJIC helps control the rates of cell births and deaths so that tissue size and activity remain constant. It also helps trigger cellular differentiation. The passage of intracellular signals from stimulated to nonstimulated cells increases the overall response of the tissue to a stimulus. In addition, gap junctions serve as electrical pathways to connect muscle cells; for example, heart, uterus and digestive tract and nervous tissue. Thus, GJIC is critical for normal heartbeat, birth, digestion, brain activity, and other functions. Gap junctions are also important pathways for the interchange of nutrients and waste products in tissues that are not well supplied with blood vessels, such as the lens of the eye and hardened bone.

Epigenetic Toxicity as a Result of Inappropriate Modulation of Gap Junction Function

As might be expected from the range of activities and functions associated with gap junctions, abnormalities in GJIC can lead to a variety of adverse effects. When an individual inherits a mutated form of a connexin gene, the cells that express that gene will be unable to form normal gap junction channels. Human diseases that are linked to the inheritance of a mutant connexin gene include forms of nerve degeneration, deafness, diabetes, cataracts, cancer, birth defects and skin disorders. Adverse effects can also result if exposures to environmental chemicals alter the numbers or functions of gap junctions. **Figure 6** illustrates the variety of conditions associated with defective gap junctions.

Understanding the adverse impacts of gap junction defects has increased with the availability of modern genetic study techniques. For example, mice can be produced that have one of more of their connexin genes permanently inactivated (connexin 'knock-out'

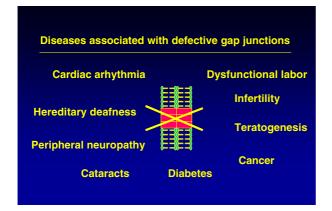


Figure 6 Disease states correlated with defective gap junction function.

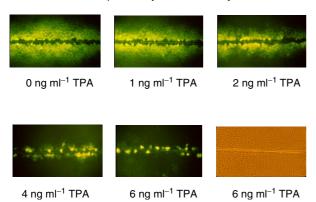
mice). Investigations using these mice have shown that inactivation of certain connexin genes leads to the death of the embryo or fetus before birth. When other connexin genes are knocked out, embryonic and fetal development occur normally but, shortly after birth, the mice die because of defects in the organs in which the knocked-out connexins should have been expressed. Finally, when yet other connexin genes are knocked out, the mice survive to adulthood but then develop diseases such as peripheral neuropathy, liver cancer, and cataracts.

Epigenetic alterations of GJIC have also been linked to toxic actions of synthetic and natural agents and human disease. Traditional toxicological studies on adverse effects of a variety of chemicals in the laboratory setting have shown that birth defects can occur from exposure to drugs that affect gap junctions; for example, alcohol and thalidomide. In addition, some carcinogenic agents alter gap junction formation in cell-and connexin-specific ways. These include natural chemicals such as Croton oil; pollutants such as polybrominated biphenyls; drugs such as phenobarbital; nutrients such as unsaturated fatty acids, retinoids, and carotenoids; pesticides such as DDT; metals such as cadmium; hormones such as estrogens; and growth factors such as epidermal growth factor. These toxic agents affect GJIC by inappropriately opening or closing gap junction channels, altering gap junction formation and connexin stability, and changing the expression of connexin genes.

These examples do not prove that chemical alteration of gap junctions is the causative factor of a disease as correlation does not mean causation. But as several known human diseases are due to the inheritance of a mutant connexin gene, it is very likely that chemical alteration of GJIC contributes to noninherited forms of these and other diseases.

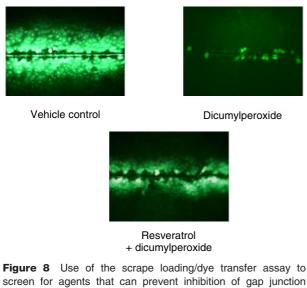
Measuring Gap Junctional Communication

Techniques have been developed to determine the degree to which cells are communicating with each other via gap junctions. One of these is called the scrape load/dye transfer assay and it is illustrated in **Figure 7**. The lower right panel is a phase contrast micrograph of normal rat liver cells through which a razor blade cut a path while the cells were immersed in a fluorescent dye solution. When the cells along the cut line were cut, they transiently open and take up the fluorescent dye. The dye then diffuses to neighboring cells through gap junction channels. The panel at the top left shows that cells that were not



Scrape load/dye transfer assay

Figure 7 Utility of the scrape loading/dye transfer assay to detect a toxin/toxicant's ability to modulate gap junctional intercellular communication. The last two images (6 ng ml⁻¹ TPA) illustrate both the epifluorescent and phase images, respectively, of the same cells to show that the complete inhibition of GJIC was not associated with the death of cells.



screen for agents that can prevent inhibition of gap junction function.

treated with any chemical could transfer the fluorescent dye to several neighboring cells. In a period of 5 min, the dye went from the cut edge cells to ~10 cells away from the edge. This demonstrates that normal, control rat liver cells communicate extensively via gap junctions. In the subsequent panels labeled with increasing concentrations of TPA (a powerful skin tumor promoter), one sees a dramatic dose-related reduction in the ability of the cell to transfer the fluorescent dye from the cells along the cut edge. This demonstrates that TPA can inhibit GJIC in a threshold, but dose-dependent fashion.

Figure 8 illustrates how this technique can be used to show that some chemicals can protect against the

inhibition of GJIC. The top left panel shows that normal, control liver cells transferred the fluorescent dye to about 10 cells away from the cut edge. The top right panel shows that treatment of these cells with noncytotoxic concentrations of another tumor promoter, dicumyl peroxide, completely inhibited the transfer of the fluorescent dye. The bottom panel demonstrates that cells treated with dicumyl peroxide and an antioxidant found in grapes and red wine, resveratrol, had higher levels of GJIC than cells treated only with dicumyl peroxide. This shows that resveratrol protected against damage to gap junctions and suggests that it might be a cancer chemopreventive agent.

Summary

In summary, GJIC enables cells to rapidly share critical ions and molecules that influence whether cells remain active, proliferate, differentiate, commit apoptosis, or adapt in response to external stimuli. In addition to effects on individual cells, this form of intercellular communication synchronizes the activities of cells within a tissue. Thus, when gap junctions are altered in inappropriate ways by endogenous or exogenous factors or conditions, a variety of toxic outcomes and diseases may result.

While extracellular and intracellular communications have long been subjects of study as part of the disciplines of physiology and biochemistry, GJIC is a relatively new area of research. Thus, it is expected that as new research findings are produced, it will be possible to identify the mechanisms by which disease-causing agents alter GJIC and to develop techniques to prevent or correct the resultant adverse effects. It is also likely that in the near future these techniques will be viewed as important tools for improving human health.

This central role of GJIC in normal physiology and disease necessitates that toxicologists understand if and how toxic agents affect intercellular communication to fully understand toxic processes and mechanisms. It is certainly important and appropriate to determine how a toxic agent impinges upon a specific molecule or intracellular process, but one cannot fully understand toxic mechanisms in a multicellular organism without studying GJIC. Fortunately within the past decade, toxicologists have begun to move away from the investigation of single molecules and discrete pathways to study global changes in gene expression, protein activities, and signaling cascades. Advances in genomics, proteomics, and bioinformatics have greatly facilitated this. Still, however, there has been relatively less study of GJIC in toxicology. Clearly, toxic agents alter GJIC, and it is likely that investigation of such actions will lead to a much greater holistic understanding of toxic mechanisms and risk.

See also: Genetic Toxicology; Toxicity Testing, Mutagenicity.

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Gasoline

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 8006-61-9
- SYNONYMS: Gas; Motor fuel; Motor spirit; Petrol; Casing head gasoline
- CHEMICAL FORMULA: Gasoline is a mixed compound that does not contain a fixed ratio of component compounds. However, alkanes constitute the largest percentage of component compounds, followed by aromatics and alkenes

Uses

Gasoline is used almost exclusively as a fuel for automobile and other internal combustion engines.

Background Information

Gasoline is a product of petroleum refining that varies in composition and often includes additives such as antiknock agents, antioxidants, lubricants, and detergents. Tetraethyl lead was one of these additives, and use of leaded gasoline as fuel was responsible for much of the human body burden of this metal for a number of years. However, the phase out of lead from gasoline during the past three decades (in the United States) has led to an over 90% reduction in human blood lead levels. More recently, other additives such as methylcyclopentadienyl manganese tricarbonyl and methyl *t*-butyl ether have been foci of concern because of possible adverse environmental impacts of these compounds.

Exposure Routes and Pathways

The major route of exposure to gasoline components and their combustion by-products is inhalation. Skin exposure may also occur during handling of gasoline. Ingestion of contaminated groundwater is another potential route of exposure but is generally not toxicologically significant since only very low levels of gasoline components have been found in drinking water.

Toxicokinetics

Since gasoline is a mixture, relevant toxicokinetics information must reflect not only the characteristics of the individual gasoline components but also any interactions among them. Thus, the toxicokinetics of various components of gasoline are not sufficient to understand the mixture. Some information on gasoline, itself, is available; e.g., percutaneous absorption is slow and absorption from the respiratory tract is more efficient than absorption from the gastrointestinal tract. However, many other pieces of toxicokinetic data are lacking so that the toxicokinetics of gasoline is presently poorly characterized.

Mechanism of Toxicity

Little information is available on most of the mechanisms of toxicity of gasoline. It has been suggested, however, that renal effects in rats are mediated by $\alpha 2\mu$ -globulin and thus of little relevance to humans who do not produce this compound.

Acute and Short-Term Toxicity (or Exposure)

Animal

Gasoline is an irritant.

Human

Gasoline is an eye irritant and may also cause damage to the skin, lungs, and the intestinal mucosa at high exposure levels. At such levels, it may also cause neurotoxicological effects such as dizziness, nausea, and headache as well as adverse effects on the cardiovascular system. At high enough levels, e.g., adult ingestion of several hundred grams, it may cause coma or even death.

Chronic Toxicity (or Exposure)

Animal

In laboratory studies, chronic gasoline exposure has been linked to renal tumors in male rats and liver tumors in female mice. However, the applicability of these results to humans is questionable.

Human

Although there have been numerous epidemiological studies of workers exposed to gasoline, the results are inconclusive with regard to a link to cardiac toxicity, neurotoxicity, or any form of cancer. However, some of the components of gasoline, e.g., benzene, are classified as known human carcinogens. In addition, intentional excessive exposure, e.g., from gasoline sniffing, can lead to adverse neurological and renal effects.

In Vitro Toxicity Data

Studies of bacterial and mammalian cells in culture indicate that gasoline is not mutagenic.

Clinical Management

Victims exposed only to gasoline vapors are not contamination risks; however, those whose clothing has been contaminated with liquid gasoline are. To decontaminate victims, exposed skin and hair should be flushed with plain water for 2–3 min and then washed with mild soap. Thorough rinsing with water should be undertaken. Exposed or irritated eyes should be irrigated with plain water or saline for 15 min. If gasoline has been ingested, emesis should not be induced or gastric lavage and activated charcoal should not be used. Catharsis with magnesium or sodium sulfate is acceptable.

If vomiting occurs, pulmonary aspiration should be watched for. In cases of respiratory compromise, airway and respiration should be secured via endotracheal entubation. Patients who have bronchospasm should be treated with aerosolized bronchodilators. Epinephrine or related substances should not be administered. There is no antidote for gasoline. Treatment should support respiratory and cardiovascular functions.

Environmental Fate

Since gasoline is a mixture, no simple summary can address the fates of all of the components. However, many of the toxicologically significant components are volatile and so are lost to the atmosphere after being released to surface soil or surface water. These compounds are then subject to photochemical oxidation. In addition, these components can leach through the soil and contaminate groundwater where they may remain for long periods of time. Under aerobic conditions, biodegradation of gasoline components can occur in soil and surface water.

Other Hazards

Gasoline is flammable at room temperature. It also poses a danger from explosion.

Exposure Standards and Guidelines

The permissible exposure limit time-weighted average for gasoline in workplace air is 900 mg m^{-3} (300 ppm). Federal limits for gasoline in drinking water and air have not been promulgated although such limits exist for some gasoline components; e.g., benzene. However, some states and municipalities have promulgated acceptable ambient air concentrations for gasoline.

See also: Manganese.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Automotive Gasoline.

Gastrointestinal System

M Joseph Fedoruk and Tee L Guidotti

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Introduction

The gastrointestinal (GI) system or tract begins with the mouth and ends in the anus. The primary function of the GI system is the digestion and absorption of food, including solids and liquids, and the provision of a barrier to many potentially harmful ingested substances. Digestion is the breakdown or hydrolysis of food into smaller molecules in preparation for absorption into the body. Absorption is the transport of nutrients across the intestinal cell. The GI system also includes the exocrine pancreas which secretes proteolytic digestive enzymes into the duodenum, which facilitates the digestion of sugars, protein, and fats. The endocrine functions of the pancreas are considered elsewhere in this encyclopedia. Bile salts produced by the liver also play a critical role in the absorption of fats and fat-soluble vitamins and the effects of xenobiotics on bile metabolism. The liver is considered an integral part of the GI tract but has a broad range of metabolic functions in addition to its role as an organ supporting digestion. With respect to blood flow, the liver is positioned in a circuit between the stomach and small intestine and the rest of the body, receiving venous blood directly from them via the portal system and draining into hepatic veins into the systemic circulation. As a consequence, the liver metabolizes many xenobiotics absorbed by the intestine before they reach the system circulation and

is a target organ for toxic or metabolically activated xenobiotics delivered to it from the digestive organs of the GI tract.

The GI tract provides the second largest surface area for direct contact of xenobiotics after the lung. Agents can contact the GI tract directly after oral ingestion and through the swallowing of particles that have been cleared from the respiratory tract by mucociliary clearance. Other potential routes of contact include direct introduction into the rectum, which is a route of administration for certain drugs. Agents metabolized by the liver and excreted in bile come into direct contact with the small intestine and can be continually recirculated because of enterohepatic circulation.

From a toxicological perspective, the GI tract is an important organ system since it is the initial site of contact of many environmental agents including food contaminants which have the potential to produce a broad array of toxicological effects. The GI tract is the target organ for a significant number of poisonings due to either inadvertent ingestion of medications, household products, and other items by children or intentional ingestion of poisons by adults during suicide attempts. GI symptoms are also common manifestations of systemic toxicity from a wide variety of toxic agents. Knowledge and recognition of such symptoms can be essential in identifying a toxic condition.

This entry provides an overview of the anatomy and physiology of the GI system and later describes the type of toxic effects that can be observed with different classes of agents.

Anatomy and Physiology

The GI tract is composed of several segments that have specialized functions with respect to digestion and absorption but share common anatomical and histological features. The GI tract can be considered as, and is embryologically derived from, a tube composed of several layers. The tube consists of (1) an inner mucosal layer that contains mucus-secreting cells and the specialized cells; (2) a submucosal layer that consists of loose connective tissue containing blood and lymphatic vessels, inflammatory cells, and nerve fibers; (3) a muscular layer containing many smooth muscles that is responsible for peristalsis and emesis; and (4) a serosal layer or covering. The specialized cell types vary in each GI tract segment and reflect the specialized function of each GI segment with respect to digestion and absorption. The individual segments are discussed below.

Oral Cavity and Pharynx

This segment of the GI tract includes the mouth and tongue, the pharynx, and oropharynx. The primary functions of the oral cavity are (1) as a processing and storage place for food to enable chewing and mixing of food with salivary enzymes, and (2) as a passage for transport to the esophagus. Salivary amylase helps break down starch. The oral cavity is lined by stratified squamous columnar epithelium, which is keratinized in areas subject to a high degree of mechanical friction such as the tongue and palate. The pharynx is also lined by stratified squamous columnar epithelium, but unlike the oral cavity it has striated muscle that is not under voluntary control. The connective tissue surrounding the cavity is loose and highly vascularized. When presented with antigenic stimuli, this tissue can become edematous quickly and lead to obstruction of the airway, a potentially life-threatening condition.

The oral cavity is also the site of a rich commensual bacterial flora that elaborates ammonia, which up to a limit neutralizes acid-forming gases inhaled through the mouth.

Esophagus

The esophagus is a musculomembranous conduit that extends from the pharynx to the stomach and is approximately 23–25 cm in length in an adult. It is lined by stratified squamous columnar epithelium and surrounded by a muscular layer composed of longitudinally arranged smooth muscle bundles. The submucosa contains many nerve fibers. It also contains submucosal glands, which are mainly present in the lower and upper portions of the esophagus and are thought to be continuations of the minor salivary glands in the oropharynx.

The primary functions of the esophagus are the transport of solid and liquids into the stomach and the prevention of retrograde flow or reflux of gastric contents. Aspiration of gastric contents into the lung can produce serious lung injury. These functions require coordinated esophageal motor activity. Manometric pressure studies have revealed that the esophagus has two areas of increased pressure or sphincters that function to prevent reflux. An upper sphincter at the level of the cricopharyngeal muscle remains closed most of the time due to its elastic properties and the tonic contraction of the cricopharyngeal and inferior pharyngeal muscles. A lower esophageal sphincter just proximal to the gastroesophageal junction also remains closed much of the time. Both sphincters must relax in response to a peristaltic wave and later increase in pressure to prevent gastric reflux.

The control mechanisms for the lower esophageal sphincter are complex and not well understood. The sphincter is innervated by preganglionic parasympathetic fibers of the vagus nerve and postganglionic inhibitory and excitatory neurons of the symptomatic nervous system. However, vagotomy or surgical cutting of the vagus nerve does not abolish sphincter tone. Many agents can decrease lower esophageal tone such as cholinergic muscarinic agonists, gastrin, and α -adrenergic agonists. Other substances, such as nicotine, β -adrenergic agents, nitric oxide, and dopamine, cause a decrease in the sphincter tone. It is not clear whether these agents play a role in the normal functioning of the tone of the lower esophagus or whether the effects are pharmacological. Smoking and high-fat meals lead to a decrease in tone and can produce symptoms of heartburn, which is due to reflux of gastric contents into the esophagus. Chronic reflux is a risk factor for esophageal cancer.

Stomach

The key function of the stomach, a bag-like widening of the digestive tract, is to receive food and to secrete gastric acid and pepsin (an enzyme to digest food); however, very little absorption of food takes place in the stomach, except for some lipid-soluble substances such as alcohol. In humans, the stomach is composed of four main segments: the cardia, the fundus, the body, and the antrum (**Figure 1**). The cardia, a narrow portion just distal to the gastroesophageal junction, is primarily lined with mucus-secreting cells. The fundus is the proximal portion that lies above the gastroesophageal junction and is largely composed of cells that secrete mucus and hydrochloric

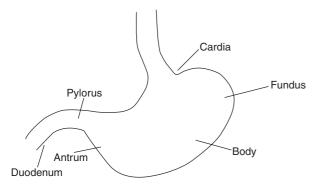


Figure 1 The stomach.

acid. The body is the part that lies proximal to the angle above the lesser curvature and contains similar cells. The antrum is located distal to the angle of the lesser curvature and is demarcated from the duodenum by the pyloric sphincter. The stomach also contains a muscular layer which facilitates mixing of the stomach contents with hydrochloric acid and pepsin. The surface of the stomach has coarse rugae, which are infoldings of the submucosa and mucosa that provide a larger area for digestion.

The stomach is lined with a mucosal surface that is punctuated by gastric pits leading to gastric glands. Foveolar cells, which secrete mucin, line the surface of the stomach and the gastric. These are tall and columnar cells that contain clear mucin-containing granules. Neck cells are located in the gastric pits, which are probably progenitor foveolar cells. Various glands empty into the gastric pits, including cardiac glands in the cardia stomach section, which mainly secrete mucus; gastric glands in the body and fundus, which contain large numbers of parietal cells; and pyloric or antral glands in the antral stomach, which contain large numbers of mucus secretory cells.

The individual cell types reflect specialized gastric digestive functions. The stomach secretes hydrochloric acid and pepsinogen, which not only digest food but can also damage gastric tissues which must be protected from these factors. Hydrochloric acid is secreted by approximately 1 billion parietal cells located in the fundus and body of the stomach secrete. The cells are interspersed along the course of mucous glands and secrete hydrochloric acid at a concentration that is approximately 3 million times that found in blood. Parietal cells contain large numbers of intracellular tubulovesicular structures derived from the endoplasmic reticulum. The endoplasmic reticular membranes contain a hydrogen-potassium ATPase that pumps hydrogen ion across a membrane in exchange for potassium.

Proteolytic proenzymes pepsinogen 1 and 11 are released by chief cells which are located at the base of

the gastric glands found also in the body and fundus of the stomach. Chief cells have morphological features of cells that synthesize protein and are characterized by an extensive endoplasmic reticulum and numerous apical secretory granules. Pepsinogen is activated by the stomach's low pH environment and is inactivated by the high pH (≥ 6) in the duodenum.

Mast cells and enterochromaffin-like cells found in the interstitium and among parietal cells contain histamine, which acts on parietal cell receptors to stimulate the release of hydrochloric acid. The histamine receptor on parietal cells is designated as H2 and is blocked by H2 blockers such as cimetidine which are widely used to treat peptic ulcers.

The factors associated with the regulation of gastric acid secretion are complex and involve chemical, neural, and hormonal influences. The stomach and small intestine contain several endocrine cells that affect the release of gastric acid. The most important factor that stimulates the release of hydrochloric acid is gastrin, a hormone that is released into the circulation by G cells which are located in the epithelial lining of the pyloric glands in the antrum of the stomach, duodenum, and proximal jejunum. Gastrin is released in response to food in the stomach and small intestine. Stimulation of the vagus nerve results in the release of acid via the muscarinic cholinergic receptors located on the parietal cells. Vagal stimulation is also thought to stimulate the release of gastrin into the circulation and lower the parietal threshold for releasing gastrin into the circulation.

Gastric acid secretion can be inhibited by several mechanisms including acid in the stomach (pH 3 inhibits gastrin release), acid in the duodenum, the presence of fat in the pancreas, and hypertonic fluids or hyperglycemia. Somatostatin, a hormone produced by antral mucosal endocrine cells (D cells), inhibits the release of gastrin by directly inhibiting the parietal cells. Somatostatin is also present in other GI tissue and the pancreas. C cells, endocrine cells in the proximal small intestine, secrete secretin in response to mucosal acidification, which also decreases gastric secretion.

The stomach has several protective mechanisms against hydrochloric acid and pepsinogen. The primary defense is the presence of gastric mucus, a large polymeric glycoprotein that is secreted by mucus glands throughout the stomach. Gastric mucous exists in two phases: (1) an insoluble mucus gel layer that coats the stomach and has a low diffusion coefficient for H⁺ and (2) in gastric juice as a soluble phase. Mucus secretion is enhanced by cholinergic stimulation of muscarinic receptors and occurs in response to mechanical and chemical irritation of the stomach. Other protective mechanisms exist, including the secretion of bicarbonate by nonparietal cells via carbonic anhydrase, and are present in foveolar and parietal cells. Gel thickness increases in response to secretion of E series prostaglandins and is decreased by nonsteroidal, anti-inflammatory medications. The other protective mechanism is tight cell junctions between surface epithelial cells, which are almost impermeable, to back diffusion of hydrochloric acid or pepsin. Prostaglandins of the E series which are in the gastric mucosa are thought to play a role by stimulating the secretion of gastric acid mucus and bicarbonate and by maintaining blood flow to the gastric mucosa which is necessary to maintain the integrity of cell surfaces and promote epithelial renewal.

Gastric acid secretion is not a primary cause of peptic ulcer disease except in extreme conditions of overproduction (the Zolinger–Ellison syndrome). However, once defenses against acid are breached, gastric acidity plays a role in perpetuating the ulcer. More significant is infection with *Helicobacter pylori*, a small flagellated gram-negative spiral bacillus that produces urease, which serves to neutralize acidity in the gastric mucus, where it is found. *H. pylori* infection, although associated with an increased risk for peptic ulcer and gastric carcinoma, is so common worldwide as to be considered commensual. Peptic ulcer is now routine treated with antibiotics directed against *H. pylori*.

Small Intestine

The primary functions of the small intestine are digestion and absorption of food. The adult small intestine is approximately 6 m in length and is composed of the duodenum, ileum, and jejunum. Digestion occurs primarily in the upper small intestine and requires the action of pancreatic enzymes such as amylase, lipase, and trypsin, which are released from the pancreas into the duodenum, and bile salts from the biliary system. Absorption largely takes place in lower portions of the small intestine.

The mucosa or lining of the small intestine is enormous. A characteristic feature of the small intestine is the mucosal lining, which is principally composed of enterocytes that contain numerous villi that serve as absorptive areas. The villi extend into the lumen and appear as finger-like projections covered with epithelial cells. The villi also contain microvilli, which are also composed of microfilaments that form a brush border. Absorption of nutrients is also enhanced by motility of the small intestine, which places food in proximity to capillaries and lymphatic lacteals that serve as absorptive channels, and by the direct movement of villi. Several types of absorptive mechanisms exist for nutrients including active transport, passive diffusion, facilitated diffusion, and endocytosis. Endocytosis occurs when the outer plasma membrane surrounds soluble or particulate nutrients in the GI tract and engulfs the contents. This process is similar to phagocytosis.

Carbohydrates, or starches, which are complex polysaccharides, are hydrolyzed to oligosaccharides and disaccharides by the action of pancreatic amylase. Disaccharides, including lactase, sucrase, and maltase, are enzymatically split by enzymes contained in the microvilli of enterocytes. Glucose and other monosaccharides are absorbed by an active transport mechanism and this action is coupled to energy derived from a sodium pump mechanism.

Proteins are initially broken down in the stomach by pepsins, but completion of digestion occurs in the duodenum by the action of pancreatic trypsin and chymotrypsin. This results in the formation of oligopeptides, dipeptides, and amino acids. Dipeptides are broken down by dipeptidases located on the microvilli and the cell cytoplasm. Amino acids are absorbed rapidly in the duodenum and jejunum by active transport mechanisms, including the generation of sodium ions.

Most dietary fat is composed of long-chain triglycerides, which contain saturated and unsaturated fats. The stomach's churning action acts to reduce the particle size of fats. In the duodenum, hydrolysis of triglycerides occurs through the action of pancreatic lipase, colipase, and bile salts, which form a ternary complex. Bile salts, which are synthesized by the liver, have detergent properties and enable the formation of micelles, which are emulsions of triglycerides or fats with bile salts. The micelles enable pancreatic lipase enzyme to access the water-fatinsoluble phase. Colipase, a pancreatic enzyme, acts to place the pancreatic lipase in close proximity to the surface of a triglyceride droplet and is necessary for the action of lipase. Lipase hydrolyzes the triglyceride to form 2-monoglycerides and fatty acids. Monoglycerides are released from micelles and come into contact with the cell surface, where they are absorbed by diffusion. Once inside the cell, the fate of fatty acid is dependent on chain length. Longchain fatty acids are esterified to triglycerides by enzymes in the endoplasmic reticulum and interact with cholesterol phospholipids and apoproteins to form chylomicrons and very low-density lipoproteins. Medium-chain fatty acids are not reesterified and enter the portal venous system, where they are transported and bound by albumen. Other nutrients absorbed in the small intestine include fat-soluble vitamins, iron, calcium, water, and sodium.

Bile salts are absorbed from the ileum or terminal portion of the small intestine and are recirculated via the portal vein. If the ileum is diseased, as in Crohn's disease, bile salts may not be absorbed, and fat absorption, including absorption of fat-soluble vitamins, may be impaired.

Endocrine cells are scattered among walls of the small intestine including the villi and crypts. The cells can release a large array of secretory products into the bloodstream. The hormones play a key role in the digestive process and exert actions through neurocrine and paracrine mechanisms. Products released in the small intestine include gastrin, somatostatin, secretin, cholecystokinin, motilin, neurotensin, enteroglucagon, vasoactive intestinal polypeptide, GI polypeptide, and other agents.

The small intestine, in addition to other portions of the mucosa and submucosa of the alimentary tract contains a large number of individual T and B cells, macrophages, and plasma cells. This lymphoid tissue becomes confluent in the ileum and forms unencapsulated nodules, which are macroscopically visible and known as Peyer's patches. The M (membrane) cells are present in the epithelial tissue overlaying the GI tract and can transcytase antigenic macromolecules from the intestine to intact lymphocytes. The tissue with lymphocyte tissue in the appendix and mesenteric lymph nodes constitutes the mucosaassociated lymphoid tissue. This system forms part of the afferent link of the intestinal immune system and is involved in the secretion of IgA, which serves as a defense mechanism against external pathogens. Other mucosal epithelial surfaces in the body (e.g., respiratory tract and genitourinary tract) contain similar populations of lymphocytes that serve to protect pathogenic organisms.

Large Intestine

The large intestine or colon is ~ 1.5 m in length. The principal function of the colon is to reabsorb water and electrolytes that are present inside a liquid luminal stream. In contrast to the small intestinal mucosa, the lining of the colon is composed of columnar absorptive cells that have shorter, flat epithelial cells with no villi, although some absorptive cells have microvilli. The mucosa is punctuated by tubular crypts that extend to the mucosal layer and contain goblet cells, which secrete mucus; Paneth cells, which secrete lysozyme; endocrine cells; and undifferentiated goblet cells. Cellular proliferation is confined to the crypts and cells differentiate and migrate to the surface to replace superficial epithelial cells lost to surface abrasion or degeneration. Lymphoid tissue is found in the mucosa and submucosa.

Cellular Replication

The GI tract has one of the highest rates of cell turnover of mitosis of any organ system. The highest rate of mitosis is in the small intestine, where between 60% and 75% of cells are turned over on a daily basis. Over 50% of the cells in the stomach pylorus are turned over daily and 10% of the cells of the colon are replaced. Agents that are known to interfere with cellular replication, such as alkylating agents or antimetabolites used in cancer chemotherapy, can have potential effects on the GI tract by interfering with normal regeneration of the cells that are undergoing rapid replacement.

Intestinal Flora

The GI tract is not sterile and bacteria are continually swallowed to the stomach with food. Hydrochloric acid limits the concentration of bacteria in the stomach (except for *H. pylori*). In contrast, the large and small intestine contain numerous bacteria. Intestinal bacteria may contain several enzymes including β -glucosidase, β -galactosidase, and β -glucuronidase. These enzymes may play a role in transforming medications to their active form or affecting their excretion. Administration of ampicillin leads to an increase in the excretion of conjugated estrogens. Diets rich in meat, which has been identified as a risk factor for cancer of the colon, also increase β -glucuronidase activity in fecal bacteria. Gastrointestinal bacteria also play a role in metabolizing some compounds into more toxic forms such as reducing azo dyes, which are used in some food additives. Some bacteria, like Lactobacilli, may serve to decrease the risk of cancer.

Exocrine Pancreas

The pancreas contains cells that have endocrine and exocrine functions. The gland is largely formed of acinar cells, which secrete digestive enzymes or their precursor into the duodenum. Exocrine function is subject to hormonal and neural regulation. Islets of Langerhans contain strands of cells including B and A cells that secrete insulin and glucagons, respectively, and form the endocrine portion of the gland.

Toxicant Effects on the GI System

Toxic effects can be mediated in the GI system by several mechanisms, which are discussed in the following sections.

Direct Mucosal or Cellular Injury

Xenobiotics that contact the mucosal or other cells of the GI tract produce irritation characterized by inflammation, degeneration, and/or proliferation. The type of toxic effect that is manifest is dependent on several factors including chemical characteristics of the agent, dose or magnitude of exposure, and type of tissue involved.

Erosions or a superficial ulceration of the mucosa can occur focally or diffusely. Erosions are due to focal necrosis of the epithelium and associated stroma and are restricted to the superficial layers. Diffuse irritation accompanied by an inflammatory reaction is called enteritis. Ulcers, in contrast, are deeper lesions extending beyond the mucosa and penetrating into the adjacent tissue layers. Chronic irritation can produce proliferative lesions including dysplasia, which potentially could become malignant.

Ingestion of strong alkali and acids has the potential to produce severe tissue destruction or liquefaction necrosis. Alkali with a pH of $\geq 11.5-12$ and acids with a pH <2 can produce significant corrosive injury. Other substances such as phenol may not be highly alkaline but can still produce corrosive injury. Alkali are found in many commercial products, such as household and industrial cleaners, dishwasher soaps and drain openers, and low-phosphate detergents. Factors affecting the degree of tissue injury or destruction include the amount ingested, the duration of contact with tissue, concentration, pH, physical form, titratable alkaline, and acid reserve.

This liquefaction process has four distinct phases:

- 1. *Inflammatory phase*: This phase lasts 1 or 2 days and consists of marked fibroblastic proliferation. In this stage, perforations may occur.
- 2. *Necrotic phase*: This phase occurs 1–4 days after injury. Cells die from coagulation of intracellular protein. Vascular thrombosis and bacterial invasion may worsen the underlying injury. The esophagus is especially vulnerable to perforation during this phase.
- 3. *Granulation phase*: This phase begins 3–5 days postinjury when necrotic tissue sloughs. Granulation tissue begins to fill in tissue defects and connective tissue begins to form in 10–12 days.
- 4. Constriction phase: This phase occurs 2.5–3 weeks following injury and is related to the formation of collagen in the healing lesion. Marked narrowing of the esophageal lumen may occur as the collagen fibers begin to contract.

The most frequent injury following alkali ingestion is esophageal burns. Diffuse circumferential esophageal burns are more common in patients ingesting liquid forms of concentrated alkaline corrosives; granular forms tend to produce more oral burns and esophageal burns that are in patches or streaks. Gastric injuries may also be more common in patients ingesting liquid alkaline corrosives or solids that have been placed in capsules. Intestinal burns, mostly duodenal, have been reported but are much less frequent. Severe duodenal injury may be more common with suicidal ingestions. From a clinical perspective, the absence of visible oral burns does not reliably exclude the presence of esophageal burns. Symptoms of stridor, vomiting, and drooling can indicate serious esophageal injury.

Sequelae associated with ingestions of caustics include a tracheoesophageal and aortoesophageal fistulae; strictures of the mouth, esophagus, and stomach; and esophageal carcinoma. In severe cases, GI bleeding or perforated viscus with mediastinitis or peritonitis may develop. Strictures are more likely to develop after second- or third-degree or circumferential burns.

Several other agents produce GI tract irritation by interfering with the gastric mucosal barrier. Gastric ulcers have been associated with the use of antiinflammatory medications including aspirin and nonsteroidal anti-inflammatory medications. The mechanism is thought to be the inhibition of cyclooxygenase, which is required for prostaglandin secretion. Prostaglandins play a key role in maintaining mucosal defenses of the stomach. Other agents that can cause severe injury to the GI tract include salicylates, heavy metals, and iron.

Several agents have been associated with producing acute pancreatitis or inflammation of the pancreas. The main causes are alcohol and a disturbance of the bile duct, which account for $\sim 50\%$ of cases. Drugs with a clear association include sulfonamides, thiazide diuretics, tetracycline, azathioprine, estrogens, and valproic acid. The mechanism for the underlying injury is not well understood. Possible associations have been reported with other medications including methyldopa, procainamide, and l-asparaginase. A relationship between corticosteriods has not been established.

The pancreas is uniquely susceptible to high concentrations of ethanol, which can induce an acute inflammatory response characterized by release of amylase into the circulation. If severe or if this evolves into chronic pancreatitis it may lead to autodigestion by exocrine enzymes and ultimately to secondary endocrine dysfunction.

Interaction with Receptors of the GI Tract

Gastrointestinal function can be affected by interaction with cellular receptors. Stimulation of cholinergic muscarinic receptors by agents such as cholinesterase inhibitors (organophosphate pesticides and carbamates) and nicotine and opioid withdrawal can lead to an increase in motility and secretions of the GI tract. This process can lead to symptoms of abdominal pain, cramps, and diarrhea. Similarly, the administration of drugs that block with cholinergic muscarinic receptor functioning (e.g., atropine, tricyclic antidepressants, opiates, and sedative hypnotic medications) can slow motility and lead to constipation.

Indirect Effect

Vomiting can occur as a consequence of the interaction of a chemical with the central chemoreceptor zone or vomiting center in the fourth ventricle of the brain. This results in GI symptoms caused by an indirect effect. Glycosides, opiates, nicotine, and possibly carbon monoxide may act in this manner. Vomiting can also occur as a consequence of local GI tract stimulation from a wide array of agents including soaps, detergents, solvents, metals (including arsenic and thallium), and toxins associated with several types of food poisonings.

Allergic Reactions

The GI tract can be a site of hypersensitivity reactions. Angioedema of the mouth including the pharynx can occur following use of several medications including ACE inhibitors. The reaction is mediated via IgE.

Carcinogenesis

Cancers of the GI tract comprise a large proportion of malignancies in the USA. Colorectal cancer is the second most common malignancy in the USA. There is strong evidence that GI cancers are affected by environmental factors since there are considerable geographical differences between cancer incidence of the same organ and migration studies have demonstrated that migrants who move to new countries over time will experience the same risks or cancer rate as the people in the host country.

Oral cavity cancers have been associated with cigarettes, alcohol, and chewing tobacco or snuff or betel nut quid (popular in parts of Asia). Cancer of the oral cavity is not common in the Western world but frequently found in some developing countries including India, where it accounts for approximately 8% of all malignancies. Risk factors associated with oral cancers include excessive alcohol consumption, although the effects of alcohol are sometimes hard to differentiate from tobacco use since persons commonly smoke and drink. Chewing of tobacco has been identified as a principal risk factor. Other factors include a history of ionizing radiation exposure and nutritional deficiencies including iron in association with Plummer–Vinson syndrome.

Esophageal cancer has been related to the use of alcohol and nitrosamines and possibly chewing betel nut (popular in parts of Asia). Other risk factors include a history of ingestion of alkaline corrosive agents, including lye. Nutritional deficiencies have also been linked to this type of malignancy.

Gastric neoplasms were among the most frequent malignancies at the turn of the century, but the incidence has decreased in the past 50 years even though there have been no major advances in diagnosis or treatment. Factors that have been linked to gastric cancer include nitrate ingestion as well as other dietary factors. Persons with atrophic gastritis who have hypochlorhydria or a relative lack of stomach acid are at greater risk. This may be secondary to the presence of bacteria, which are normally killed in an acid gastric environment and that transform nitrates to nitrites, which can eventually form carcinogenic nitrosamines.

Colon cancer has been associated with several factors including radiation exposure, limited physical activity, dietary fat intake, high meat intake, and nitrosamines. Fats may increase the risk of cancer by changing the intestinal flora or increasing the concentration of bile acids or because of a secondary effect on metabolism of xenobiotics. Negative associations have been associated with intake of fresh vegetables and meats. Other risk factors include family polyposis, chronic ulcerative colitis, and familial cancer syndrome. Cancer of the rectum not only shares some risk factors with the colon cancer overall but also has distinct characteristics possibly related to sexually transmitted infections, chronic inflammation, and cigarette smoking.

See also: Absorption; Acids; Alkalies; Carbamate Pesticides; Carcinogenesis; Corrosives; Endocrine System; Liver; Metals; Organophosphates; Poisoning Emergencies in Humans.

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Generally Recognized as Safe (GRAS)

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Generally Recognized as Safe (GRAS) is a regulatory category created for a group of food additives that were exempted from the more rigorous regulatory requirements for food additives in the 1958 Food Additives Amendment to the (US) Food, Drug, and Cosmetics (FD&C) Act of 1938. If a substance was accorded GRAS status, it was generally recognized by experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures or experience based on common use in food, to be safe under the conditions of its intended use.

The statutory definition of 'food additive' covers only a substance that "is not generally recognized ... to be safe under the conditions of its intended use." Thus, in the peculiar meaning of the term as it is used in the statute, a substance that becomes a component of a food (even as an ingredient) would not be a 'food additive' if it is generally recognized as safe. Congress further defined GRAS as requiring that a substance used in food be generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common used in food) to be safe under the conditions of its intended use.

Thus, GRAS status may exist if some level of scientific agreement about a substance's safety exists based either on appropriate testing or common use in food prior to 1958.

The GRAS exception obviously raises a number of serious interpretive difficulties. The statute provides little further elaboration about the required degree of scientific agreement, the types of scientific procedures that could provide the necessary predicate for such agreement, or, in the case of substances in widespread used before 1958, the required nature and extent of such prior use. For instance, how could a substance be GRAS without experience based on common prior use? Did Congress thereby intend to give manufacturers of new food-use substances the option of submitting test results to non-Food and Drug Administration (FDA) scientists for their evaluation and possible stamp of approval? At least one witness at the 1957 congressional hearings apparently thought so, suggesting that a company could seek the advice of private or academic consultants on the question of whether there was general recognition of safety based on existing data. As discussed below, this has become a common practice.

Because the GRAS exception became a common feature of every one of the numerous bills on the subject considered by Congress, the legislative history sheds some additional light on these and other questions. Although not technically a 'grandfather clause' (which would permanently exempt from coverage all substances used in food prior to the enactment date), the GRAS exception attempts to minimize the potentially significant and unnecessary burden that would otherwise be placed on both the industry and the FDA if the agency had to evaluate and formally approve common substances used in food. In addition, for substances that were not regarded as GRAS and therefore subject to regulation as food additives, Congress initially provided a transitional period of up to 30 months for compliance with the new premarket approval requirements, but it subsequently extended this phase-in period by almost five additional years.

The FD&C Act included similar GRAS language in defining the term 'new drug,' as did the Pesticide Residues Amendment of 1954. Although conceding during the congressional hearings that the language was inherently ambiguous, the agency thought that it could apply this flexible GRAS exception to food additives in a sensible manner. (Interestingly, just 2 years after enacting the Food Additives Amendments.) As subsequently construed by reviewing courts, the exception applicable to drugs is quite narrow, in part because the statute requires that a drug be both GRAS and GRAE (generally recognized as effective). In 1973, the Supreme Court held that the exception in the definition of new drug required an 'expert consensus' of both safety and effectiveness.

Both the FDA and reviewing courts sometimes have struggled to make sense of the GRAS exception. All agree that there must be a fairly high level of scientific agreement. The FDA's implementing regulations, finally promulgated almost two decades after passage of the Food Additives Amendment, provide as follows:

• Generally, recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies that may be corroborated by unpublished studies and other data and information.

• Evidence of GRAS must relate to the conditions of intended use; general recognition of the safe use of a substance in a different product or at a different level would not suffice to escape the food additive definition. The exception turns not on safety itself so much as on recognition of safety by scientific experts. Testimony of an absence of any evidence of a health hazard would not suffice to establish GRAS status, at least not unless coupled with evidence of common prior use. If GRAS status is premised on common use prior to 1958, then such use must have been fairly extensive.

Originally, the FDA categorically refused to recognize use outside of the United States. This policy did not, however, survive a subsequent judicial challenge. The revised regulations provide that prior foreign use may support GRAS status, but only if the information about such use is readily available and corroborated. In addition, GRAS status based on prior foreign use must satisfy domestic conceptions of safety. If GRAS status is based on prior foreign use, the FDA urges the manufacturer to seek its concurrence. Other sections of the regulations continue to define eligibility for GRAS status by reference to common use in the United States.

During the congressional hearing leading up to enactment of the Food Additives Amendment, the FDA submitted a 'partial' list of what it would regard as GRAS substances including items such as butter, coffee, cream, gelatin, lard, lemon juice, margarine, molasses, mustard, olive oil, paprika, pepper, salt, sugar, vinegar, and wine. During the first several years after enactment of the Food Additives Amendment, the FDA listed in its regulations hundreds of ingredients as GRAS. The original GRAS lists included, for example, ascorbic acid, calcium chloride, caramel, and sodium phosphate.

Because these inventories emerged without any detailed scientific assessment of the original safety data, much less of the data subsequently generated with constantly improving detection and safety assessment methods (as underscored by the discovery of evidence linking an artificial sweetener mixture containing cyclamate to cancer), the FDA initiated a systematic review in 1969 in order to settle the GRAS or food additive status of a number of substances commonly added to food. The agency designated several categories of food ingredients for this review: substances of natural biological origin that were widely consumed as food before 1958 but subsequently were modified in certain respects by new production processes or selective breeding; distillates, isolates, extracts, and reaction products of GRAS substances; and substances not of natural biological origin or intended for consumption for other than their nutrient properties.

The (US) National Academy of Sciences (NAS) undertook ingredient usage surveys, and, in 1972, the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) established a Select Committee on GRAS Substances (SCOGS) to conduct reviews of the available scientific literature. Over a period of 10 years, SCOGS forwarded to the FDA detailed reports on 468 food substances (of which 422 were direct ingredients). The Select Committee first created an array of five standardized recommendations, and it concluded that 72% of the food substances under review should remain GRAS and only 1% should immediately become subject to food additive requirements. Although the FDA planned to review each of these reports and pursue appropriate rulemaking, it has not completed its GRAS list review many years after receiving the last SCOGS report.

A number of substances currently appear on the GRAS affirmation list that emerged from the FDA's comprehensive review. At present, almost 200 separate ingredients are included as GRAS for direct use in food. The FDA concedes, however, that its GRAS lists are not exhaustive: "Because of the large number of substances the intended use of which results or may reasonably be expected to result, directly or indirectly, in their becoming a component or otherwise affecting the characteristics of food, it is impracticable to list all such substances that are GRAS". Thus, a substance "of natural biological origin that has been widely consumed for its nutrient properties in the United States prior to January 1, 1958, without known detrimental effects, which is subject only to conventional processing ... will ordinarily be regarded as GRAS without specific inclusion" in one of the GRAS lists. More specifically, "by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, vinegar, baking powder, and monosodium glutamate as safe for their intended use."

GRAS status does not free a substance of FDA controls. At a minimum, a GRAS substance must comply with any applicable food grade specifications appearing in the FOOD CHEMICAL CODEX, and it must perform an appropriate function (and be used at a level no higher than necessary to achieve its intended purpose) in the food or food-contact article in which it is used. In addition, a substance must comply with any specific usage limitations appearing in any GRAS affirmation regulation. If no specific

limitation applies, GRAS status is lost only if the conditions of use differ significantly from those providing the basis for eligibility. Finally, '[n]ew information', and any revision of an existing GRAS regulation would be accomplished by the FDA through notice-and-comment rulemaking procedures. In contrast, any revision of a food additive regulation would require more cumbersome procedures.

Unless the FDA previously has decided otherwise, a person may take the position that a particular fooduse substance is GRAS and, therefore, exempt from food additive approval requirements. In fact, there is no present requirement that the agency be advised of such private GRAS determinations. A few manufacturers have commissioned safety reviews by reputable scientific organizations, and FASEB has conducted a handful of private GRAS reviews during the last several years. For example, the Procter & Gamble Company asked the Federation to review the safety of caprenin, a reduced calorie fat substitute; on the basis of FASEB's report, the company determined that this substance was GRAS, filed a GRAS affirmation petition with the FDA, and began selling it to food processors. Similarly, Nabisco Foods sought a FASEB review of salatrim, another fat substitute subsequently brought to market on the basis of a GRAS self-determination. Some have suggested that the National Center for Food Safety and Technology, an organization recently established in the Chicago area with private and government support, might play a similar role in the future.

Whether undertaken for the FDA or a private entity, FASEB's LSRO assembled an ad hoc panel of experts from several different scientific disciplines to conduct the requested reviews. These experts usually are drawn from among FASEB's > more than 40 000 members, and they are hired by LSRO to serve as independent consultants. The expert panels prepare study reports that are "peer-reviewed by an independent internal FASEB committee for clarity, objectivity, and scientific integrity..., [and] the reports of each study are published in scientific journals or are made available publicly."

Similarly, a few industry associations have created their own expert panels to review the possible GRAS status of food ingredients, as the Flavor and Extract Manufacturer's Association (FEMA) has done for the last few decades. FEMA's project began in 1959, initially surveying the industry about the usage of different flavoring substances. The association then established a permanent panel – composed of six to eight recognized and independent experts from various disciplines including toxicology and biochemistry – to evaluate scientific literature reviews (SLRs) assembled for its consideration and then assess the GRAS status of those flavoring substances. Over the last 30 years, the expert panel's reports have been published periodically in the journal '*Food Technology*'. Furthermore, the SLRs underlying the Panel's GRAS determinations were made available to the public and forwarded to the FDA.

In the case of a new flavoring substance, a company seeking an opinion about the flavor's potential GRAS status must submit an application form and literature search to FEMA's staff which, after a preliminary check for completeness, forwards the request and information to the expert panel for consideration at its next regularly scheduled meeting. The available literature is evaluated against FEMA's published criteria, and a GRAS designation requires a unanimous vote by the panel; otherwise, the flavoring substance will be placed in a hold category for further study or be designated as not GRAS.

Since the inception of this project, FEMA has considered > 2000 flavoring substances. The expert panel's initial set of reviews identified 1118 substances as GRAS based on prior safe use and six more as GRAS based on the available scientific information. The FDA incorporated only 277 of these flavors in its own GRAS list, but it also designated another 846 of these FEMA-reviewed substances as approved food additives on the strength of the existing safety data and without the need for filing separate petitions. In 1985, FEMA finished a comprehensive reevaluation aimed at updating its original GRAS determinations, dropping three flavoring substances from the list. In 1993, FEMA began a second reevaluation process, coupled with an effort to update and reformat all existing SLRs, which it hopes to complete in 5 years.

The FDA has not challenged the marketing of flavors the FEMA had identified as GRAS, whether or not it has incorporated them into its own lists of GRAS substances or approved food additives. In extending the date by which persons would have to comply with its bulk flavoring requirements, the agency described the FEMA list as one of the "reliable industry association GRAS lists." The FDA also occasionally refers to FEMA's GRAS listing of a flavor to support a GRAS affirmation proposal.

Another potentially important source of food safety expertise resides in the Joint Expert Committees on Food Additives (JECFA), first organized in 1956 by the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) and now associated with these organizations' Codex Alimentarius Commission. JECFA reports have influenced decisions by the FDA and other regulatory bodies, and its recommendations concerning particular additives might be relied upon by companies in making GRAS self-determinations. Under the FDA's GRAS criteria, a report by JECFA or a comparable group certainly could qualify as 'general recognition' of safety, even if a substance has never before been used in food.

Although nothing prevents such GRAS self-determinations, the strategy carries obvious regulatory risks. On occasion, the agency has pursued enforcement proceedings, disagreeing with a company's belated claim that a substance is GRAS.

FDA has an 'EAFUS' Food Additive Database website. This is an informational database maintained by the FDA Center for Food Safety and Applied Nutrition (CFSAN) under an ongoing program known as the Priority-based Assessment of Food Additives (PAFA). It contains administrative, chemical, and toxicological information on over 2000 substances directly added to food, including substances regulated by the US Food and Drug Administration (FDA) as direct, 'secondary' direct, and color additives, and Generally Recognized as Safe and prior-sanctioned substances. In addition, the database contains only administrative and chemical information on less then 1000 such substances. The more than 3000 total substances together comprise an inventory often referred to as 'Everything' Added to Food in the United States (EAFUS). This list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS. Nevertheless, it contains only a partial list of all food ingredients that may in fact be lawfully added to food, because under federal law some ingredients may be added to food under a GRAS determination made independently from the FDA. The list contains many, but not all, of the substances subject to independent GRAS determinations.

The Research Institute for Fragrance Materials (RIFM) uses an expert of academic dermatologists,

toxicologists, and environmental scientists safe use determinations of fragrance materials. The Expert Panel uses a decision tree approach to assessing the dermal, systemic, and environmental endpoints. Conclusions of the Expert Panel on safe use, drawn from critical evaluation of all available hazard data, and exposure information provided by industry, form the basis for industry-wide standards issued by the International Fragrance Association.

See also: Flavor and Extract Manufacturers Association; Food Additives; Food and Drug Administration, US; International Fragrance Association (IFRA); Joint FAO/ WHO Expert Meetings (JECFA and JMPR); Research Institute for Fragrance Materials (RIFM).

Further Reading

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Relevant Website

http://vm.cfsan.fda.gov – US Food and Drug Administration (FDA). CFR 21 (part 184) (includes a list of direct food additives affirmed as generally recognized as safe). See also the link to the 'EAFUS' Food Additive Database.

Genetic Ecotoxicology See Ecotoxicology, Genetic.

Genetic Toxicology

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Overview of Genetic Toxicology

Genetic toxicology is the study of the toxic effects of chemicals and radiations on the hereditary material, or the deoxyribonucleic acid (DNA), of cells. Genetic toxicology therefore involves the study of DNA single-strand breaks and double-strand breaks, damage to DNA, mutations in DNA, and recombinational events in DNA mediated by exogenous agents in bacteria, yeast, cells of the fruit fly, plant cells, and mammalian cells. In plant cells and mammalian cells, genetic toxicology also encompasses micronucleus formation, chromosomal aberrations, chromosomal aneuploidy, and morphological and neoplastic transformation. In addition, genetic toxicology also encompasses chemical carcinogenesis in lower animals and in humans. The importance of genetic toxicology is that it allows investigators to measure the DNAdamaging, mutagenic, and carcinogenic effects of chemical carcinogens and ultraviolet (UV) and ionizing radiations and it also allows investigators to study genetic damage and mutations in lower animals and humans.

DNA Damage

Many chemicals, and UV and ionizing radiations, can cause damage to DNA bases. This can result in a labilization of the DNA base-sugar phosphate bond. Bases can then depurinate, or dissociate from the sugar phosphate backbone of DNA. This can leave that DNA strand which is lacking a DNA base susceptible to attack by nucleases, leading to cleavage of that strand by endonucleases. This will result in a break in a single strand of DNA. Single-strand breaks in DNA are easily recognized by centrifugation in alkaline sucrose gradients. This separates the DNA strands, and also allows visualization of the separated and broken strands. Using gel electrophoresis and specific plasmids treated with chemical mutagens or radiations, also allows investigators to detect singlestrand breaks in DNA. If these DNA single-strand breaks are not repaired correctly by DNA repair enzymes, this can lead to a cytotoxic event.

Similarly, a number of chemical agents and ionizing radiations can lead to one or more breaks in both strands of DNA simultaneously. This is called a DNA double-strand break. Ionizing radiations, such as X-rays and neutrons, are very effective in depositing a sufficient amount of energy into DNA to result in DNA double-strand breaks. DNA doublestrand breaks are easily recognized by centrifugation in neutral sucrose. These types of breaks of the DNA strands are difficult to repair by the DNA repair machinery of the cell. If they are not repaired correctly, these DNA double-strand breaks can lead to cytotoxicity.

UV light (254 nm) and certain chemical mutagens can also efficiently induce cross-links in DNA. These can be manifested as either intra-strand DNA crosslinks, that is, occurring within one strand of DNA, or inter-strand DNA cross-links, which occur between two strands of DNA. These types of DNA cross-links can inhibit DNA replication and transcription. This can cause the cell to remain in a static state, where it is trapped and cannot proceed through mitosis. In such a state, a cell can become degraded by nucleases and proteases, leading to cell death. Hence, it is very important for the cell to repair these types of DNA cross-links, so the cell can complete DNA synthesis and mitosis, commence transcription, and survive. Examples of agents that are efficient at inducing DNA cross-links are UV light of 254 nm, nitrogen mustard, and psoralen plus UV light.

In addition, ionizing radiations (X-rays and gamma rays) and certain chemicals that generate active oxygen species, such as superoxide, can cause oxidative damage to DNA bases. Bleomycin and adriamycin are examples of chemicals that can generate superoxide. Superoxide can then dismutate in the presence of the enzyme, superoxide dismutase, which can lead to the generation of hydrogen peroxide. The reaction of hydrogen peroxide and additional superoxide in the presence of ferrous iron can then lead to generation of hydroxyl radicals. Hydrogen peroxide and hydroxyl radicals can oxidize DNA bases.

A fifth type of DNA damage results from the covalent binding of chemical mutagens and mutagenic chemical carcinogens to DNA bases. An example of this is the chemical mutagen and mutagenic chemical carcinogen, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG). MNNG is thought to generate methyl carbonium ions, which can bind covalently to the O-6 position of guanine, and to the N-7 position of guanine in DNA. The chemical carcinogen, benzo(*a*)pyrene (BaP), is metabolized to diol epoxide metabolites. One of the metabolites, the antibenzo(*a*)pyrene 7,8-dihydroxydiol-9,10-epoxide can bind covalently to the exocyclic amine of guanine in

DNA, leading to a covalent adduct. Covalent mutagen/carcinogen-DNA base adducts are very stable, and can lead to cytotoxicity, mutation, or carcinogenesis if they are not repaired properly.

DNA Repair

We now know that in bacteria, yeast, and mammalian cells, there are enzymatic systems that can repair damaged DNA. These systems are known as DNA repair systems.

Much of the repair that takes place in bacteria and in mammalian cells whose DNA has been damaged by chemical mutagens or by ionizing radiations, proceeds with a high degree of fidelity, and repairs the DNA damage correctly. However, a certain fraction of this repair proceeds incorrectly, and this misrepair leads to mutations. While some of this misrepair generates mutations and can be cytotoxic, a fraction of this misrepair is beneficial by generating mutations that can lead to genetic diversity in organisms and hence provides new organisms that can lead to evolution of various species.

In bacteria, we now recognize a number of DNA repair systems. The first repair system, which has been the most intensively studied and the best understood, is the system of photoreactivation repair, or direct repair. This repair system is very efficient at repairing thymine dimers formed between thymine bases in DNA by absorption of UV light of 254 nm by the thymine bases. In this type of repair, an antenna pigment, methylene tetrahydrofolate (MTHF), absorbs near UV light of wavelength 350 nm. MTHF then transfers the energy of this photon by Forster resonance energy transfer to reduced flavin adenine dinucleotide (FADH). FADH then transfers an electron to the thymine dimer, which decomposes it, returning it to its original state of two separate thymine bases in DNA. This repair takes place in the presence of the photoreactivating enzyme, which contains a pocket that binds to and holds the thymine dimer in place. Since this repair system returns the thymine dimer to its original separate thymine bases in DNA, no mutations occur during this process. Hence, this repair is said to be 'error-free', and it does not induce mutations. The photoreactivating enzyme has been cloned and sequenced, and X-ray crystallographic analysis of the photoreactivating enzyme has revealed the structure of this enzyme.

A second DNA repair system in bacteria is designated excision repair. This repair system efficiently repairs DNA strands that have been irradiated with UV light or ionizing radiations, oxidatively damaged, or that have chemical-DNA adducts in them. This system involves the steps of incision by an incision endonuclease proximate to the site of the damage, followed by excision of the damaged DNA bases by DNA polymerase I. Next, DNA polymerase I fills in the resulting nucleotide gaps by adding nucleotides complementary to the undamaged strand, using the undamaged strand as a template. Finally, DNA ligase seals the phosphodiester chain. This repair proceeds with a high degree of fidelity, and therefore only induces a very low frequency of mutations. Some authors refer to this as 'error-free' DNA repair, although a low frequency of mutations are created by this repair system.

A third type of DNA repair is called the SOS response. The SOS response involves the induction of two different types of DNA repair. In this situation, where there are thymine dimers in DNA due to UV irradiation of the DNA, or other DNA damage, a normally quiescent molecule, called the rec A protease, binds to the site of this DNA damage. The binding of rec A to damaged DNA causes the rec A protease to become catalytically active. The rec A protease then binds to various molecules of the lex A repressor that are already bound to the bacterial genome. Lex A repressor molecules normally bind to the SOS boxes of genes in the genome that encode endonucleases, exonucleases, helicases, DNA polymerases, and other molecules important in DNA repair. When the activated rec A protease binds to the Lex A repressors, this causes the Lex A repressors to autocatalytically cleave themselves. This results in the induction of the synthesis of ~ 50 protein molecules involved in DNA repair, among them an error-prone DNA polymerase. This error-prone DNA polymerase causes nucleotide synthesis to occur opposite the thymine dimers, with a low degree of fidelity. This leads to mutations in the DNA. In addition, during the SOS response, the rec A protease also acts as a recombinogenic enzyme. In this case, at a replication fork containing a thymine dimer, rec A-mediated recombination can occur to generate a situation in which there is at least one good template for DNA synthesis on each strand of the replication fork. While allowing DNA repair and hence DNA synthesis to proceed, rec A-mediated recombination is also a process that proceeds with a low degree of fidelity, with error rates of 1/1000, leading also to mutations. Similar types of DNA exist in mammalian cells.

Mutagenesis

Overview of Mutagenesis and Its Biological Significance

What is mutagenesis and why should we be concerned with this process? Mutagenesis is the process by which mutations are induced in the cells of organisms. Mutations are changes in the hereditary material of cells, their DNA, that cause observable changes in hereditary traits in offspring. These changes are transmitted to the ribonucleic acid (RNA), which is synthesized according to the instructions carried by the DNA, and then to proteins, which conduct chemical (enzymatic) reactions in the cell, or serve as structural materials, giving a cell its shape.

Mutations can have beneficial effects, deleterious effects, or no consequences in organisms. Certain mutations have a positive effect on the organism. The sickle cell mutation in the hemoglobin gene and hence the hemoglobin protein molecule, for instance, is thought to give humans in Africa an ability to survive malaria better. The resulting mutated hemoglobin aggregates in the red blood cells, leading them to assume a sickled shape, which makes it difficult for the malarial parasite to enter and infect the red blood cells. Many mutations are neutral and have no significant effect on the organism at all. However, certain types of mutations can have deleterious consequences in organisms. An example of a deleterious mutation in humans is one that destroys the activity of an enzyme called adenosine deaminase, leading to a deficient immune system and a consequent inability to fight disease, as occurred in the famous 'bubble boy'. Other deleterious mutations, such as mutations in the germ cells (sperm or egg cells), can lead to a predisposition to cancer, such as the Li-Fraumeni syndrome, and still other mutations can be lethal and result in nonviable offspring. Mutation is an inevitable process, and it is occurring all the time spontaneously. Mutations that lead to beneficial traits in an organism will be selected for, and mutations that lead to defects in critical cellular properties or to the death of the organism will be selected against, during the course of evolution.

During the past 100 years, we have learned a significant amount about the nature and effects of mutations on cell growth and survival and on the growth and survival of various organisms. Mutations have been most intensively studied in bacteria because bacteria grow very rapidly, and the mutations are rapidly expressed. From experimental studies, we now know that UV and ionizing radiations and specific chemicals called mutagens can induce mutations in bacteria, in yeast, in plants, in the fruit fly Drosophila, in mice, in single mouse cells in culture, in single human cells in culture, and in humans, although the last has been less well studied. We know a significant amount concerning mutations in the fruit fly, Drosophila melanogaster, and a significant amount concerning the effects of mutations in mice.

Using recently developed assays to detect mutations in humans by analyzing white blood cells, we are beginning to acquire knowledge concerning the induction of mutations in humans caused by ionizing radiations (e.g., the atomic bomb survivors in Hiroshima and Nagasaki), mutations caused by mutagenic cancer chemotherapeutic agents, and mutations caused by cigarette smoke. With current methods using techniques of molecular biology, we can detect the presence of certain mutations believed to be deleterious in humans, and genetic counseling can occasionally help certain families.

Scientists have also learned a substantial amount regarding cancer induction by mutagenic chemical carcinogens and mutagenic UV and ionizing radiations. A very important finding in this field is that UV and ionizing radiations and specific chemical mutagens induce mutations in specific cellular genes called proto-oncogenes, which activate them to oncogenes. Chemical mutagens and ionizing radiations can also cause amplification of the proto-oncogenes, leading to higher steady-state levels of the protein products of proto-oncogenes. These agents can also cause chromosomal breakage and translocation of a part of the chromsome(s) bearing proto-oncogenes to other chromosomes, where the proto-oncogenes can be placed under the control of different promoters of gene expression or fused with other genes, leading to aberrant proto-oncogene products. Mutagens also induce deleterious or inactivating mutations in other genes called tumor suppressor genes, inactivating them, or cause partial or full deletions of these genes, leading to loss of the tumor suppressor gene protein products. Combinations of activating mutations in proto-oncogenes and inactivating mutations in tumor suppressor genes, on the order of five to eight such mutations, in somatic (nongermline) or germ line cells play a key role in carcinogenesis, or the process of cancer induction caused by mutagenic chemical carcinogens, in humans. Specific details of the types of mutations that occur in organisms and their biological significance follow.

Definition and Description of Mutations

As has been well known since the pioneering experiments of Griffith, Avery, MacLeod, McCarty, Watson, and Crick, DNA is the genetic material of bacterial and mammalian cells. DNA encodes information in a triplet code which specifies the sequence of amino acids in proteins. Proteins are the enzymatic and structural polymers of cells. DNA consists of two antiparallel chains of nucleotides. Hydrogen bonds between bases on one strand and bases on the opposite strand constitute in the aggregate sufficient bond strength to keep the double helix of DNA intact. However, at room temperature, sufficient energy is deposited in the DNA helix that the hydrogen bonds constantly break and reform, leading the DNA base pairs to occasionally separate, and the DNA structure to 'breathe'. DNA is replicated in a semiconservative fashion, as shown by the original experiment of Drs. Meselson and Stahl, such that each original strand serves as a template on which a new complementary strand is replicated. In this replication, complementary DNA bases are added to bases on the original strand, such that guanine pairs with cytosine and thymine pairs with adenine, as accomplished by DNA polymerases. In bacteria, DNA polymerase accomplishes a large fraction of DNA synthesis, aided by DNA polymerase I, which works on the lagging strand.

The sequence of DNA is specified very precisely. A mutation is any change in the sequence of DNA bases from the original sequence of DNA bases. In the simplest form, during replication, the DNA polymerase enzymes can accidentally substitute an adenine for a guanine opposite a cytosine during DNA replication. This simple kind of mutation would be called a transition mutation, in which one purine, guanine, was instead replaced by another purine, an adenine base. Similarly, if during replication a pyrimidine base, such as thymine, was supposed to be inserted opposite an adenine base on the template strand, but instead a cytosine was inserted opposite the adenine, this would also be called a transition mutation. A transversion mutation is one in which a purine base substitutes for a pyrimidine base (guanine for thymidine) or a pyrimidine base substitutes for a purine base (cytosine for adenine). These types of mutations are also called base substitution mutations.

The next more complex type of mutation is referred to as an addition or deletion mutation. In a deletion mutation, one or more bases are removed from the DNA. In an addition mutation, one or more bases are added to the DNA. Addition mutations are also called insertion mutations. Deletion mutations are called small deletions if only a few bases are deleted from the DNA, or large deletions if many bases are deleted from the DNA. The same considerations hold for small-addition and largeaddition mutations.

So far, we have only considered mutations in which the genetic code is kept in strict register. As is commonly known, the genetic code is read in triplets, such that three nucleotides are read together to specify one specific amino acid. With base substitution mutations, only one base is changed, so the amino acid specified by the new triplet nucleotide specifies a new amino acid. However, the rest of the nucleotide sequence remains the same, so the protein specified only has one amino acid changed in it. This situation is similar in the case of addition and deletion mutations, provided the addition or deletion is three bases or a multiple of three bases. Of course, in this situation, there is gain or loss of one or more amino acids, and this can have severe consequences for the resultant protein, depending on where in the protein the amino acids are inserted or deleted. However, beyond the site at which the three base addition or deletion is induced, the genetic code remains in register, and the rest of the protein, beyond the addition or deletion mutation of three or a multiple of three nucleotides, will remain normal.

A special circumstance arises when one or two bases, or any multiple of one or two bases, but not three bases, are deleted or inserted into a DNA sequence. In this case, the sequence of bases encoding amino acids is now shifted. The original amino acids in the encoded protein are changed, and the code is shifted out of register at and beyond the site of this type of mutation. Hence, a new or 'scrambled' protein is produced from the site of the mutation onward. Such a special type of mutation is called a frameshift mutation, since the coding frame is shifted out of its original alignment. In this case, the structure of the protein is 'scrambled' from the site of the deletion or insertion on through the rest of the protein. In this case, the protein can have an altered structure, and if the protein is an enzyme, the enzymatic activity of the protein may be decreased or abolished.

Another simple type of mutation that needs to be considered is gene amplification. In this case, a gene is copied into many more replicas of that same gene. The extra copies of this gene are then inserted into the DNA. A further type of mutation is due to a translocation of a gene sequence. In mammalian cells, there is the additional complication that the DNA and its genes are arranged on discrete chromosomes. These chromosomes can be broken, and pieces from one chromosome attached to another chromosome, to form a structure known as a translocation. This can result in a deletion mutation if the sequences are not joined correctly and can also result in the translocated gene being placed next to a strong promoter element, which can cause the gene to be read more frequently, affecting expression of this gene.

Consequences of Specific Types of Mutations

The consequences of transition mutations and transversion mutations depend on where they occur in a gene coding for a protein. If they occur in a site that does not significantly change the shape of a protein used to maintain the structural integrity of a cell, or in a site that does not affect the structure of an enzyme, then they do not have a significant effect on the structure of the cell or on the enzymatic activity of a protein. If, however, the transition or transversion mutation occurs in a part of the protein that significantly changes its structure or decreases its enzymatic activity, then the mutation can have severe negative consequences for the survival of the cell.

Three base additions and deletions similarly may not have severe consequences if they occur in a region of the protein that does not affect the structure of the protein or its enzymatic activity. Of course, if these deletions and additions occur in critical parts of the protein that affect its structure, or in the active site of an enzyme, they can have significant effects on cell survival and the phenotype of the cell.

In general, the frameshift type of mutation is usually deleterious to protein structure and enzymatic activity. Such mutations 'scramble' the structure of the protein downstream from the mutation, and hence destroy the structural integrity of structural proteins and destroy the enzymatic activity of enzymes.

General Types of Mutagens: The Concept of Metabolic Activation

Broadly speaking, there are six general types of mutagens. First, there are mutagens that are 'fraudulent' DNA bases. These are bases whose structures are similar to but somewhat different in structure than the normal bases. An example of such a base is 5-bromouracil, which is similar in structure to the normal base thymidine and can substitute for thymidine in DNA, but which has different hydrogen properties and hence different base-pairing properties than thymidine. Approximately 33 large base analogs have been synthesized and their mutagenic properties studied.

Second, there is a group of mutagens called frameshift mutagens. These mutagens are, in general, large, planar aromatic molecules that can intercalate into the DNA. In the process of intercalation, the intercalators slip into the DNA and lie flat between two adjacent base pairs, with the plane of the intercalator lying flat upon the planar aromatic rings of the base pairs. New bonds are formed between the II electron clouds of the DNA bases and the intercalating aromatic molecules, which stabilizes the new intercalated structure. Treatment of cells with this type of mutagen increases the frequency of occurrence of frameshift mutations. Examples of such mutagens include acridine orange, acriflavine, and ethidium bromide. These molecules are highly fluorescent, and are commonly used to stain DNA in agarose gels, due to their intercalating and fluorescent properties.

The third group of mutagens are the direct alkylating agents. These mutagens generate methyl and ethyl carbonium ions, which are chemically reactive and readily bind covalently to nucleophilic groups on the bases of DNA, including but not limited to, the O-6 atom of guanine and the N-7 atom of guanine. Examples of these alkylating mutagens are MNNG, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and epoxides such as ethylene oxide. There are also alkylating agents that have two reactive groups on the same molecule, such as nitrogen mustard. Nitrogen mustard and similar molecules can bind to both strands of DNA, leading to a cross-link between them, or to two places within one strand of DNA, leading to an intrastrand cross-link. UV light can also cause formation of intrastrand or inter-strand cross-links in DNA.

Fourth, there is a large group of mutagens referred to as premutagens or promutagens. These compounds are chemically inert and usually very hydrophobic. All organisms must metabolize these hydrophobic compounds to make them sufficiently water soluble for excretion from the cell membrane and from the organism. Otherwise, these hydrophobic molecules will bioaccumulate in the hydrophobic regions of membranes of cells, causing inhibition of the functioning of enzymes in membranes, inhibition of membrane transport functions, and damage to the integrity of the membrane, which delineates the cell from its environment. Examples of such hydrophobic molecules are the polycyclic aromatic hydrocarbons (PAHs), such as the ubiquitous environmental pollutant and carcinogen, benzo(a)pyrene. In order to remove these hydrophobic molecules from cells, organisms utilize cytochrome P450 enzymes plus atmospheric oxygen, plus reducing equivalents such as reduced nicotinamide adenine dinucleotide phosphate (NADPH) and reduced nicotinamide adenine dinucleotide (NADH) to epoxidate the hydrophobic molecules, in the case of PAHs, activating them to mutagens. This step results in the generation of an active mutagen, in the form of an epoxide. This active mutagen can cause mutation in the organism, although most of this reactive molecule will subsequently react with water via the enzyme epoxide hydrase or with glucose, sulfate, or glutathione via enzymatic processes to form water-soluble conjugates that can be excreted in the urine. Examples of such promutagens or premutagens are the PAHs (e.g., benzo(*a*)pyrene) and the aromatic amines (e.g., β -naphthylamine). An additional unique promutagen is a metabolite of the fungus, Aspergillus flavus, called aflatoxin B1. Aflatoxin B1 serves as a biocide against other microorganisms to preserve the ecological niche of *Aspergillus flavus*. Other examples of promutagens include the large group of nitrosamines. Dimethyl-nitrosamine, or DMN, is one example of a potent mutagenic, carcinogenic nitrosamine.

Fifth, it must be pointed out that the PAHs can intercalate into DNA and also are metabolized to active alkylating agents such as epoxides. Hence, PAH and similar promutagens form a fifth set of complex mutagens that can intercalate into and alkylate DNA bases, therefore inducing base substitution and frameshift mutations. Other compounds that bind specifically to DNA in a physical sense, such as aflatoxin, and are metabolically activated to epoxides that bind covalently to DNA bases, are also included in this group of complex mutagens, which can both bind to DNA and generate alkylating moieties upon metabolism by cytochrome P450 enzymes.

A sixth type of agent that is active at the DNA level is metal salts. These include compounds of the elements nickel, chromium, and arsenic (a metalloid). Some metal salts are carcinogenic when inhaled by lower animals, cause chromosomal damage in cultured murine and human cells, and are carcinogenic to occupationally exposed humans. Soluble hexavalent chromium compounds are mutagenic and clastogenic. Particular insoluble chromium compounds containing hexavalent chromium induce lung tumors when inhaled by lower animals. Specific insoluble chromium compounds can also be inhaled by humans in the chrome plating or chromium-manufacturing industries, and can induce lung cancer. Specific insoluble nickel compounds, such as nickel subsulfide and black and green nickel oxides, are carcinogenic in animals. In nickel refinery operations in the past, humans have inhaled mixtures of soluble and insoluble nickel compounds, and have shown increased incidences of nasal and lung cancer. Specific insoluble nickel compounds are phagocytosed into cultured murine and human cells and cause oxygen radical generation, chromosomal breakage, and inhibition of DNA methylation in these cells. Arsenic compounds are carcinogenic in humans, in the context of copper smelting operations, where arsenic compounds contaminate copper ores. Roasting copper ores leads to generation of arsenic trioxide, which induces lung cancer in humans. Drinking water contaminated with arsenic compounds also leads to lung cancer and other cancers in humans in Taiwan, where the artesian wells are contaminated with arsenic compounds. Arsenic compounds induce lung cancer, urinary bladder cancer, skin cancer, liver cancer, and leukemias. Arsenic compounds are believed to generate oxygen radicals, which can induce DNA damage.

Molecular Mechanisms of Mutagenesis

Mechanistically, the simplest type of mutagenesis occurs when the enzyme DNA polymerase is copying one strand of DNA into its complementary strand and places the incorrect nucleotide into the newly synthesized strand of DNA. Although it is thermodynamically favored that the correct base will be inserted, there is a lesser but real probability that the incorrect base will be inserted during DNA replication. An example would be placement of the wrong base, adenine (A), opposite the DNA base cytosine (C), instead of inserting the correct base guanine (G) opposite the base C. This results in what is described as a G/C to A/T transition mutation, and it is called a spontaneous mutation.

A second type of induced mutagenesis occurs when an alkylating agent, such as MNNG, reacts with the base guanine in DNA and places a methyl group on the oxygen in the 6 position of guanine to form an ether linkage. During the normal replication of the DNA, guanine pairs with cytosine, with which it makes three hydrogen bonds. When guanine is methylated on the oxygen in position 6, due to treatment with MNNG, this methylated guanine now incorrectly pairs with an incoming thymine instead of the cytosine with which a normal guanine would pair. This results in a G/C to A/T transition mutation.

Deletion and addition mutations are caused when the DNA 'breathes' or opens its structure and occasionally a piece of this DNA loops out. This occurs because the energy of room temperature (kT, where k is the Boltzmann constant), is sufficient to break apart some of the hydrogen bonds which hold the two DNA strands together. When a part of a DNA strand loops out, and when an intercalator molecule, such as acridine orange or ethidium bromide, subsequently intercalates into the DNA strand at a looped out structure, it can stabilize this looped out structure. DNA repair enzymes can then recognize this looped out structure as an aberrant structure, and can then excise this structure out of the DNA by use of incision endonucleases and DNA polymerase I. This process would then result in a deletion mutation. Addition mutations may be caused by insertion of extra DNA bases during DNA replication. This can happen spontaneously. It is also thought to happen when a DNA polymerase either slips along runs of GC base pairs, or when the polymerase incorrectly recognizes the intercalator molecule as a DNA base.

As mentioned previously, the planar PAHs are very complex molecules. They can intercalate into DNA. In addition, by virtue of their being metabolized into epoxides, they can also covalently bind to DNA. Hence, they can cause base substitution mutations (transitions and transversions) and can also cause frameshift mutations when they are copied by the DNA polymerase incorrectly and incorrect nucleotides are inserted into the DNA, leading to mutations in the DNA. PAH bound to DNA also cause a labilization of the DNA base-sugar bond, leading to depurination of the PAH-adducted base. During DNA repair, the DNA polymerase occasionally adds the correct base back at the depurinated site, but often adds an incorrect base, leading to frequent transversion mutations at the depurinated site.

Bacterial Mutagenesis Detection Systems

Two assays in bacteria are commonly used to detect and study the molecular mechanisms of mutation in bacteria and also to screen chemicals to determine whether they are mutagenic to bacteria. The most commonly employed assay is that of reversion of mutant Salmonella typhimurium bacteria back to wild type or normal bacteria, developed by Dr. Bruce Ames and colleagues and the University of California at Berkeley. In this assay, suspect mutagens plus a source of metabolic activation in the form of S-9 are added to and incubated with S. typhimurium bacteria. S-9 is a preparation of rat liver, in which the rat liver has been homogenized, then centrifuged at 9000 g, and the supernatant is utilized. Rat liver S-9 contains cytochrome P450 and other xenobiotic metabolizing enzymes. The specific Ames' strains of S. typhimurium contain mutations in the genes encoding enzymes that biosynthesize histidine, such that these mutant bacterial strains cannot grow unless exogenous histidine is provided. In this assay, the investigator counts the number of mutated bacteria that can now grow in medium lacking histidine, and scores these as reverted mutant bacteria. This assay is very effective at detecting the mutagenicity of chemicals. Since reversion is very specific, it is common for investigators to use two Ames' strains to detect base substitution mutations, two Ames' strains to detect frameshift mutagens, and one strain of bacteria to detect mutagens that generate oxygen radicals. This assay also identifies 50% of all chemical carcinogens that are mutagenic carcinogens by detecting their mutagenicity. This assay is commonly used by industrial firms such as pharmaceutical companies, by governmental agencies charged with regulating the containment of carcinogenic substances, and by researchers interested in identifying new chemical mutagens and in understanding the molecular mechanisms of chemical mutagenesis. It is a rapid assay that takes only 2 or 3 days to complete and is relatively inexpensive (\$500 or less per assay in the commercial sector).

A second mutagenesis assay, formulated by Dr. William Thilly and colleagues at the Massachusetts Institute of Technology is based on the Ames' assay. In this assay, the Ames' Salmonella bacterial strains are used, but the assay is a forward mutation assay in which bacteria are treated with chemical mutagens plus or minus S-9 metabolic activation. Then, the number of bacterial colonies resistant to the toxicity of 8-azaguanine are scored as mutant colonies. This assay has been claimed to be more sensitive than the original Ames assay because the entire genes should be the targets for mutagenesis, as opposed to small parts of histidine-synthesizing genes in the Ames' reversion assay. However, this assay is not employed as frequently as the Ames' assay. A large body of work has been done to characterize and understand the molecular bases by which the Ames' assay functions, and a very large number of mutagens have been detected and studied in the Ames assays. Hence, today, the Ames' assay remains the assay of choice to detect bacterial mutagens.

In both bacterial mutagenesis assays, one plots the number of mutant colonies on the ordinate (y-axis) versus the concentration of test compound on the abscissa (x-axis). The plots are usually linear up to the point at which cytotoxicity overwhelms mutation induction, and the number of mutants passes through a maximum and then declines. Hence, bacterial mutagenesis studies are usually conducted with low concentrations of chemical compounds or radiations that induce a linear frequency of mutations but do not cause significant cytotoxicity.

Mammalian Cell Mutagenesis Assays

In mammalian cells, it is common to utilize mutagenesis assays that measure induction of mutants that are resistant to the cytotoxicity of toxic drugs. One of the most frequently employed mutation assays in mammalian cells is the assay detecting mutation conferring 6-thioguanine resistance. This assay is most frequently employed in the Chinese hamster ovary (CHO) cell line or in the V79 Chinese hamster lung fibroblast cell line. In this assay, the normal or 'wild-type' cells are killed by the cytotoxic drug 6-thioguanine, or its closely related analog, 8-azaguanine. These drugs enter mammalian cells and react with the cellular metabolite, 5'-phosphoribosyl-pyrophosphate (PRPP), to form a toxic nucleotide which is incorporated into DNA and RNA, leading to cell death. In the mutation assays, the cells are treated with the suspect mutagen plus and minus S-9 (cytochrome P450) metabolic activation. Next, the cells are reseeded into new cell culture medium containing 6-thioguanine or 8-azaguanine, which is called a mutant-selective agent. 6-Thioguanine or 8-azaguanine (they have similar effects) kills all the wild-type cells at sufficiently high concentrations and therefore selects against the wild-type cells. However, both spontaneous and mutagen-induced mutant cells are resistant to the cytotoxicity of 6-thioguanine and continue to grow and form discrete colonies. These mutant colonies are then fixed to the dishes with methanol or 70% ethanol, stained with the nuclear stain, Giemsa, and then scored with a microscope. One then plots the mutant frequency (number of viable mutants/cell culture dish)/(plating efficiency of cells x number of cells seeded per dish) on the ordinate (y-axis) versus the concentration of the mutagen on the abscissa (x-axis). For a strong mutagen, there is usually a dose-response effect, that is, the mutant frequency increases, usually linearly, as a function of increasing concentration of mutagen added to cells.

The mutant colonies are resistant to the cytotoxicity of 6-thioguanine because they have been mutated at the gene encoding the enzyme hypoxanthine guanine phosphoribosyl-pyrophosphate (HGPRT), such that the activity of this enzyme is decreased or abolished. This enzyme carries out a reaction between the DNA bases hypoxanthine or guanine and PRPP to form inosine and guanosine, which are then incorporated into DNA and RNA. In mutant cells, the gene encoding the HGPRT enzyme, hence the enzyme itself, is mutated. Therefore, the enzyme has a substantially reduced ability, or no ability, to carry out this condensation. The mutant cells cannot react toxic 6-thioguanine with PRPP to form a toxic nucleotide and are therefore resistant to the cytotoxicity of 6-thioguanine. They therefore survive and form colonies even in the presence of 6-thioguanine. Plotting the mutation frequency versus the concentration of mutagen added yields linear curves. This is a general assay and, as a forward mutation assay, it detects base substitution, addition, deletion, and frameshift mutations. It is one of the most widely used mutation assays in mammalian cells.

A similar assay is one in which L5178Y mouse lymphoma cells containing one active and one inactive gene encoding thymidine kinase are selected in trifluorothymidine. This treatment is lethal to wildtype cells. However, spontaneous mutants, and those induced by mutagens, have mutations in the second copy of the thymidine kinase gene, and hence do not phosphorylate trifluorothymidine and are resistant to the toxicity of this drug. This mutation assay is very valuable, because it detects point mutations, and it also detects mutations involving large amounts of damage to the chromosome, measured as resistant colonies with a small size.

A third assay that has been used to detect mutations in mammalian cells induced by chemical carcinogens and UV and ionizing radiations is that of mutation to ouabain resistance. Ouabain is a cardiac glycoside that binds specifically to the sodium, potassium adenosine triphosphatase ((Na,K) ATPase). The (Na,K) ATPase is an enzyme located in the plasma membrane of mammalian cells. This enzyme hydrolyzes ATP, and uses the resultant energy liberated to drive electrogenic transport of sodium ion out of the cell and transport of potassium ion into the cell. The transport is not equal, and three sodium ions are transported out of the cell, while two potassium ions are transported into the cell. The resultant electrogenic gradient is used to drive the transport of glucose and certain types of amino acids into the cell and to regulate cell volume, which are all crucial for cell survival. When cells are treated with the cardiac glycoside - ouabain, ouabain binds to a specific binding site on the (Na,K) ATPase, and inhibits the activity of this enzyme. As a result of this binding, the enzymatic activity of the (Na,K) ATPase is inhibited, and the transport of glucose and certain amino acids, and the regulation of cell volume, which are dependent on the activity of the (Na,K) ATPase, are inhibited, and cells die. Hence, ouabain at a concentration of 3 mM will kill murine fibroblasts down to spontaneous mutant frequencies (one mutant/one million wild-type cells). Treating murine fibroblasts, such as C3H/10T1/2 Cl 8 mouse embryo cells, with mutagens such as MNNG or BaP, induces mutations in the gene encoding the (Na,K) ATPase, such that the ouabain binding site is mutated. Hence, ouabain will no longer bind to the (Na,K) ATPase, and these mutant cells are resistant to the cytotoxicity of ouabain and will form mutant colonies in the presence of ouabain. This assay detects a restricted set of base substitution mutations which will mutate the ouabain binding site, but otherwise not affect the other enzymatic properties of the (Na,K) ATPase. It is thought that frameshift mutagens produce ouabain-resistant mutants that have a sufficiently damaged (Na,K) ATPase that these mutants are not viable.

Chromosome Breakage and Micronucleus Formation

In mammalian cells, the genes are arranged on discrete chromosomes. There are many assays that have been developed in mammalian cells to measure the ability of specific chemicals and ionizing and UV radiations to induce damage to these chromosomes, referred to as chromosomal aberrations. In these assays, the cells are seeded, treated with the chemical

or radiation of interest, then treated with colcemid to arrest the cells in metaphase. The cells are then swelled in hypotonic potassium chloride. The swelled cells are then dropped onto microscope slides, which bursts the cells and produces what is called a metaphase spread, or display of chromosomes. The chromosomes in these metaphase spreads are then stained with the nuclear stain, Giemsa stain, and examined by microscope to quantitate the numbers of chromosome aberrations present in the treated cells. Typical chromosomal aberrations that are scored include gaps, breaks, dicentrics (where parts of two chromosomes fuse, such that the resultant structure bears two centromeres), satellite associations, and ring chromosomes. Most mutagenic chemical carcinogens and ionizing and UV radiations have the ability to induce chromosome aberrations in mammalian cells. Translocations, in which one chromosome is broken and one or more parts of it are fused to another chromosome, can also be recognized with this assay. At sufficiently low concentrations, where cytotoxicity exerted by a chemical is sufficiently low, the induction of chromosome aberrations by chemical mutagens is linear as a function of the concentration of the chemical mutagen tested. Chemicals and radiations that can induce chromosome aberrations are referred to as clastogens.

A second type of damage that can be induced by chemical mutagens and ionizing and UV radiation is called micronucleus formation. In this assay, cells are treated with the chemical or radiation of interest, and then the cells are treated with cytochalasin B to inhibit cytokinesis. The cells are then visualized by staining with acridine orange, and micronuclei can be visualized under the microscope. Micronuclei are vesicles containing one or more whole chromosomes or pieces of chromosomes. The implication of observing micronuclei is that on further rounds of cell division, these micronuclei containing chromosomes or pieces of chromosomes can be lost from cells. Hence, formation of micronuclei indicates that large amounts of genetic material can be lost from cells, as much as that contained on an entire chromosome.

Induction of Morphological and Neoplastic Transformation in Mammalian Cells: Cell Culture Models for Chemically Induced Cancer Proceeding through Mutagenesis

There is a group of assays that can be utilized to study the ability of chemical carcinogens or UV or ionizing radiations to convert normal cells into cancerous (transformed or tumor) cells when normal cells are grown in cell culture and treated with these carcinogens. One property of normal cells, particularly fibroblasts, is that they divide, grow, and eventually fill a surface such as that of a tissue culture dish, and then stop growing. This is because cells in contact transmit signals through their membranes, on through the cytoskeleton (cell skeleton), and on to the cell nucleus, which triggers a negative feedback signal, which forces cells to stop growing. This property is referred to as contact inhibition of cell division. In cell culture, this property is manifested when the cells grow and fill the cell culture dish with a layer of cells one cell thick. However, when fibroblastic cells are treated with a chemical carcinogen that is already activated to an electrophile, or that can be activated by the specific types of cytochrome P450 enzymes the cell possesses to an electrophile, then 1% or less of the cells are 'transformed', or changed. They have lost contact inhibition and now grow on top of one another in arrays where the cells 'criss-cross' over one another. Since the fibroblastic cells are changed in shape or morphology, we refer to this as 'morphological transformation', or change in cell shape. This change in cell shape manifests itself as an overgrowth of the cells above the monolayer, in small piles, referred to as foci of transformed cells. Foci are easily seen when cells are stained with specific dyes, such as Giemsa stain or crystal violet. The stained foci can then be counted under the microscope. Scoring foci is an assay for chemical carcinogens since it detects one of the five steps that must be accomplished for fibroblastic cells to become able to form tumors when injected into immunosuppressed mice.

A second property of most normal cells is that they need to anchor to a surface, such as that of a tissue culture dish, in order for them to replicate their DNA and divide. This property is referred to as 'anchorage dependence'. Normal cells cannot grow in suspension in liquid media. An exception to this is white blood cells, which can grow in liquid suspension because this is their normal milieu in the circulating blood. However, most cells, particularly connective tissue cells like fibroblasts, cannot grow in liquid suspension and are anchorage dependent. When fibroblasts are treated with chemical carcinogens that are already activated to alkylating agents, or if the cells themselves have the cytochrome P450 enzymes to activate the carcinogens they are treated with, then a small fraction, on the order of 1 in 1 million, of the normal cells will be converted into anchorageindependent cells. Anchorage independent cells can grow and form colonies in liquid suspension, in a semi-solid medium such as 0.5% agar, which has the approximate consistency of jello. Anchorage independence is another property that must be acquired by fibroblasts before they can become tumorigenic.

Normal cells also have a finite lifespan and undergo ~ 60 population doublings, then senesce or die through a process of programmed cell death. When cells are treated with chemical carcinogens or UV or ionizing radiations, a small fraction of them (1% in mouse cells and far less in human cells) can become transformed to immortality, such that they now grow forever. Acquisition of cellular immortality is a third step on the road to tumorigenicity, and can be acquired when cells are treated with chemical carcinogens or UV or ionizing radiations. Cellular immortality is relatively easy to induce in cultured murine cells, and much more difficult to induce in cultured human cells, likely due to the greater stability of the chromosomal complement of human cells compared to that of murine cells.

A final step on the route to development of tumor cells involves conversion of fibroblastic cells that have become immortal, morphologically transformed, and anchorage independent into tumor cells. This can occur spontaneously or upon treatment of cells with chemical carcinogens or UV or ionizing radiations.

What changes in the DNA result in the induction of the changes in cell properties (phenotypes) that we refer to as morphological transformation, anchorage independence, cellular immortality, and tumorigenicity? There are two broad classes of genes that control cell growth in a positive way: the proto-oncogenes, which control cell growth and signal transduction, and the tumor suppressor genes, which inhibit cell growth. Chemical carcinogens can react with and cause mutations in, amplification of, or translocation of proto-oncogenes, converting them into active celltransforming genes known as activated oncogenes. There are ~50 proto-oncogenes that can be converted into activated oncogenes.

There are four broad classes of proto-oncogenes. One class of proto-oncogenes encodes protein products localized in the cell nucleus which act as factors that activate transcription of specific genes. This group is exemplified by genes such as *c-myc* and *c-jun*. A second group is located in the cell membrane or the cytoplasm and transfers biochemical signals in the cell. A prominent example of this group is the *c*-Ha-*ras* proto-oncogene. The protein product of this gene binds the high-energy molecule guanosine triphosphate, which activates this protein such that it transfers signals to other proteins and eventually toward the nucleus. The protein products of the last two groups of proto-oncogenes are located at the membrane and are growth factors and receptors for these growth factors. The *c-sis* gene is an example of this type of proto-oncogene. The significance of these genes is that mutational or other types of activations of two or more of these genes play a role in inducing transformed phenotypes and contribute to formation of a tumor cell.

There are ~ 50 tumor suppressor genes that have been identified to date. Chemical carcinogens cause mutations in these genes to inactivate them, or can break the chromosomes on which these genes are located, or can cause loss of an entire chromosome bearing a tumor suppressor gene from the cell. Inactivation of the two copies of a tumor suppressor gene renders it inactive in the cell. Inactivation by chemical carcinogens of two or more tumor suppressor genes, in concert with activation of two or more proto-oncogenes into oncogenes, cumulatively leads to formation of a tumor cell. Examples of tumor suppressor genes include the retinoblastoma (Rb) gene and the p53 tumor suppressor gene. Inactivations of tumor suppressor genes and activations of specific oncogenes have been found in all human tumors that have been studied intensively to date.

Tier Concept for Screening and for Detecting Chemical Mutagens and Mutagenic Chemical Carcinogens

Genetic toxicology, the science by which chemicals and agents that cause mutation and other changes to DNA are studied, has evolved substantially during the past 50 years. It is now standard practice to detect chemical mutagens by utilizing the Ames' bacterial mutagenesis assay and other bacterial mutagenesis assays, both with and without S-9 metabolic activation, and to utilize assays detecting mutation to 6-thioguanine resistance in CHO or V79 mammalian cells. There are also assays that detect the ability of chemicals to cause chromosome breakage and micronucleus formation in Chinese hamster V79 cells or CHO cells by examining chromosomes of these cells under a microscope after chemical carcinogen treatment.

Assays that detect the ability of chemical carcinogens or UV or ionizing radiations to induce unscheduled DNA synthesis, or DNA repair, are also commonly used. In one of these, autoradiography is used to measure the incorporation of tritiated thymidine into the DNA of cells treated with these carcinogens. All the assays mentioned above are commonly used at the same time to detect the genotoxic properties of chemicals or radiation in what is referred to as a battery of tests. This battery of tests usually constitutes what is termed a primary screen for mutagens, and is used to determine whether a specific chemical is a mutagen.

In addition to detecting chemical mutagens, these methodologies can also be used to detect chemical carcinogens. The most certain way to detect chemical carcinogens is to paint the skin of animals with solutions or suspensions of carcinogens, to feed carcinogens to animals, or to add carcinogens to the animals' drinking water, and observe whether the animals develop tumors. Unfortunately, treating mice or rats with chemical carcinogens and assaving for tumor induction takes >2 years and costs \sim \$5 million per chemical tested. This price is simply too large and the time too long to permit scientists to use animal bioassays to screen widely and routinely for the carcinogenicity of the hundreds of thousands of chemicals that need to be studied. During the past 20 years, an alternate strategy to detect carcinogens has arisen. This strategy is referred to as a tier screening strategy and relies on the fact that $\sim 50\%$ of the known carcinogens are mutagens. Hence, the current strategy relied on by most government, regulatory, and academic laboratories is to use inexpensive, rapid assays to test the large number of chemicals in use today in what is referred to as a primary screen using a battery of genetic toxicology assays, as outlined above. In this screen, one would use the Ames' bacterial mutagenesis assay, an assay for chromosome breakage, occasionally an assay to detect micronucleus formation, an assay to detect induction of DNA repair, and a mammalian cell mutagenesis assay. Chemicals that cause mutagenesis, chromosome breakage, or DNA repair in these assays would be considered suspect carcinogens. Further product development on these chemicals would likely be halted or the chemicals would be modified in their structure. Chemicals negative in this primary screen that were proposed to be used for human applications, such as food additives or cosmetics, would then be tested in cell transformation assays for the ability to induce morphological or anchorage-independent transformation, in what is considered a secondary screen. Chemicals positive in cell transformation assays are then eliminated from further development or chemically modified so they no longer cause cell transformation. Finally, chemicals to be marketed as cosmetics or food additives would then be tested in whole animal carcinogenesis assays. Only those that were not carcinogenic would ideally then be marketed to the public or used in commerce where large numbers of people would be exposed to them.

See also: Ames Test; Analytical Toxicology; Carcinogen Classification Schemes; Carcinogen–DNA Adduct

Formation and DNA Repair; Carcinogenesis; Cell Proliferation; Chromosome Aberrations; Dominant Lethal Tests; Host-Mediated Assay; Molecular Toxicology– Recombinant DNA Technology; Mouse Lymphoma Assay; Radiation Toxicology, Ionizing and Nonionizing; Reproductive System, Female; Reproductive System, Male; Sister Chromatid Exchanges; Toxicity Testing, Developmental; Toxicity Testing, Reproductive.

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Genetically Engineered Foods

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Introduction

Genetically engineered foods are a subset of genetically modified organisms (GMOs). The definition of a GMO may vary depending on the source of the information. However, the World Health Organization (WHO) specifically defines GMOs as "...organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally."

Farmers began using GMOs for the growth of soy-beans, potatoes, and corn in the mid-1990s finding benefits ranging from reduced operating cost to enhanced crop production. However, the use of GMO technology had not been without controversy then and is still debated now. Concern over the use of GMOs became heightened by the inadvertent introduction of a genetically modified corn (Star-LinkTM) into the human food supply. Ostensibly registered by the US Environmental Protection Agency for use as an insect resistant plant pesticide and animal feed, the StarLinkTM controversy propelled its producer to voluntarily cancel its registration and cease production. This episode in the evolution of GMO production has since then prompted citizen's watch groups, nongovernmental organizations (NGOs), and governmental agencies worldwide to address concerns regarding the use of GMOs ranging from specific toxicity to humans, plants, fish, and livestock to fear of large scale ecosystem disruption.

Current Technologies

In its simplest terms, the production of GMOs is derived from manipulation of the subject genome in order to achieve some desired trait or end result. Therefore, traditional agricultural practices intended to manipulate breeding or reproduction to select for desired traits over a somewhat protracted period of time can be thought of as genetic modification practiced over thousands of years. While drawing on these long used natural selection methods, current technologies have now been developed to achieve rapid and more dramatic results such as crop resistance to insects, production of novel pharmaceuticals, and increased animal growth and milk production. Thus, a brief outline of the essential gene manipulation technologies is fundamental to further understanding of potential global health concerns.

Transgenic Manipulation

Transgenic manipulation refers to the insertion, removal, and modification of the plant genome for placement into an individual of the same species, or across species to achieve the desired results in a relatively rapid period of time.

Two widely used techniques developed to achieve these ends are termed *in vitro* and vector-based techniques. *In vitro* techniques mechanically insert or inject a specific protein, gene, or genetic sequence into the subject organism. Within that general category, three methods are often defined; microinjection, particle or microprojectile bombardment, and DNA uptake directly into organism. Alternatively, vector-assisted techniques use live viral or bacterial carriers (i.e., vectors) to facilitate transfer of genetic material into the subject organism. In Vitro Methods Microinjection in vitro methods use a fine needle and microscopic manipulation to directly inject the gene material into protoplasts of the subject organism. The difficulty and cost of the microinjection process may be circumvented by DNA transfer via protoplast mixing. In this method, the 'protoplast-associated' DNA of one individual is allowed contact with that of another individual in a polyethylene glycol environment that is favorable to DNA exchange. Subsequent replication processes of the genetic material facilitate incorporation of the desired gene into the subject organism for an increase in copy number and favorable trait selection. Temporary disruption of the cell membrane via electrical stimulation (i.e., electroporation) can be used to enhance the transfer efficiency of this process.

Another often-used *in vitro* technique is 'particle bombardment'. In this method, microparticles of tungsten or gold are coated with the transgenic material and are literally 'shot' into the plant cell with compressed helium gas or an electrical discharge. Recombination and replication of the DNA material transpires within the subject organism DNA prior to manipulations to increase copy number and trait selection.

Vector-Facilitated Methods Vector-facilitated methods depend on the use of nonpathogenic viruses, or sections of bacterial DNA to transfer portions of the transgene or the transgene in its entirety using the biological invasive capabilities of the vector. Essentially, the vector DNA is modified and subsequently allowed to 'infect' the subject DNA to facilitate DNA transfer to the host. These vectors are frequently engineered to eliminate their virulence yet retain the DNA transfer capability of the original pathogen.

Marker Gene Incorporation

The mere delivery of the transgene into the host species or individual does not guarantee that the production of a GMO will be successful. This is due to the relative inefficient transfer process and the somewhat random selection process that must subsequently be used to enhance the number of individual copies of the transgene for further trait development. In order to address this limitation, genes or DNA segments coding for known and readily observable phenotypes (i.e., marker genes) are 'coinserted' with the transgene to better detect successful transformations. Such marker genes are now commonly used to detect color expressions or other visible attributes for better identification and subsequent selection and enhancement of the desired trait.

Commonly Produced GMOs

GMO technologies discussed above and others have been used to create a variety of modified crop, fish, and animal species for crop prey resistance, pharmaceutical development, and increased production from livestock. It is these applications that have attracted public and scientific interest over the years. As will be noted below, several organisms are currently used, though it should be anticipated that additional organisms will be employed in the future.

Genetically Modified Crops

The use of genetically modified corn and cotton has increased over 10-fold from 1992 to 1999 and as of 2002, 50 crop species have been evaluated for uses by the US Food and Drug Administration (FDA). In the development of transgenic crops, genes isolated from several varieties of the bacterium, *Bacillus thuringienses* (Bt) are probably the best known and most often cited example of GMO development.

The use of this bacterial species has been deemed to be ideally suited for GMO use due to possession of toxins known as delta endotoxins. The structure of the delta endotoxin, is complex, containing three major regions or sections that each connote differential characteristics to the ultimate toxin (Figure 1).

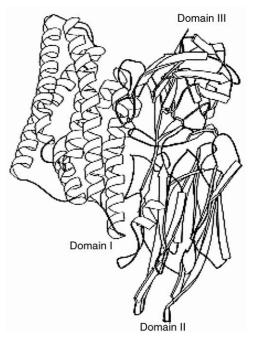


Figure 1 Molecular structure of *Bacillus thuringienses* delta endotoxin. (Reproduced from Li J, Carroll J, and Ellar DJ (1991) *Nature* 353: 815–821, with permission of the Nature Publishing Group.)

The domains of the molecule have markedly different conformations that control key aspects of 'Bt' toxicity to target insects. The α -helical bundles of the domain I region can be inserted into the gut cell membrane of the target species, thus, facilitating ion leakage. The domain II region consists of three β -sheet conformations that are structurally similar to immunoglobulin antigen binding regions, suggesting that the key characteristic section resides in the gut area. Domain III is a densely packed structure that is believed to protect the exposed end of the toxin from cleavage by digestive proteases; thus helping to ensure structural integrity and subsequent toxicity to the target species.

By incorporating these characteristics of the Bt toxin into valued crop species, crop resistance to insects feeding on crop species may be enhanced helping to increase vitality, size, and acreage yields.

Coded genes for the Bt delta endotoxin production have been isolated for insertion into a variety of vegetables including potatoes, field corn, sweet corn, and soybeans.

Genetically Modified Fish

No genetically modified fish have been approved for entry into the US human food supply as of this writing although growing pressure for such use is anticipated. However, as with crop species, the use of GMO technologies in fish has been deemed useful for obtaining an increase in production, size, disease resistance, optimal food consumption, and pharmaceutical development. Common species selected for research into the application of transgenic manipulations include salmon, tilapia, channel catfish, and medaka.

Alternatively, very active research in the use of transgenic fish as research models has been ongoing for several years with ever increasing focus on their use in the investigation of mutagenicity and environmental toxicology. Typical organisms for such applications tend to be small and easily cultured species such as medaka, mummichog, zebra fish, and others displaying favorable genetic attributes.

Transgenic fish were noted as being first introduced into the research community in 1985. Since that time, the use of transgenic fish to refine methods of transgene manipulation and application as potential analytical tools has grown substantially in the assessment of mutation frequencies. Several mutation assays using bacteriophage and plasmid vectors have been developed. Examples are shown in **Table 1**. As can be noted, detection methods may be based on modifications in enzyme systems that become evident in subsequent growth of bacterial species on selective growth media.

In concert with the mutation assays that are shown in **Table 1**, the potential use of transgenic fish in the assessment of environmental health risk assessment offers opportunities to further assess the effects of water- and sediment-associated contaminants in the environment.

Genetically Modified Livestock

The genetic modification of livestock stems from a desire to enhance growth, increase production of high protein milk and cheese, facilitate biomedical research, as well as potentially protect against incidental toxicity via exposure to pesticides that may be associated with food crops. Animal genomes that have been successfully modified include sheep, pigs, cows, rabbits, and chickens. Two key research areas applied to livestock are discussed below.

Enhanced Production Growth, as a primary metric of production, has been and is the subject of much research. Of substantial interest is the potential for use of transgenes in stimulating 'overproduction' of growth hormones to enhance animal growth. Several test protocols have been evaluated with moderate success in various livestock models.

For instance, blood levels of zinc-induced growth hormones have been increased in pigs and sheep. However, such activity was minimal in pigs and negligible via attempted phosphoenolpyruvate carboxykinase induction. Enhanced growth, via increased production of growth hormones also has been used to modify the qualitative nature of the animal such as lean muscle mass. However, such manipulations were not without negative effects such as renal failure and gastric ulcers of the subject animal.

Table 1Various transgenic fish mutation assays

Assay	Vector/fish species	Detection method
lacl Mutation assay	Bacteriophage λ LIZ vector/medaka	α-Galactosidase functionality
cll Mutation assay	Bacteriophage λ/medaka	Plaque formation
rpsL Mutation assay	pML4 Plasmid vector/zebra fish	Kanamycin and streptomycin resistance
lacZ Mutation assay	Plasmid pUR288 vector/medaka and mummichog	Galactose-sensitivity

Pharmaceutical Development As of 2001, three companies were engaged in the manufacture of ~ 30 pharmaceuticals to produce proteins and antibodies derived from transgenic procedures. The transgenic-mediated production of pharmaceuticals is primarily achieved by facilitating construction of proteins that can then be expressed in milk of organisms are often termed 'bioreactors'. Examples of biologically active proteins that have been produced include antitrypsin, tissue plasmogen activator, and human blood clotting factor.

Human Health Risk

Although potentially beneficial, the development of GMOs is fraught with concern over the potential for adverse effects on human health. While human health effects of popular concern consist of increased toxicity, decreased nutritional value of food, and increased antibiotic resistance, possibly the most widely recognized concern centers on the potential increase in allergic reactions of individuals consuming transgenically modified food.

Approximately 1–2% of the population and 5–8% of children experience food allergies that are the result of natural selection processes that have occurred over thousands of years. Elevations in these frequencies may suggest allergic reactions to GMOs. Such allergic reactions are of two general types, immuoglobulin E (IgE)-mediated and non-IgE mediated reactions.

IgE-mediated allergenicity requires that individuals must first be exposed to an allergen in a sensitization dose prior to showing overt signs of the allergy. In this reaction, antigen specific binding of IgE to mast cells and basophils, followed by release of pharmacologically active chemicals such as histamines, cytokines, chemokines, and arachidonic metabolites are ultimately responsible for the classic rapid allergic reaction often termed 'immediate hypersensitivity reactions'. Toxic responses may range from, dermatitis, urticaria, and itching, to fatal anaphylactic shock and may be induced by proteins associated with a variety of foods including peanuts, grains, and fish.

Non-IgE mediated allergic reactions do not require a sensitization exposure and may occur especially in infants and children consuming milk protein and grains, and are characterized by a delayed onset of symptoms after exposure to the food. Food-induced enterocolitic syndromes caused by milk protein ingestion may result in vomiting, diarrhea, and general deterioration of the individual. Celiac disease is a specific example of such a wasting syndrome stemming from a reaction to cereal or grain (i.e., wheat, rye, barley, oats, and spelt) 'glutens'. Individuals experiencing this disease may show weight loss, diarrhea, abdominal cramps, gas and bloating, general weakness, oily stool, and stunted growth in children. Though significant in terms of effects to an afflicted individual, only ~ 1 in 300–3000 individuals in a population seem to be affected by celiac disease.

To date, no confirmed cases of increased allergic reactions to GMOs have been documented and thus the extent to which genetically modified food contributes to significant allergic reactions in the population is not accurately known. The only reported incident of potential GMO allergenicity occurred after production of soybeans modified with Brazil nut protein. Allergenicity to the genetically modified soybean was detected and the product was not marketed, precluding exposure and toxicity.

The previously discussed StarLinkTM episode is perhaps the most widely evaluated incidence potentially leading to potential health effects associated with GMOs. Using enzyme-linked immunosorbent assay (ELISA) in a retrospective study, the US FDA hypothesized that exposure to the Bt Cry9c protein could be cause for allergic responses. However, the results of the FDA research concluded that there was no evidence of GMO-mediated allergenicity subsequent to potential exposure to the StarLinkTM corn.

Because the lack of evidence of health effects cannot conclusively show that GMOs are not a human health concern, current global efforts are focused on the development of protocols to proactively and systemically detect and assess potential adverse effects.

Environmental Health Risk

The American Medical Association (AMA) has estimated that in 1999, 200 million acres of land had been planted worldwide with transgenic crops. The AMA further indicated that over 25 000 field trials for environmental effects of GMOs had been performed in 45 countries without noted adverse environmental consequences. Despite these conclusions, the limited geographical size and comprehensiveness of such trials confounds definitive conclusions regarding the potential for adverse effects such as enhanced crop pest resistance, out crossing with weedy relatives of crops, reduced biodiversity, nutritional deficiency of food sources, and toxicity to nontarget species.

Concerns over the safety of transgene introduction into environment was sensitized early in the GMO debate with significant focus on the potential toxicity of Bt endotoxins to monarch butterfly (*Danaus plexippus*) larvae exposed to transgenic pollen. Early data suggested that Bt corn pollen could result in potentially significant reactions in the monarch gut. However, at that time (mid-1990s), the general regulatory consensus in the United States suggested that the adverse effects of Bt toxins were negligible when actual exposure conditions in the field were considered. In 1999, laboratory tests were conducted to determine if Bt transformed corn pollen consumed by monarch larvae at environmentally significant concentrations could result in adverse effects under well-controlled conditions. The widely published data resulting from this testing, indeed showed that larvae consuming Bt corn pollen experienced a decreased growth rate, expressed as the concentration of protein $(0.76 \text{ ng ml}^{-1})$ required to result in growth reduction of 50% of the population (EC₅₀). Mortality, expressed as the LD₅₀, was determined to occur at $3.3 \text{ ng protein ml}^{-1}$ diet. Estimated pollen densities of ~10 and 50–100 grains cm^{-2^-} were deemed to potentially result in these adverse effects.

As a potentially significant environmental and ecological risk, such Bt pollen toxicity notably stirred the interest and emotions of the scientific and public interest communities. In 2001, a collaborative research effort of Canadian and US scientists developed a weight of evidence risk assessment to address the concern. The risk assessment results suggested that the likelihood of an adverse effect on the monarch population was less than 1 in 10000 (or less than 1×10^{-4}) when the toxicity information and exposure estimates were integrated into a risk analysis. While these data support the conclusion that an adverse effect on the monarch receptor was unlikely, it should be noted that the absence of data suggesting risk under given assumptions and circumstances should be interpreted carefully as a general conclusion of safety in all instances.

The concern for adverse ecological effects is not limited to plant or insect communities and direct toxicity. For instance, experimental data collected using fish suggests that the incorporation of transgenes may actually benefit the transgenic individual. For instance, mathematical models have been developed and evaluated to assess population effects of the transgenic fish of wild populations. Using the same data that show that the transgene may benefit the individual, models have predicted that the inadvertent release of the transgenically modified fish into the environment may result in significant adverse environmental effects such as invasion by genetically modified fish resulting in increased competition with and displacement of native species, reduced hardiness of the modified fish offspring in the wild, and potential extinction of the naturally occurring population. Although such effects have not been conclusively supported by field evaluations or verifying laboratory data, the concerns for long-term adverse

ecosystem effects remains a subject of continued research.

As noted earlier in this article, transgenically modified growth hormones have been administered to cattle to increase milk production. However, it has been noted that the administration of growth hormones can result in adverse effects in the exposed animal including, fertility reduction, changes in bone growth, increase chance of mastitis, and reductions in endocrine function. The long-term effects of inadvertent exposures to the transgene-modified growth hormone on nontreated cattle herds, livestock, or breeding stock is poorly understood.

Safety Evaluation Methodology

The complexity of GMO introduction to the human food chain as well as uncertainties regarding potential ecosystem effects has prompted governmental agencies and NGOs to recommend highly structured and systematic safety evaluation protocols prior to GMO release. While several protocols have been proposed, developed, and are being adopted worldwide, those of the European Union and the WHO provide reasonable and illustrative examples for reference.

Substantial Equivalence

A fundamental concept essential to the currently defined approaches for determining GMO safety is that of 'substantial equivalence' (SE). Used as the basis for establishing a comparative benchmark, SE depends on two key concepts. First, existing traditional foods are assumed to be safe as evidenced by their longterm use. Second, the response to traditional foods can be used as a basis for comparison to transgenically modified foods which are often derived from traditional foods.

Predicated on the definition of substantial equivalence, a decision process for further evaluation of the GMO can be defined according to the general rules shown in Table 2.

While the decision matrix shown in Table 2 suggests a rather simple testing approach, in reality, adherence to the SE concept requires that it be applied to a multitude of characteristic variables including chemical SE, biological SE, SE of potential exposure routes, and the SE of possible overall safe use.

The WHO Decision Tree for Assessment of Potential Allergenicity

Allergenicity is the current overriding concern regarding potential human exposures to GMOs. Thus, the Table 2 Simplified decision matrix for evaluation of GMO safety according to the concept of substantial equivalence

If the GMO demonstrates	Then
Substantial equivalence to traditional food Substantial equivalence to traditional food but also demonstrates evidence of a specific new trait No substantial equivalence to traditional food	No further testing or evaluation is necessary The assessment must focus on the potential effects of the new trait or gene Comprehensive toxicological and nutritional testing is required

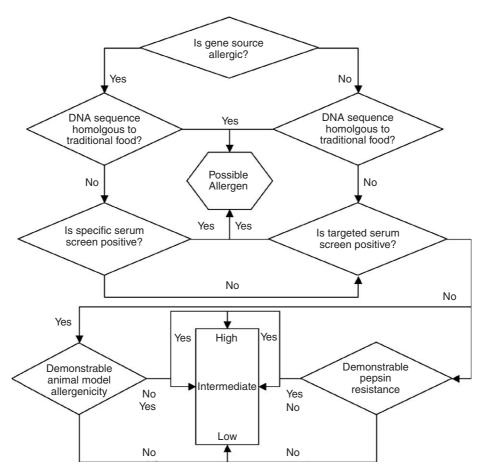


Figure 2 Decision tree for the evaluation of allegenic potential associated with GMO. (Adapted from FAO/WHO (2000) Safety Aspects of Genetically Modified Foods of Plant Origin. Geneva, Switzerland: World Health Organization.)

WHO has developed a decision tree approach to determine the human health risk from exposure to GMOs.

The decision tree and associated rationale and documentation is best reviewed in WHO records. However, Figure 2 shows the general approach (with modifications for presentation purposes) suggested by WHO to determine if a GMO is a potential allergen.

The decision tree is not a prescriptive and mandated method with substantial recommended technical protocols but rather is a conceptual model to help the evaluator determine the potential that the GMO may trigger allergic reactions in greater proportion than the unexposed populations. As noted by examination of **Figure 2**, the incorporation of comparisons with traditional foods coupled with an allowance for graded responses ensures that the risks of allergic reactions are assessed in the context of exposures and the substantial equivalence concept.

Concluding Remarks

The development of GMOs, and genetically modified foods in particular, is, in large part, a direct response to ever increasing global food demands. Current information suggests that GMOs are unlikely to have substantial adverse near term effects on human health and the environment. However, the assessment of the potential for longer-term adverse effects poses a greater challenge.

As a result of perceived needs, the technology inspired over the last 20 years and continues to develop; both in methodology and application. As a result of increased concerns over health effects, such activities will be scrutinized with growing vigilance on the part of citizens, scientists, regulatory bodies, and advisory commissions; ultimately to assure nearand long-term protection of global human health and the environment.

See also: Food and Drug Administration, US; Toxicity Testing, Mutagenicity; Transgenic Animals.

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Genetically Modified Organisms See Genetically Engineered Foods.

Genomics, Toxicogenomics

Kartik Shankar and Harihara M Mehendale

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Toxicogenomics refers to the application transcriptonomic (high-throughput analyses of gene expression) techniques to the field of toxicology. Classically, toxicologists examine potential adverse outcomes and putative mechanisms due to xenobiotic exposure by biochemical and histopathological markers of toxicity. In the current healthcare and regulatory environment, chemicals suspected to have potential for significant adverse health effects are selected to undergo subsequent testing for carcinogenicity and chronic toxicity. However, long-term studies are typically labor intensive and time consuming and can cost \$2-4 million. As of 2002, the National Toxicology Program had tested or is currently testing 505 chemicals in long-term studies, 66 in short-term studies, and only a single chemical in a subchronic study. Given that almost 70 000-85 000 chemicals

are used in commerce today, it is evident that alternative high-throughput methods for screening the toxic potential of chemicals is needed. This would allow for prioritization of untested chemicals in the classical approach of toxicity testing. Concurrent with rapid advances in bioinformatic tools and classification algorithms toxicogenomic analyses is fast becoming a viable option in high-throughput screening of potentially hazardous chemicals.

Applications of Toxicogenomics

Predictive Toxicology

The underlying premise of toxicogenomics is that toxicity is associated with changes in the global gene expression. Since toxicity by itself is resultant due to some form of cellular dysfunction or cell death, it will either be preceded or followed by some level of changes in gene expression. By monitoring the global gene expression changes a characteristic 'signature' change can be attributed to a particular phenotype of toxicity. Based on known gene expression signatures for established toxicants predictive models can be designed that will judge the toxic potential of untested chemicals based on their gene expression fingerprints. The progress in toxicogenomics as a tool for predictive toxicology is coupled to the advances in bioinformatics. Statistical techniques used to build predictive models range from linear and nonlinear discriminant analysis, Bayesian classification, nearest neighbor approaches, and neural networks.

Understand Mechanisms of Toxicity

The current mainstay for understanding mechanisms of toxicity involve use of *in vivo* model systems, including the rat and the mouse and other *in vitro* approaches involving a wide variety of cell and tissue culture techniques. Molecular mechanisms are explored based on hypothesis-driven experiments that, via appropriate interventional designs, test the involvement of a particular mechanism in mediating toxic outcome. The ability to scan genome-wide changes in expression of thousands of genes *ex vivo* or *ex vitro* now presents the unique ability to create novel hypotheses. It is naive to expect that microarrays and toxicogenomic investigations will replace traditional mechanistic approaches. On the other hand, toxicogenomic approaches complement and enhance the hypothesis generating capacity and allow probing a larger window of potential mechanisms.

See also: Bioinformatics; Microarray Analysis.

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GF

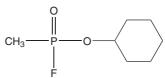
Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 329-99-7
- SYNONYMS: Cyclosarin; Cyclohexyl methylphosphonofluoridate; CMPF; Cyclosin; Methylphosphonofluoridic acid; cyclohexyl ester; Methyl cyclohexylfluorophosphonate; Cyclohexyl ester of methylphosphonofluoridic acid; Cyclohexylmethyl-fluorophosphonate; Methylfluorocyclohexylphosphonate; Nerve gas; Nerve agent
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: GF is a colorless, liquid, organophosphate human-made nerve agent with intermediate persistence. The evaporation rate is ~ 1/20th that of water. It has a

nondescript odor described as sweet, musty, peaches, and shellac

- Chemical Formula: C₇H₁₄FO₂P
- CHEMICAL STRUCTURE:



Uses

Cyclosarin is a nerve agent used in chemical warfare.

Exposure Routes and Pathways

Exposure can occur by inhalation, skin absorption of liquid or vapor, as well as by ingestion.

Toxicokinetics

Cyclosarin can be absorbed into the body by all routes of exposure. It is usually liquid in its normal

^{*}The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

state, but will volatilize if heated to form vapor or aerosol. It is considered to have an intermediate persistence with an evaporation rate approximately onehalf of water. GF is only slightly soluble in water, and the liquid in large amounts can layer out at the bottom of pools.

Mechanism of Toxicity

GF, an organophosphate, is a lethal cholinesterase inhibitor similar in action to sarin (GB). Limited data suggest delayed neuropathy such as postural sway and impaired psychomotor performance. Miosis has been noted for up to 62 days. Like sarin and the other nerve agents, GF and GB inhibit the enzymes butyrylcholinesterase in the plasma, the acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. During recovery, however, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzymes is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood-brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and their behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is ~1% per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent–enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotinic acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system (CNS).

Recent investigations have focused on organophosphate nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on γ -amino butyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline, acetylcholine following inhibition of brain cholinesterase activity have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of the nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

The onset of toxicity is usually rapid, occurring within minutes of exposure. Reduced acetylcholine levels are indicators of nerve agent exposure. Signs and symptoms are dependent on the degree of intoxication and may include the following:

- nervousness/restlessness;
- miosis (contraction of the pupil);
- rhinorrhea (runny nose), excessive salivation;
- dyspnea (difficulty in breathing due to bronchoconstriction/secretions);
- sweating;
- bradycardia (slow heartbeat);
- loss of consciousness;
- convulsions;
- flaccid paralysis;
- loss of bladder and bowel control; and
- apnea (breathing stopped).

The LD_{50} in humans has been estimated as $35 \,\mu g \, kg^{-1}$ by inhalation, $0.350 \, m g \, kg^{-1}$ percutaneously, and $1.0-1.4 \, m g$ per person intravenously.

Animal Toxicity

Subcutaneously, the reported LD_{50} values are 56.5–110 µg kg⁻¹ in guinea pigs, 130 µg kg⁻¹ in hamsters, 100 µg kg⁻¹ in mice, 100 µg kg⁻¹ in rabbits, and 225 µg kg⁻¹ in rats.

Intramuscularly in mice, the LD_{50} was reported as $224 \,\mu g \, kg^{-1}$ and in the rhesus monkey as $46.6 \,\mu g \, kg^{-1}$.

Clinical Management

The immediate treatment for nerve agent intoxication is intravenous injection of 2 mg atropine sulfate (intramuscular injection should be considered if the patient is hypoxic and ventilation cannot be initiated, as there is a risk of ventricular fibrillation). This should be followed by additional injections of atropine at 10–15 min intervals, continuing until bradycardia has been reversed (e.g., until the heart rate is at 90 beats per minute). If breathing has stopped, a mechanical respirator should be used to ventilate the patient. Mouth-to-mouth resuscitation should not be attempted. If possible, oxygen or oxygen-enriched air should be used for ventilation. If possible, cardiac activity should be monitored.

Oximes (pralidoxime salts, obidoxime) may be of use in restoring acetylcholinesterase activity. Obidoxime may be used to treat GF intoxication; however, it may cause liver damage. Animal studies indicate that the oxime Hi-6 may be significantly superior to other oximes in the treatment of GF intoxication, but it is not widely available. Therefore, pralidoxime salts should be used, with a slow intravenous infusion of 500 mg to 1 g being given initially.

Diazepam should be administered to control convulsions. It also has value in controlling fear on the part of the patient. An initial dose of 5 mg may be followed by additional doses at 15 min intervals up to a total of 15 mg.

Protective equipment (self-contained breathing equipment or gas mask, barrier suit) must be used. Medical personnel treating casualties should avoid direct (skin-to-skin) contact: protective gear including breathing protection should be worn when treating casualties prior to decontamination. Latex gloves are not adequate protection. Casualties should be decontaminated as rapidly as possible. Casualties should be removed from exposure as rapidly as possible, but must not be moved into clean treatment areas where unmasked/ungloved personnel are working until decontamination is complete.

Decontamination of victims is accomplished by removing the victim from the contaminated area, removal of clothing, and removal or neutralization of agent present on the skin (skin decontamination may be unnecessary if the exposure was only to GF vapor). Any visible droplets should be blotted (not wiped) away using an absorbent material (e.g., paper towels and facial tissues); if available, towelettes moistened with a neutralizing solution should be used. Adsorbent powders may also be used for removal of droplets (in the absence of standard adsorbents, field expedients such as flour may be useful). A solution of 0.5% hypochlorite bleach may be used for skin decontamination. Hair should be thoroughly cleaned using soap and water, with care being taken to prevent wash water from contacting skin.

Surface decontamination may be accomplished using hypochlorite bleach slurries, dilute alkalis, or DS2 decontaminating solution. Steam and ammonia may be used for the decontamination of confined spaces.

See also: Acetylcholine; Cholinesterase Inhibition; Nerve Agents.

Relevant Websites

- http://www.bt.cdc.gov US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.
- http://sis.nlm.nih.gov US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

GI Tract See Gastrointestinal System.

Ginger Jake

Robin C Guy

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 78-30-8 (Tri-o-cresyl phosphate, TOCP)
- SYNONYMS: Jamaican ginger paralysis; Ginger Jake paralysis; Ginger Jake walk; Jake leg; Organophosphorus ester-induced delayed neurotoxicity (OPIDN)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organic phosphate compounds, primarily the *o*-isomer of tritolyl phosphate
- CHEMICAL FORMULA: Example: C₂₁H₂₁O₄P

Uses

Tri-o-cresyl phosphate (TOCP) is used as a plasticizer in lacquers and varnishes. It is also used in hydraulic fluids, as a flame retardant, gasoline additive, heat exchange medium, waterproofing agent, and as a solvent for nitrocellulose.

Background Information

Discovered in the 1920s prohibition era in the United States, the name coming from the use of Jamaican ginger ('Jake') to flavor batches of cheap ('bath tub') gin. The ginger having been grown (and therefore being contaminated) with the help of pesticides, for example, TOCP, a potent organophosphate. The result was an axonal dying-back neuropathy affecting mainly large muscle groups. Jake poisoning struck thousands of adults in the United States, leaving many crippled by irreversible paralysis. TOCP was also a contaminant in cooking oil in Morocco in the late 1950s.

Exposure Routes and Pathways

TOCP's industrial uses may result in its release to the environment through various waste streams. If released to the atmosphere, TOCP will exist in both the vapor and particulate phases in the ambient atmosphere. Occupational exposure may occur through inhalation of dust particles and dermal contact with TOCP. The general population may be exposed to TOCP via ingestion of contaminated drinking water.

Toxicokinetics

TOCP can be absorbed after ingestion, through the skin, and by inhalation.

Mechanism of Toxicity

TOCP produces a delayed neurotoxicity, by inhibiting a nonspecific neuronal carboxylesterase, neuropathic target esterase. The neuropathic target esterase appears to have a role in neuronal lipid metabolism. Neuropathic target esterase enzymatic activity is highest in nervous tissue.

Acute and Short-Term Toxicity (or Exposure)

Animal

Table 1 lists the LD_{50} and LD_{Lo} values based on the mode of administration of TOCP in different animal species.

Table 1 LD_{50} and LD_{Lo} values, based on mode of administraionof TOCP, for different animal species

	Mode	Species	Amount (m kg $^{-1}$)
LD ₅₀	Oral	Rat	1160
LDLo	IP	Mouse	50
LDLo	SC	Dog	500
LDLO	SC	Cat	185
LDLo	Oral	Rabbit	500
LDLO	IP	Rabbit	100
LDLO	SC	Rabbit	100
LDLo	IV	Rabbit	100
LDLO	IM	Rabbit	135
LDLo	SC	Guinea pig	300
LD _{Lo}	SC	Dog	300

LD₅₀, lethal concentration in 50% fatality of those tested; LD_{Lo}, lowest published lethal concentration; Oral, oral administration; IP, intraperitoneal administration; SC, subcutaneous administration; IV, intravenous administration; IM, intramuscular administration.

Human

Inhalation of TOCP may cause headache, nausea, vomiting, and muscle pain. Ingestion may lead to abdominal pain, nausea, and vomiting. Dermal exposure causes redness and pain. Symptoms may be delayed.

Chronic Toxicity (or Exposure)

Animal

Species differ in responses to TOCP. The chicken and cat have been used extensively especially because the responses in those species are very similar to those of man. Rabbit, dog, monkey, and guinea pig react inconsistently while rats and mice are reported to be resistant to paralysis although they still have nervous tissue damage.

Histological examination of hens exposed to TOCP revealed a wallerian 'dying-back' degeneration of the larger diameter axons and myelin sheaths. If the neuropathic target esterase is inhibited by 70%, the typical organophophorus ester-induced delayed neurotoxicity (OPIDN) will follow after an approximate 7–14 day delay.

Human

Main symptoms involve the nervous system. The initial symptoms are characterized by muscle weakness in the arms and legs that may occur days or weeks after exposure. Eventually, symptoms include symptoms of spinal cord injury, including a clumsy shuffling gait, spasticity, hyperreflexia, and permanent damage to the pyramidal tracts and upper motor neuron syndrome. This syndrome is known as OPIDN.

Clinical Management

No specific therapy is known as a treatment for victims presenting signs of Ginger Jake paralysis; as this is a delayed neurotoxic effect. It would be prudent to determine the cause of exposure to ensure that the victim is no longer exposed to TOCP and provide supportive care. The victim should be transported to a hospital for evaluation.

Environmental Fate

An estimated bioconcentration factor value suggests that bioconcentration in aquatic organisms is very high; however, depuration half-lives ranging from 4 to 6 days for TOCP isomers indicate that bioconcentration may not be an important process. TOCP is not expected to volatilize from water surfaces. Biodegradation of TOCP in river water and bottom sediment followed first-order kinetics; the half-life in river water and bottom sediment at 25° C was 10 days. Vapor-phase TOCP is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life in air is

Ginseng

Michael Wahl

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• SYNONYMS: *Panax quinquefolium*; American ginseng

Uses

Ginseng is used for many medicinal and rejuvenating reasons domestically and abroad. Traditional Chinese medicine values ginseng as a nerve and cardiac stimulant, a treatment for impotence, to promote metabolism, and to moderate blood pressure and blood sugar levels. Two or three grams are considered therapeutic. Fifty grams has been prescribed but has resulted in adverse side effects.

Background Information

Ginseng is a long-stemmed herb with palmate leaves. Flowers are yellowish green and bloom in groups of two to four. Red drupe berries appear in clusters. This herb is native to the Orient but widely cultivated in the United States for export. estimated to be \sim 1.2 days. Particulate-phase TOCP may be physically removed from the air by wet and dry deposition.

See also: Food and Drug Administration; Food Quality Protection Act, US; Food, Drug, and Cosmetic Act, US; Organophosphate Poisoning, Delayed Neurotoxicity.

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Relevant Website

http://www.inchem.org – Tricresyl Phosphate (Environmental Health Criteria Number 110 – from the International Programme on Chemical Safety), 1990.

Exposure Routes and Pathways

Exposures occur via ingestion (e.g., teas and soups). Ginseng cigarettes are also available. A topical preparation is used to approximate wound edges. In the United States, it is widely accepted as an effective demulcent.

Mechanism of Toxicity

All plant parts contain 13 capon glycosides. Two of these agents have a prolonged anti-inflammatory action similar to that of nonsteroidal anti-inflammatory medications. Another component is thought to affect corticosteroid secretion in the central nervous system. The fusiform roots are recognized for their vitamin and mineral content.

Acute and Short-Term Toxicity (or Exposure)

Human

Limited information exists for acute ingestions of ginseng. Case reports have described cerebral arthritis following large acute ingestions of ginseng extract.

Chronic Toxicity (or Exposure)

Human

In large therapeutic doses, patients may suffer insomnia, depression, and nervous behavior. When used in chronically excessive doses, patients may suffer from hypertension, insomnia, dermal blemishes, and morning diarrhea. Hypertension and depression may result from abrupt withdrawal after chronic use.

In Vitro Toxicity Data

Studies of the ginseng extract ginsenocide Rb1 in a rat peritoneal mast cell model have demonstrated potent antihistaminic effects.

Clinical Management

Treatment of ginseng exposures is supportive. The use of the herbal preparation is discontinued. Most cases of ginseng overdose do not require aggressive management. Supportive care for symptoms that develop is the mainstay of treatment.

Further Reading

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Global Environmental Change

Thomas Wilbanks

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To say that the global environment is changing is to state the obvious. The global environment always changes, and life on earth has always been affected by those changes. Why, then, has 'global environmental change' become a subject of special interest in recent decades?

Throughout the earth's history, environmental change has reflected the interplay of natural atmospheric and geological forces, with infrequent interventions from outer space. In recent millennia, however, human use of the earth has increasingly reshaped physical and ecological environments locally and regionally through such agencies as fire and irrigation. In recent centuries, human transformations of the earth have come to have truly global impacts, and it is at least possible that some of these impacts – if they continue – could threaten the improved quality of life that is the aim of most human economic development.

The growing dominance of human actions as an aspect of global environmental systems stems from a combination of human population growth, economic growth (which implies increased demands for materials and services), and technological change. Since 1000 BC, the total human population has grown from ~ 50 million to ~ 6 billion. Just since 1970, the total output of the global economy has increased from \sim \$4 trillion (in US dollars) to \sim \$40 trillion, which means both vastly increased requirements for physical and biological building-blocks to support

economic production and also vastly increased waste streams from that production. In the past century, technological change – spurred by economic competition – has changed resource requirements and the composition of the by-products of production, in many cases making the resource requirements more intrusive and the by-products more hazardous to human and ecological health.

The most visible evidence of this transformation is changes in land uses: natural ecosystems being replaced by managed ones, natural landscapes by built ones, uses related to local demands replaced by uses related to distant demands. As a global phenomenon, these changes have been pushed in part by resource extraction for materials and food to support economic growth in centers of global economic and political power, not only in remote source areas but also within the major powers themselves. Results have included land degradation from mining, deforestation for agricultural development, and in some cases desertification in marginal areas from such practices as overgrazing. Associated economic production activities, such as agroprocessing and manufacturing, generate wastes that must be absorbed by environmental systems: the air, water bodies, and/or the land and subsurface.

These activities and impacts arise from a complex interplay of processes that operate at a variety of geographical scales from global to local, and temporal scales from very immediate to very long term. Chains of causality work both from the global scale to the local, as in the case of economic globalization, and from the local to the global, either systemically (where local actions result in changes in global systems, such as ozone depletion) or cumulatively (where local actions result in local or regional changes of global significance, such as species extinction).

Some observers are convinced that the growing volume and intensity of such activities threaten nature-society balances on which our very survival depends. Waste production and disposal poses hazards from toxic and radioactive substances, which even casual observers can relate to land-surface degradation in countries including the United States and Russia; and many water bodies have been degraded as well. Carbon emissions due to fossil fuel use appear to be the primary cause of global climate change. Deforestation and environmental pollution are reducing global biological diversity, with implications still being studied. Moreover, biological mutations pose the possibility of new dangers to people, animals, and plants, as we find that epidemics such as HIV/AIDS are still imaginable in a modern world.

Other observers argue that it is not environmental 'preservation' that is the primary issue. Sustainable development depends at least as much on economic progress for human societies. The issue is adaptive environmental management, utilizing technological and institutional change to sustain economic growth while reducing adverse impacts on the environment. In order to avoid potentially radical social transformations in the future, economic and environmental progress need to be closely coupled.

Particularly important are issues of technological and institutional change. Technological change offers breakthroughs in environmental monitoring and waste control, prospects for substituting new alternatives for depletable resources, and new tools for analysis and assessment. At the same time, it continues to introduce new substances, some of which could have unintended consequences. Institutional change in this era of the information technology revolution offers new opportunities for information access and participative decision-making, but it raises questions about information control and privacy.

In many cases, progress is being made. In many nations, air and water pollution is being reduced and the degradation of land areas is being reversed. A new awareness of environmental risks and hazards, combined with new tools for monitoring and control, is being reflected in both public policy and stakeholder participation. Even at the international scale, where progress can be complicated by differing national styles and agendas, the Montreal Protocol on substances that deplete the ozone layer shows that action is possible and environmental standards for international trade are being developed and implemented.

But many challenges remain as the world contemplates continued population and economic growth, new risks of unintended consequences from technological developments such as bioengineering, and a tendency for decisions to be shaped by narrow economic and political interests. Unless an 'environmental ethic' becomes widespread, it is likely that global environmental change will remain a pressing concern for both society and science.

See also: Ecotoxicology; Pollution, Soil; Pollution, Water.

Further Reading

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Global Warming See Global Environmental Change.

Glutathione

Shayne C Gad

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Glutathione, also referred to as GSH, is an endogenous component of cellular metabolism, a tripeptide composed of glycine, cysteine, and glutamic acid. It is normally present in the liver at a concentration of 10 mmol l^{-1} . It is an integral part of the biotransformation of xenobiotic substances, and serves to protect the body from reducing agents.

Unlike amino acids, glutathione conjugation involves electrophilic substrates. Some of this is done none-nzymatically. Glutathione *S*-transferases, or ligandin, aids the enzymatic conjugation by catalyzing the reaction, converting GSH to GS^- . Glutathione *S*-transferases comprise ~10% of total cellular protein.

Glutathione can conjugate with xenobiotics in many ways. It may displace an electron-withdrawing group, putting GS^- in its place. It may add itself (GSH) to the substrate. It may also respond to a substrate formed from earlier metabolism. A xenobiotic may stereoselectively conjugate, removing one or more of the peptides.

Glutathione conjugation helps contribute to detoxification by binding electrophiles that could otherwise bind to proteins or nucleic acids, resulting in cellular damage and genetic mutations. Exaggerated presence of glutathione *S*-transferase may indicate resistance to chemical toxicity. Different glutathione *S*-transferase responses to different chemicals between species may add to differences in susceptibility to toxic effects. Glutathione *S*-transferases are a super family of enzymes that provide protection against many electrophilic compounds by catalyzing the conjugation of these compounds with glutathione to excretable water-soluble forms.

On the other hand, glutathione conjugation may activate the toxic moiety within a xenobiotic. Activation mechanisms involving glutathione include:

- Toxic metabolites released from conjugation with haloalkanes, organic thiocyanates, and nitrosoguanides.
- Electrophilic sulfur mustards formed from conjugation with vicinal dihaloalkanes.
- Conjugates of halogenated alkenes activated by enzymatic activity in the kidney.

 Toxic metabolites produced from γ-glutamyltranspeptidase degradation of quiriones, quinoneimines, and isothiocyanates.

A classic example of glutathione-related toxicity is acetaminophen. Phase 1 metabolism of acetaminophen by P450 results in a toxic metabolite. Glutathione conjugation breaks down and detoxifies the metabolite and excretes it as mercapturic acid. Sufficient glutathione is a key player in this protective biotransformation. If as much as 70% of endogenous glutathione is already consumed, toxic activation may take place. It takes only 15.8 g of acetaminophen to reduce glutathione levels to the point where hepatotoxicity may occur.

Glutathione conjugates have two routes of excretion – via bile or via urine. Conjugates eliminated in the urine are first converted to mercapturic acids in the kidney. Mercapturic acid is defined as *N*-acetylated, *S*-substituted cysteine conjugates arising from conjugation of a xenobiotic with glutathione. Its biosynthesis involves conjugation of the GSH itself. Glycine and glutamic acid are removed; then cysteine is conjugated further by interaction with *N*-acetyltransferase. This last step converts the substance to mercapturic acid.

Conjugation with mercapturic acid may also activate hepatotoxins. It may cleave with γ -glutamyl-transpeptidase, an enzyme implicated in the degradation quinones, quinoneimines, and isothiocyanates. Acetaminophen also increases the urinary excretion of mercapturic acid and cysteine conjugates, enabling the formation of its own hepatotoxic metabolites. Treatment with mercaptamine, a synthetic mercapturic acid, can reduce acetaminophen intoxication.

It has been reported that binding sites provided with true specificity for GSH exist in the central nervous system, and this satisfies the main requisite for considering GSH as a neuromediator in addition to its functions noted above.

See also: Biotransformation; Kidney; Metallothionein; Oxidative Stress.

Further Reading

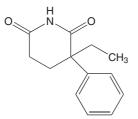
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Glutethimide

Rebeca Gracia

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 77-21-4
- SYNONYMS: Doriden; Dorimide; Doridene, Glimid, Elrodorm glutarimide, 2-Ethyl-2-phenylglutarimide; 3-Ethyl-3-phenyl-2,6-piperidinedione; 2-Phenyl-2ethylglutaric acid imide; α-phenyl-α-ethylglutarimide
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Piperidinedione hypnotic and sedative
- CHEMICAL FORMULA: C₁₃H₁₅NO₂
- CHEMICAL STRUCTURE:



Uses

Glutethimide was once used as a sedative–hypnotic agent. Its use has generally been abandoned because of its acute and chronic toxicity, abuse potential, and the availability of more favorable alternatives.

Background Information

Glutethimide in combination with codeine was commonly abused and was referred to by various slang or street names including: Sets, Loads, Three's and Eight's, Fours and Doors. Glutethimide was changed from a Schedule III to Schedule II Controlled Substance in 1991.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposure to glutethimide. It is available as 125, 250, and 500 mg tablets.

Toxicokinetics

Glutethimide is erratically absorbed from the gastrointestinal tract, but peak serum concentrations generally occur within 1–6 h following a therapeutic dose. Glutethimide is metabolized by the liver to conjugated and unconjugated metabolites, two of which are active. These active metabolizes, 4-hydroxy-2-ethyl-2-phenyl-glutarimide (4-HG) and α -phenyl- γ -butyrolactone, accumulate in overdose patients and contribute to the toxic effects of glutethimide. 4-HG has been found to be twice as potent as the parent compound. Measured plasma levels of glutethimide do not correlate well with toxicity.

Protein binding is 50%. The volume of distribution is 1.71 kg^{-1} . Glutethimide is highly lipid soluble and accumulates in the brain and adipose tissue. The elimination half-life for glutethimide is ~10–12 h but is prolonged in overdose.

Mechanism of Toxicity

Glutethimide depresses the central and autonomic nervous systems. The pharmacologic mechanism of glutethimide is not well understood. It produces effects comparable to those of phenobarbital. In addition, it possesses marked antimuscarinic activity.

Acute and Short-Term Toxicity (or Exposure)

Human

Ingestion of a single 500 mg tablet is likely to produce toxicity in a child. The potentially toxic and lethal doses of glutethimide in adults are generally accepted to be 3 and 10 g, respectively. Acute overdose with glutethimide results in central nervous system depression ranging from lethargy to profound coma. Prolonged and fluctuating coma may occur due to redistribution of active metabolites from adipose stores and from enterohepatic recirculation. Hypotension and respiratory depression may develop. Anticholinergic manifestations, such as decreased gastrointestinal motility and urinary retention, may complicate the clinical course. Pulmonary and cerebral edema, cardiovascular shock, and seizures may develop in severe cases.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in pregnant rats at doses up to 0.4% did not result in fetal toxicity.

Human

Chronic use of high doses of glutethimide may produce psychological and physical dependence. Abrupt discontinuation of therapy may result in withdrawal signs and symptoms such as nausea, vomiting, tremulousness, tachycardia, fever, delirium, hallucinations, and seizures. Unlike opioid withdrawal, glutethimide withdrawal can be life threatening.

In Vitro Toxicity Data

Studies of glutethimide in *Drosophila melanogaster* have not demonstrated genotoxicity.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Activated charcoal can be used to adsorb glutethimide if given within 1 h of exposure. Respiratory support including oxygen and ventilation should be provided as needed. There is no antidote for glutethimide. If hypotension occurs it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and pressors by intravenous infusion. Standard measures for the management of seizures and cerebral edema should be employed. Hemodialysis and hemoperfusion may be effective for the active removal of glutethimide but should be reserved for severe cases when standard supportive measures are inadequate. The occurrence of withdrawal signs and symptoms indicates the need to reinstitute glutethimide therapy and gradually reduce the dose until it is discontinued. A barbiturate such as phenobarbital or a benzodiazepine may be substituted for glutethimide.

See also: Benzodiazepines; Charcoal; Codeine.

Further Reading

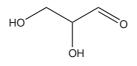
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- Hansen AR, Kennedy KA, and Ambre JJ (1975) Glutethimide poisoning: A metabolite contributes to morbidity and mortality. *New England Journal of Medicine* 292: 250–252.

Glyceraldehyde

Stephen R Clough

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 367-47-5
- SYNONYMS: DL-Glyceraldehyde; 2,3-Dihydroxypropanal; α,β-Dihydroxypropionaldehyde; Glyceric aldehyde; Glycerose; 2,3-Dihydroxypropionaldehyde (glyceraldehyde can exist as two different isomers, D-glyceraldehyde and L-glyceraldehyde, which are 'mirror images' of each other)
- Chemical Formula: $C_3H_6O_3$
- CHEMICAL STRUCTURE:



Uses

Glyceraldehyde is used: in nutrition; in the preparation of polyesters and adhesives; as a cellulose modifier; and in the tanning of leather. It is also used in biochemical research; the two isomers are used as 'reference' chemicals because each is one of the simplest molecules to compare against other molecules (such as sugars and amino acids). The conformation of the alcohol and aldehyde groups around the central carbon of D-glyceraldehyde helps scientists evaluate the structure and nomenclature (identity) of other simple sugars, such as glucose. This makes glyceraldehyde an important tool and reference standard for the biochemist.

Exposure Routes and Pathways

Only persons involved in the manufacture and production of glyceraldehyde would be expected to be exposed to significant concentrations of this compound. Because it is a solid at room temperature, exposure would be anticipated to occur only through contact with the skin or by inhalation of airborne dust. Skin exposure may also occur from contact with aqueous solutions (40%) of glyceraldehyde.

Acute and Short-Term Toxicity (or Exposure)

Animal

The median lethal dose (LD_{50}) for glyceraldehyde in rats is $2 g k g^{-1}$, which places it in the category of

slightly to moderately toxic. The chemical is also slightly toxic by the intraperitoneal route. No other data are available on animal toxicity.

Human

Because glyceraldehyde is a normal metabolic intermediate in humans, this chemical cannot be readily categorized as a 'toxic' chemical. Given a large enough exposure or dose, any chemical can result in toxic injury. As with any aldhyde/alcohol, very high air concentrations or accidental ingestion of large amounts would be expected to overwhelm the body's natural defenses and produce an adverse effect (e.g., eye, nose, lung irritation from airborne dust; and stomach ache/nausea following ingestion).

Chronic Toxicity (or Exposure)

No information could be found on the chronic toxicity of glyceraldehyde.

Clinical Management

Persons who have been overcome by high concentrations or doses of glyceraldehyde should be removed from the area of high exposure. Medical attention should be sought. Treatment should be similar to first aid following any high-level chemical exposure; irrigation of the eyes with copious amounts of water, washing of exposed skin with soap and water, and supportive therapy following ingestion.

Ecotoxicology

No records are available in the US Environmental Protection Agency ECOTOX database for glyceraldehyde. Because this chemical is a metabolic intermediate and could easily be utilized by microorganisms, a release or spill of this compound into the general environment would not be expected to have any long-term adverse effects as the half-life in soil or water would be expected to be very brief (days).

Miscellaneous

Glyceraldehyde is a chemical that occurs naturally in living organisms, including humans. It is an intermediate in the metabolism of fructose. In the liver, fructose is converted to fructose-1-phosphate by the enzyme fructokinase. Fructose-1-phosphate is then converted to glyceraldehyde and dihydroxyacetone phosphate by the enzyme fructose-1-phosphate aldolase. Glyceraldehyde is then converted to glyceraldehyde-3-phosphate by the enzyme glyceraldehyde kinase. Glyceraldehyde-3-phosphate is a high-energy intermediate that may then move into the glycolysis cycle, which provides the body with a way of extracting energy to make ATP, which can then be used to power other metabolic functions, such as muscle contraction.

Further Reading

British Industrial Biological Research Association (1990) *Toxicity Profile of Glyceraldebyde*. Carshalton, UK: British Industrial Biological Research Association.

Glycerol

Kathryn A Wurzel

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 56-81-5
- SYNONYMS: Glycerin; Glycerine; 1,2,3-Propanetriol; 1,2,3-Trihydroxypropane; Glyceritol; Glycyl alcohol
- Chemical Formula: C₃H₈O₃

Uses

Glycerol is used as a solvent for flavors and food colors. It is also used as a humectant, plasticizer, emollient, sweetener, and filler in low-fat food products such as cookies. It is used in the manufacture of dynamite and propellants (nitroglycerol), cosmetics, candy, liqueurs, printing and copying inks, lubricants, pharmaceuticals (suppositories, cough syrups, elixirs, expectorants, and cardiac medications), personal care products (toothpaste, mouthwashes, skin care products, hair care products, and soaps), and antifreeze. Glycerol is also used to keep fabrics pliable and cellophane and special quality papers flexible and tough. Glycerol is a common energy yielding food and is widely distributed in food, both as a natural constituent and as a GRAS (generally recognized as safe) additive. Glycerol is used therapeutically to reduce intraocular pressure due to glaucoma and for cerebral edema.

Exposure Routes and Pathways

Inhalation, dermal contact, ocular contact, and ingestion are the exposure pathways for glycerol.

Toxicokinetics

Oral exposure results in rapid absorption through the gastrointestinal tract with rapid distribution in the blood. Most glycerol is incorporated into the body fat. Seven to 14% is excreted unchanged in the urine within 2.5 h of ingestion with ~80% of metabolism occurring in the liver and 10–20% occurring in the kidneys. Glycerol metabolism in the liver is initiated by glycerokinase, with glycerol further metabolized to carbon dioxide and water or utilized in glucose or glycogen synthesis. Some glycerol may combine with free fatty acid to form triglycerides. The elimination half-life of glycerol is ~30–40 min.

Mechanism of Toxicity

The medicinal action of glycerol as a laxative is a result of an increase in water absorption and irritation effects that cause evacuation of the bowel. Certain medical conditions such as cardiac, renal, or liver disease and/or diabetes may be exacerbated by shifts in body water as a result of exposure to glycerol.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rhabdomyolysis (muscle necrosis) in experimental animals may cause death as a result of acute renal injury. Intramuscular injection in rabbits results in necrosis of muscle fibers and disruption in cell plasma membrane. Extensive regeneration is apparent 7– 14 days following exposure: changes are similar to muscular dystrophy. The glycol myopathy may be a good model for pathophysiological studies of Duchennes muscular dystrophy. An oral LD₅₀ of 12 600 mg kg⁻¹ and dermal LD₅₀ of 10 000 g kg⁻¹ has been reported in rats and rabbits, respectively.

Human

Glycerol is of a low order of acute oral and dermal toxicity. Toxicity following acute ingestion of excessive amounts of glycerol-based laxatives is generally minimal and limited to the gastrointestinal tract. Aspiration may result in pneumonitis. Adverse effects following oral administration include mild headache, dizziness, nausea, vomiting, thirst, and diarrhea. Glycerol dropped on the human eye causes a strong stinging and burning sensation with tearing and dilation of conjunctival vessels, but no obvious injury. Hemolysis, hemoglobinuria, and renal failure may occur at very large doses and is a function of concentration and route of administration (oral or parenterally). Severe dehydration, cardiac arrhythmias, and hyperosmolar nonketoic coma may be fatal.

Chronic Toxicity (or Exposure)

Animal

Chronic oral exposure to glycerol may cause mild irritation of the gastrointestinal tract. In a 2 year study, no systemic or local effects were reported at a dose of $10\,000\,\text{mg}\,\text{kg}^{-1}$ body weight. Inhalation of glycerol aerosols may cause irritation of the respiratory tract.

Glycerol did not produce statistically significant effects in chromosome aberrations and dominant lethal assays. It is not thought to be genotoxic.

In Vitro Toxicity Data

Glycerol has not been shown to induce mutations in bacterial assays, chromosomal effects in mammalian cells, or cause primary DNA damage *in vitro*.

Clinical Management

Due to the generally low toxicity of glycerol and potential aspiration hazard, emesis is not generally recommended following ingestion. Activated charcoal should only be used in the event of very large ingestions due to the potential for induction of vomiting. Dehydration, electrolyte imbalance, hyperglycemia, and acidosis or alkalosis require management by the clinician as appropriate. Excessive diarrhea should be treated with high fluid intake and monitoring of fluid and electrolyte status.

Environmental Fate

Glycerol is neither expected to bioconcentrate in fish and aquatic organisms nor expected to adsorb readily to sediment. In soil, glycerol undergoes rapid biodegradation under aerobic conditions, is highly mobile, and demonstrates low volatility. In water, it rapidly degrades under aerobic conditions. Biodegradation in seawater and under anaerobic conditions is expected.

Ecotoxicology

Glycerol has low toxicity to algae and fish. The threshold toxicity is reported as greater than $3000 \text{ mg} \text{ l}^{-1}$.

Other Hazards

Glycerol is combustible. Upon combustion, carbon monoxide and carbon dioxide are formed. If heated or in a fire, compressed air or oxygen apparatus and gas-tight suit may be needed.

It may polymerize as temperatures increase. It reacts violently with strong oxidizers and reacts with some acids. Contact with some oxidizers and acids may present an increased risk of fire or explosion.

Exposure Standards and Guidelines

Current data are not sufficient to determine the carcinogenicity of glycerol.

Occupational Safety and Health Administration Standards – permissible exposure limit, 1998: 15 mg m^{-3} mist, total dust.

Eight-hour time-weighted average (TWA): 5 mg m^{-3} mist respirable fraction.

American Conference of Governmental Industrial Hygienists threshold limit value, 2002: 8 h TWA 10 mg m^{-3} mist. Excursion limit three times the TWA for no more than a total of 30 min per workday.

See also: Eye Irritancy Testing; Kidney.

Further Reading

Roberts PR, Black KW, and Zaloga GP (1997) Enteral feeding improves outcome and protects against glycerolinduced acute renal failure in the rat. *American Journal of Respiratory and Critical Care Medicine* 156(4 Pt 1): 1265–1269.

Relevant Website

http://www.jtbaker.com – Mallinckrodt Chemicals. J.T. Baker (2004) MSDS for Glycerol.

Glycol Ethers

Linda A Malley

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• SYNONYMS: This is a large and diverse group of compounds that can be divided into two general classes: the ethylene glycol ethers and the propylene glycol ethers

Uses

Glycol ethers are extensively used in industrial applications as solvents for the manufacture of lacquers, varnishes, resins, printing inks, and textile dyes; as antiicing additives in brake fluids; and as gasoline additives. In addition, they are used in consumer products such as latex paints and cleaners.

Background Information

Glycol ethers used in industrial applications are generally colorless, and miscible with water and organic solvents. They are produced by reacting ethylene oxide or propylene oxide with anhydrous alcohol in the presence of a catalyst. This process produces mixtures that are separated by fractional distillation.

Exposure Routes and Pathways

Dermal contact and inhalation of vapor, aerosol, and/or mist are the primary routes of exposure. Although the oral route of exposure would not be expected during proper use of the material, there is the potential for accidental ingestion to occur.

Toxicokinetics

Glycol ethers are absorbed readily after oral, dermal, or inhalation exposure. In addition, for the ethylene series, the ratio of the oral LD_{50} to the dermal LD_{50} is ~1, indicating that an equivalent amount of material can be absorbed by either route. The differences in toxicity between the ethylene series and the propylene series appears to be due the metabolites produced. The parent glycol ethers are substrates for alcohol dehydrogenase (ADH). Further conversion by aldehyde dehydrogenase produces alkoxyacetic acids. Conversion by ADH can be inhibited by pyrazole, alcohol, and other ADH inhibitors. Higher molecular weight glycol ethers are also partially metabolized by P450 isozymes. The ethylene glycol ethers are metabolized via the alkoxyacetaldehyde to the respective alkoxyacetic acid. For example, ethylene glycol monomethyl ether (EGME) is metabolized to methoxyacetic acid, which has been shown to produce the same biological effects as the parent compound, EGME. However, in the propylene glycol ether series, propylene glycol monomethyl ether (PGME) is metabolized to propylene glycol, which is further metabolized to carbon dioxide. The alkoxyacid metabolites appear to be responsible for the toxic effects reported in the testes, bone marrow, and embryo.

Mechanism of Toxicity

The alkoxyacid metabolites appear to be responsible for the toxic effects reported in the testes, bone marrow, and embryo. The testes, bone marrow, and embryo contain large numbers of rapidly dividing and differentiating cells, and it is possible that one or more processes of cell division and differentiation are affected. It has been hypothesized that alkoxyacetic acids may be introduced into the Krebs cycle by formation of methoxy- or ethoxyacetyl-CoA and by formation of methoxy- or ethoxycitrate by mitochondrial enzymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Glycol ethers as a class are not acutely toxic by the oral route. Inhalation exposure to high concentrations of compounds in the ethylene series can cause lethality. However, exposure to compounds in the propylene series was not lethal to rodents even at nearly saturated concentrations.

The monoalkyl ethylene glycol ethers have been shown to possess a wide spectrum of biological activity, with some variation in the range of effects and potency among the individual compounds. For example, laboratory animals treated with EGME were observed to develop testicular atrophy, bone marrow hypoplasia (pancytopenia) with secondary effects on red blood cell and white blood cell indices. EGME was teratogenic, embryotoxic, and fetotoxic in pregnant animals. Ethylene glycol monoethyl ether (EGEE) produced a similar pattern of toxicity, while ethylene glycol monopropyl ether (EGPE) and ethylene glycol monobutyl ether (EGBE) caused hemolysis and embryotoxicity/fetotoxicity without causing teratogenicity or effects on the bone marrow and testes. Therefore, it appears that the testicular and bone marrow effects decrease with increasing size of the alkoxy group, with the maximal effects observed for EGME. In general, the hemolytic effects appear to increase with the size of the alkoxy group. In addition, the hemolytic effects are more pronounced in mice, rats, and rabbits compared to dogs and man, which are less affected. The testicular effects have been observed in mice, rats, rabbits, and dogs, and the hematological effects have been observed in mice, rats, rabbits, cats, dogs, and man. However, propylene glycol ethers do not cause the testicular or hematological effects and are not teratogenic, although fetotoxicity has been reported in some studies at concentrations that also produced maternal toxicity. There are also reports that EGEE and EGBE cause kidney enlargement without functional impairment. EGME has been reported to affect conditioned avoidance behavior in trained rats, and PGME has been reported to cause central nervous system (CNS) depression. In general, the acetates derived from the glycol ethers have the same toxicological activity as the parent glycol ether. However, the acetate of PGME does appear to have teratogenic potential in rabbits, in contrast to the parent compound PGME.

In rats, EGME and its metabolite have also been reported to cause thymic involution in the absence of effects on body weight or spleen weight, reduced response to T cell mitogens, depressed production of interleukin-2, and altered response to primary antibody plaque-forming cells. Mice were not affected.

Human

Acute effects of overexposure include CNS changes (depression, ataxia, dysarthria, somnolence, tremor, personality change, and blurred vision); irritation of the eyes, nose, and throat; renal failure (including albuminuria, hematuria, and oxaluria); hemorrhagic gastritis; metabolic acidosis; and macrocytic anemia. An oral dose reported to cause lethality was $3 \,\mathrm{g \, kg^{-1}}$. There have been a number of reports in which workers were exposed to glycol ethers in the workplace. Fatigue, weakness, lethargy, anemia, bone marrow hypoplasia, and other abnormalities of hematological parameters (immaturity of neutrophils with some abnormal cells and low platelet concentration) have been reported in workers at exposure concentrations ranging from ~ 60 to 4000 ppm. There does not appear to be an association between exposure to glycol ethers and adverse effects on human testes.

Chronic Toxicity (or Exposure)

Animal

A 2 year inhalation study was conducted in F344/N rats and B6C3F1 mice. Exposure concentrations in rats were 0, 31.2, 62.5, and 125 ppm, and in mice the concentrations were 0, 62.5, 125, and 250 ppm for 6 h day^{-1} , 5 days week⁻¹. No oncogenic effects occurred in male rats. Pheochromocytomas were increased in 125 ppm females; however, the increase was not statistically significant. In male and female mice, forestomach squamous cell papillomas or carcinomas were increased in the 250 ppm group. Hemangiosarcomas were also increased in 250 ppm male mice. However, EGEE was not oncogenic in a 2 year rat feeding study at dietary concentrations of up to 0.9 g kg⁻¹ day⁻¹.

Human

Painters at a large shipyard exposed to EGEE and EGME (time-weighted average $0-80.5 \text{ mg m}^{-3}$ and $0-17.7 \text{ mg m}^{-3}$, respectively) had an increased prevalence of oligospermia and azoospermia. In addition, a significant proportion of the painters were

anemic and granulocytopenic. Workers exposed to EGEE in a metal castings process at concentrations of 0–24 ppm had significantly lower sperm count per ejaculate; however, mean sperm concentrations were similar to unexposed workers.

In Vitro Toxicity Data

A number of the glycol ethers have been evaluated with respect to potential mutagenicity and, in general, they were not mutagenic in a variety of test systems.

Clinical Management

If ingestion has occurred, emesis or gastric lavage may be useful if initiated within 30 min. If acidosis is present, it can be treated with intravenous sodium bicarbonate as needed. Hemodialysis may be indicated in cases of severe acid-base and/or fluid-electrolyte abnormalities or in cases of renal failure. Animal data suggest that ethanol therapy may inhibit the formation of toxic metabolites.

Table 1 Names, molecular formulas, structures, and exposure standards for typical ethylene glycol ethers

Compound	CAS number	Molecular formula	Chemical structure	Exposure standard
Ethylene glycol monomethyl ether	109-86-4	$C_3H_8O_2$	HOCH ₂ CH ₂ OCH ₃	TLV = 5 ppm NIOSH = 0.1 ppm
Ethylene glycol monoethyl ether	110-80-5	$C_4H_{10}O_2$	HOCH ₂ CH ₂ OC ₂ H ₅	IDLH = 200 ppm TLV = 5 ppm OSHA PEL = 200 ppm NIOSH = 0.5 ppm IDLH = 500 ppm
Ethylene glycol monobutyl ether	111-76-2	C ₆ H ₁₄ O ₂	HOCH ₂ CH ₂ OC ₄ H ₉	IDLH = 500 ppm OSHA = 50 ppm TLV = 25 ppm NIOSH = 5 ppm Possible human carcinogen
Ethylene glycol monopropyl ether	2807-30-9	$C_5H_{12}O_2$	HOCH ₂ CH ₂ OC ₃ H ₇	None
Ethylene glycol monophenyl ether	122-99-6	$C_8H_{10}O_2$	HOCH ₂ CH ₂ OC ₆ H ₅	None
Ethylene glycol monohexyl ether	112-25-4	$C_8H_{18}O_2$	HOCH ₂ CH ₂ OC ₆ H ₁₃	None
Diethylene glycol monomethyl ether	111-77-3	$C_5H_{12}O_3$	HOCH ₂ CH ₂ O-CH ₂ CH ₂ OCH ₃	None
Diethylene glycol monoethyl ether	111-90-0	$C_6H_{14}O_3$	HOCH ₂ CH ₂ O-CH ₂ CH ₂ OC ₂ H ₅	WEEL = 25 ppm
Diethylene glycol monobutyl ether	112-34-5	C ₈ H ₁₈ O ₃	HOCH ₂ CH ₂ O-CH ₂ CH ₂ OC ₄ H ₉	None
Diethylene glycol monopropyl ether	6881-94-3	C ₇ H ₁₆ O ₃	HOCH ₂ CH ₂ O-CH ₂ CH ₂ OC ₃ H ₇	None
Diethylene glycol monohexyl ether	112-59-4	C ₁₀ H ₂₂ O ₃	HOCH ₂ CH ₂ O-CH ₂ CH ₂ OC ₆ H ₁₃	None
Triethylene glycol methyl ether	112-35-6	C ₇ H ₁₆ O ₄	HOCH ₂ CH ₂ O- CH ₂ CH ₂ OCH ₂ CH ₂ -OCH ₃	None
Triethylene glycol ethyl ether	112-50-5	$\mathrm{C_8H_{18}O_4}$	HOCH ₂ CH ₂ O– CH ₂ CH ₂ OCH ₂ CH ₂ –OC ₂ H ₅	None
Triethylene glycol butyl ether	143-22-6	$C_{10}H_{22}O_4$	$\begin{array}{c} HOCH_2CH_2O-\\ CH_2CH_2OCH_2CH_2-OC_4H_9 \end{array}$	None

TLV, threshold limit value for an 8 h day; OSHA PEL, Occupational Safety and Health Administration permissible exposure limit; WEEL, workplace environmental exposure level for an 8 h day; NIOSH, National Institute of Occupational Safety and Health exposure level for a 10 h day; IDLH, Immediately Dangerous to Life or Health.

Compound	CAS number	Molecular formula	Chemical structure	Exposure standards
Propylene glycol monomethyl ether	107-98-2	$C_4H_{10}O_2$	CH ₃ CH(OH)CH ₂ –OCH ₃	TLV = 100 ppm NIOSH = 100 ppm
Propylene glycol monoethyl ether	1569-02-4	$C_5H_{12}O_2$	CH ₃ CH(OH)CH ₂ –OC ₂ H ₅	None
Propylene glycol monopropyl ether	1569-01-3	$C_{6}H_{14}O_{2}$	CH ₃ CH(OH)CH ₂ –OC ₃ H ₇	None
Propylene glycol isopropyl ether	3944-36-3	$C_6H_{14}O_2$	CH ₃ CH(OH)CH ₂ –OCH(CH ₃) ₂	None
Propylene glycol n-butyl ether	5131-66-8	C ₇ H ₁₆ O ₂	CH ₃ CH(OH)CH ₂ –OC ₄ H ₉	None
Propylene glycol t-butyl ether	57018-52-7	$C_7 H_{16} O_2$	CH ₃ CH(OH)CH ₂ –OC(CH ₃) ₃	None
Propylene glycol butoxyethyl ether	124-16-3	C ₉ H ₂₀ O ₃	CH ₃ CH(OH)CH ₂ –OC ₂ H ₅ OC ₄ H ₉	None
Propylene glycol phenyl ether	770-35-4	$C_9H_{12}O_2$	CH ₃ CH(OH)CH ₂ –OC ₆ H ₅	None
Dipropylene glycol methyl ether	34590-94-8	$C_7H_{16}O_3$	CH ₃ OCH(CH ₃)–CH ₂ OCH(CH ₃)–CH ₂ OH	TLV = 100 ppm NIOSH = 100 ppm IDLH = 600 ppm
Dipropylene glycol ethyl ether	15764-24-6	$C_8H_{18}O_3$	C ₂ H ₅ OCH ₂ CH ₂ –CH ₂ OCH ₂ CH ₂ –CH ₂ OH	None
Dipropylene glycol butyl ether	29911-28-2	C ₁₀ H ₂₂ O ₃	C ₄ H ₉ OCH ₂ CH–(CH ₃)OCH ₂ CH–(CH ₃)OH	None
Tripropylene glycol methyl ether	20324-33-8	C ₁₀ H ₂₂ O ₄	CH ₃ OCH ₂ CH–(CH ₃)OCH ₂ CH– (CH ₃)OCH ₂ CH–(CH ₃)OH	None
Tripropylene glycol ethyl ether	20178-34-1	$C_{11}H_{24}O_4$	$C_2H_5OCH_2CH(CH_3)OCH_2CH$ (CH_3)OCH_2CH(CH_3)OH	None
Tripropylene glycol butyl ether	57499-93-1	$C_{13}H_{28}O_4$	$C_4H_9OCH_2CH(CH_3)OCH_2CH$ (CH_3)OCH_2CH(CH_3)OH	None
Butylene glycol methyl ether	53778-73-7	$C_5H_{12}O_2$	CH ₃ OCH ₂ CH–(OH)CH ₂ CH ₃	None
Butylene glycol ethyl ether	111-73-9	$C_6H_{14}O_2$	HOCH ₂ CH ₂ CH ₂ -CH ₂ OCH ₂ CH ₃	None

 Table 2
 Names, molecular formula, structures, and exposure standards for typical propylene glycol ethers

TLV, threshold limit value for an 8 h day; OSHA PEL, Occupational Safety and Health Administration permissible exposure limit; WEEL, workplace environmental exposure level for an 8 h day; NIOSH, National Institute of Occupational Safety and Health exposure level for a 10 h day; IDLH, Immediately Dangerous to Life or Health.

Environmental Fate

In the aquatic ecosystem, glycol ethers are estimated to remain in the water and are not expected to bioconcentrate in aquatic organisms or adsorb to sediment; however, they are expected to be removed by aerobic biodegradation. In the atmosphere, glycol ethers are expected to react with hydroxyl radicals. In the soil, they are expected to be mobile, and may volatilize from dry soil surfaces. Hydrolysis and photolysis are not expected to significantly contribute to their removal. However, aerobic degradation is expected to occur rapidly in soil.

Ecotoxicology

The 24 h LC₅₀ in fish was greater than $5000 \text{ mg} \text{l}^{-1}$ and the 96 h LC₅₀ was greater than 10 000 ppm for EGEE. For EGBE, the 96 h LC₅₀ was 1250–1490 ppm for fish and the LC₅₀ for shrimp was $800 \text{ mg} \text{l}^{-1}$.

Other Hazards

Some glycol ethers are incompatible with strong oxidizers and caustic agents.

Exposure Standards and Guidelines

See Tables 1 and 2.

See also: Ethylene Glycol; Ethylene Glycol Monoethyl Ether; Ethylene Glycol Mono-*n*-Butyl Ether; Polyethylene Glycol.

Further Reading

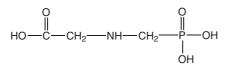
- Bioassay Systems Corporation (1983) Determination of the Reproductive Effects in Mice of Nine Selected Chemicals. EPA Document No. FYI-OTS-0483-0240.
- Doe JE (1984) Further studies on the toxicology of the glycol ethers with emphasis on rapid screening and hazard assessment. *Environmental Health Perspectives* 57: 199–206.
- Miller RR (1987) Metabolism and disposition of glycol ethers. *Drug Metabolism Reviews* 18(1): 1–22.
- Toxicology and Carcinogensis Studies of 2-Butoxyethanol in F344/N Rats and B6C3F1 Mice. Technical Report Series No 484 (2000). NIH Publication No. 00-3974.
- US EPA (1984) Health Effects Assessment for Glycol Ethers. EPA/540/1-86/052.
- US EPA (2000) Integrated Risk Information System (IRIS) for Ethylene Glycol Monobutyl Ether (111-76-2), March 15.

Glyphosate

Kevin N Baer

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 1071-83-6
- SYNONYMS: N-Phosphonomethyl glycine; Roundup (41%); Accord; Rodeo; Gliialka; Sonic; Glifinox; Glycel
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organic phosphate herbicide
- CHEMICAL STRUCTURE:



Uses

Glyphosate is the active ingredient in several commercial herbicides for nonselective weed control.

Exposure Routes and Pathways

The primary route of exposure to glyphosate is through accidental or intentional ingestion. Dermal exposure is not typically associated with systemic effects. Most incidents reported in humans have involved skin or eye irritation in workers after exposure during mixing, loading, or application.

Toxicokinetics

The major breakdown product of glyphosate is aminomethyl phosphonic acid (AMPA). The oral absorption of glyphosate and AMPA is low, with both compounds being eliminated essentially unchanged. Dermal absorption is also very low. High concentrations have been found in the kidneys, liver, brain, and blood following intentional oral ingestion. Glyphosate is rapidly excreted in the urine in large amounts. Usually within 24–48 h, glyphosate is undetectable in the urine. Neither glyphosate nor AMPA exhibit any tendency for bioaccumulation.

Mechanism of Toxicity

Several mechanisms have been proposed for glyphosate, such as uncoupling of mitochondrial oxidative phosphorylation, inhibition of aryl hydrocarbon hydroxylase activity, and inhibition of cytochrome P450 activity. However, surfactants present in many commercial preparations (i.e., Roundup), are considered to be responsible, in part, for the observed toxicity. In contrast to organophosphate insecticides, glyphosate is not a significant inhibitor of acetylcholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Glyphosate is a compound of low mammalian toxicity. Oral LD_{50} values for laboratory rodents are >4 g kg⁻¹. The dermal LD_{50} value in rabbits is ~5 g kg⁻¹. Direct contact with eyes of concentrated solutions of glyphosate can lead to transient irritation.

Human

The primary effects following ingestion include mucous membrane irritation, abdominal pain, vomiting, diarrhea, hypotension, oliguria, and anuria. Esophageal and gastric erosions have occurred after ingestion of concentrated solutions (41% glyphosate). In fatal cases, hypovolemic shock, cardiac arrhythmias, metabolic acidosis, and pulmonary edema have been reported. However, glyphosate has relatively low toxicity, with mortality rates of only 17% in suicidal cases. Ingestions of 150 ml or less have not resulted in deaths.

Chronic Toxicity (or Exposure)

Animal

Glyphosate and AMPA exhibit little potential for chronic toxicity. Multigeneration feeding studies failed to detect any tumorigenic potential. Glyphosate and AMPA were not teratogenic and did not lead to developmental toxicity. Two reproductive toxicity studies failed to detect any significant alterations. Glyphosate and AMPA were negative in endocrine modulation assays.

Human

Dermatitis resembling sunburn has been reported following prolonged skin exposure. There is no evidence of carcinogenicity in humans and only one tumorigenic response has been observed in experimental animals.

Clinical Management

There is no specific antidote; symptoms should be treated. For exposure to the eyes, the eyes should be flushed with plenty of water for at least 15 min. Due to the possibility of esophageal erosion, emesis is not recommended. Activated charcoal and a cathartic should be administered following ingestion of large amounts of glyphosate. Oral irrigation and dilution may be sufficient for smaller ingestions. In severe cases, basic life support, such as fluid replacement for hypovolemic shock, should be provided.

Gold

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7440-57-5
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Metals
- CHEMICAL FORMULA: Au¹⁺, Au³⁺

Uses

Gold has found many industrial uses because of its excellent electrical and thermal conductivity properties. It is used for plating other metals and as an alloying metal. It is used in the manufacture of jewelry, dental inlays, art, currency, electronic components, and in some medical devices to provide radio opacity. Gold compounds have also found use in medicine in the treatment of certain cancers, rheumatoid arthritis, discoid lupus (a rare skin disease), and in specialized surgical procedures.

Background Information

Gold is probably the first pure metal known to man and is chemically nonreactive.

Exposure Routes and Pathways

The most common exposure pathway is through dermal contact. Inhalation and oral exposure to gold dust may occur in occupational settings. Hemodialysis is indicated in patients with renal failure.

See also: Pesticides.

Further Reading

Williams GM, Kroes R, and Munro IC (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regulatory Toxicology and Pharmacology* 31: 117–165.

Toxicokinetics

Gold dust and gold salts are poorly absorbed from the gastrointestinal tract.

Mechanism of Toxicity

The main mechanism believed to be responsible for gold salt toxicity is the formation of gold–protein complexes that elicit immune reactions. That is, gold salts may act as a hapten with subsequent antibody production against the gold–protein complex. The gold–protein–antibody complexes may in turn accumulate in the glomerular subepithelium. A second possible mechanism of gold salt toxicity is that antibodies may be formed against kidney tubular cells damaged by gold.

Acute and Short-Term Toxicity (or Exposure)

Human

Not known to be acutely toxic, though some of its salts are.

Chronic Toxicity (or Exposure)

Animal

Animal experiments have shown that gold dust is not carcinogenic to rats. However, subcutaneous implantation of gold sheets was able to induce tumors.

Human

Oral administration of excessive amounts of gold salts has been found to produce pancytopenia in

certain individuals. In addition, therapeutic doses of gold salts given for the treatment of rheumatic disease may produce adverse side effects such as dermatitis, immune complex hypersensitivity, nephrotoxicity, and peripheral neuropathy. Gold can also cause aplastic anemia and kidney damage.

Clinical Management

If toxicity occurs, further exposure to gold or gold salts should be prevented. Dimercaprol may be used as a chelating agent. A physician should be consulted if gold is being used as a therapeutic agent. Supportive treatment should be provided.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MD (1995) Metal Toxicology. San Diego, CA: Academic Press.

Merchant B (1998) Gold, the noble metal and the paradoxes of its toxicology. *Biologicals* 26: 49–59.

Good Clinical Practice (GCP)

Sharmilee P Sawant and Harihara M Mehendale

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Good clinical practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording, and reporting the results of clinical trials that involves participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and wellbeing of trial subjects is protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible. The guidance was developed with consideration of the current GCP of the European Union, Japan, and the United States, as well as those of Australia, Canada, and the World Health Organization. The purpose of these guidelines is to set globally applicable standards for the conduct of biomedical research on human subjects.

Traditionally, GCP has been a term used by those in government and industry to identify a collection of related regulations and guidelines that, when taken together, define the clinical study-related responsibilities of sponsors, clinical investigators, monitors, and institutional review boards (IRBs).

Principles of GCP

The principles of GCP are as follows:

- 1. Before a trial is initiated, risks should be weighed against the anticipated benefits for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits outweigh the risks.
- 2. The rights, safety, and well-being of the trial subjects are the most important considerations

and should prevail over interests of science and society.

- 3. The available nonclinical and clinical data on an investigational new drug (IND) should be adequate to support the proposed clinical trial.
- 4. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
- 5. A trial should be conducted in compliance with the protocol that has received prior IRB approval.
- 6. The medical care given to, and medical decisions made on behalf of, trial subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.
- 7. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
- 8. Freely given informed consent should be obtained from every subject prior to clinical trial participation.
- 9. The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality in accordance with the applicable regulatory requirement(s).
- 10. INDs should be manufactured, handled, and stored in accordance with applicable good manufacturing practice.

Specific responsibilities for investigators, sponsors, and the IRB/Independent Ethics Committee (IEC) detailed in the GCP provisions include the following.

Responsibilities of Investigators

A clinical investigator is the individual who conducts, or who is the responsible leader of a team that conducts, a clinical investigation. Federal regulations state that "an investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation."

Investigator responsibilities include:

- 1. *Control of the product*: The investigator can administer the IND only to subjects under his or her personal supervision.
- 2. *Record keeping and record retention*: The investigator must maintain adequate drug usage records, and must prepare and maintain, for each subject, adequate and accurate records of all observations and data pertinent to the clinical trial. The records must be kept for a minimum of 2 years till the marketing application's approved or a sponsor has discontinued an IND and notified the Food and Drug Administration (FDA).
- 3. *The investigator must provide to the sponsor*: (1) progress report of the clinical study; (2) safety reports of all adverse experiences that may reasonably be regarded as caused by, or probably caused by, the drug; and (3) adequate reports shortly after the completion of the investigator's participation in the study.
- 4. Assurance of IRB review: The investigator must assure that an IRB complying with regulatory requirements will be responsible for the initial and continuing review and approval of the proposed clinical trial.
- 5. *Handling of controlled substances*: The investigator should take adequate precautions to prevent theft or diversion of the product subjected to the Controlled Substances Act.

Responsibilities of Sponsor

Federal regulations define 'sponsor' as "a person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization."

In general, the term 'sponsor' refers to a commercial manufacturer that has developed a product in which it holds the principal financial interest. A sponsor may also be a physician, commonly called a 'sponsor-investigator', which federal regulations define as "an individual who both initiates and conducts an investigation and under whose immediate direction the investigational drug is administered or dispensed." The FDA defines sponsor responsibilities in Part 312, Subpart D in the Code of Federal Regulations (CFR).

The sponsor responsibilities can be divided into the following general areas:

- 1. *Selecting qualified investigators and monitors*: The sponsor must select qualified investigators-physicians and other professionals to conduct the clinical trial. According to the guideline, the sponsor may assign one or more appropriately trained and qualified individuals to monitor the progress of the clinical investigation.
- 2. *Informing investigators*: The sponsor should give complete information to the investigators about the investigational drug.
- 3. *Trial design and review of ongoing studies*: The sponsor should closely monitor the conduct and progress of clinical trails to determine if the investigator is conducting the trial in compliance with the protocol previously approved by IRB/ IEC, applicable federal regulations, and an acceptable standard of GCP and whether the IND study is presenting unreasonable risk to the human subjects.
- 4. *Record keeping and record retention:* The sponsor should maintain adequate records showing the receipt, shipment to the investigator or disposition of the IND and keep records of the quantity of IND, date of shipment to the investigator.
- 5. Ensuring the return or disposition of unused IND and related supplies.

Institutional Review Board/Independent Ethics Committee

IRB/IEC is an independent body constituted of medical, scientific, and nonscientific members, whose responsibility is to ensure the protection of the rights, safety, and well-being of human subjects involved in a trial by reviewing, approving, and providing continuing review of trials, of protocols and amendments, and of the methods and material to be used in obtaining and documenting informed consent of the trial subjects. IRBs that approve studies of FDA regulated products must be established and operated in compliance with 21 CFR Part 56.

The responsibilities of IRB/IEC are to

- 1. Safeguard the rights, safety, and well-being of all trial subjects.
- 2. See if selection of subjects is equitable and informed consent is sought and documented in accordance with federal regulations.

3. Review the investigator brochure (IB), safety information, information about payments and compensation to subjects, the investigator's current curriculum vitae, and/or other documentation evidencing qualifications, and any other documents that the IRB/IEC may require to fulfill its responsibilities.

An IRB must have at least five members, chosen by the institution. FDA regulations allow institutions that do not have IRB/IECs to use 'independent' or other institutions' IRB/IECs to review their studies. IRB/IEC members are often physicians, pharmacologists, toxicologists, and administrative managers from the parent institution. Generally, drug sponsors have limited direct contact with an IRB/IEC. Aside from safety concerns, an IRB/IEC may address several issues including specific standards of the institution, state, and locality in reviewing a study. Any research program that the board approves must meet several criteria specified in FDA regulations:

- 1. Risk to subjects must be minimized and should be reasonable in relation to anticipated benefits and importance of the knowledge that may be expected to be gained.
- 2. Subject selection must be equitable.
- 3. Informed consent must be sought from each prospective subject or the subject's legally authorized representative and should be appropriately documented.
- 4. The research plan must make adequate provisions to monitor the collected data to ensure safety of subjects.

The sponsor and investigator will prepare the protocol for conducting clinical trials of the IND and the IB and get it approved by the IRB/IEC before the conduct of the clinical trial.

Clinical Trial Protocol

The contents of a trial protocol should generally include the following topics. However, specific information may be provided in separate information sheets such as the IB. The clinical trial protocol briefly contains:

- 1. *General information*: This includes protocol titles, name, title and address of sponsor, investigator, and qualified physician.
- 2. Background information: Name and description of investigational products, justification for route of administration, dosage and treatment

period, description of population studied, and references.

- 3. Trial objective and purpose.
- 4. *Trial design*: The scientific integrity of the trial and credibility of the data from the trial depends on the trial design (e.g., double-blind, placebo-controlled, parallel design) and can be randomized and/or blinded to minimize/avoid bias.
- 5. Selection and treatment of subjects.
- 6. Assessment of safety, efficacy, and statistics.
- 7. Quality control and assurance.
- 8. Ethics.
- 9. Data handling and record keeping.

Investigator Brochure

The IB is a compilation of the clinical and nonclinical data on the investigational product(s) that are relevant to the study of the products in human subjects. Briefly, it contains:

- 1. *Summary*: A brief summary highlighting the significant physical, chemical, pharmaceutical, pharmacological, toxicological, pharmacokinetics, metabolic, and clinical information available that is relevant to the stages of clinical development of the investigational product.
- 2. *Introduction*: Introductory statement about the investigational drug and general approach followed for evaluating the investigational drug.
- 3. Physical, chemical, and pharmaceutical properties and formulation.
- 4. Nonclinical studies' data and results should be provided in summary form.
- 5. Effects in humans.
- 6. Summary of data and guidance for the investigator.

Summary

FDA's GCPs regulations are designed to accomplish two primary goals: (1) to ensure the quality and integrity of the data obtained from clinical studies so that the FDA's decisions based on these data are informed and responsible; (2) to protect the rights and, to the degree possible, the welfare of clinical subjects. To summarize, GCPs are regulations and guidelines that, when taken together, define the clinical studyrelated responsibilities of sponsors, clinical investigators, monitors, and IRB/IEC for the conduct of clinical trials, and define and monitor the clinical trials as per FDA regulations. Following clinical studies and based on the results of nonclinical and clinical studies, the drug sponsor formally proposes a new drug application (NDA) to the FDA for approval of a new drug for marketing and sales in the United States. To obtain this government authorization, a sponsor submits in an NDA thousands of pages of nonclinical and clinical test data and analyses, drug chemistry information, and description of manufacturing procedures. Traditionally, the FDA has required that regulatory submissions, such as NDAs, be submitted as paper documents. Regulations in 21 CFR Part 314 provide the requirements and procedures for submitting applications to the Center for Drug Evaluation and Research to obtain approval for the marketing of new drugs. Since August 1997 these applications can be submitted electronically. *See also:* Food and Drug Administration, US; Good Laboratory Practices (GLP); Investigative New Drug Application; Safety Testing, Clinical Studies.

Further Reading

Mathieu M (1997) Good clinical practices (GCP). In: *New Drug Development: A Regulatory Overview*, 5th edn., pp. 163–184. Waltham, MA: PARAXEL International Corporation.

Relevant Website

http://www.fda.gov – Food and Drug Administration website.

Good Laboratory Practices (GLP)

Robin C Guy

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Background Information

Good Laboratory Practices (GLPs) are a standard compliance monitoring program that assures the quality and integrity of nonclinical test data submitted to the (US) Food and Drug Administration (FDA) and the (US) Environmental Protection Agency (EPA). These were originally developed by the FDA to develop minimum research standards for laboratories to protect the quality and integrity of studies as a result of procedures conducted in some studies that were not conducted according to conventional laboratory procedures. As a result, FDA promulgated the GLP Regulations, 21 CFR Part 58, on December 22, 1978 (43 FR 59986). The regulations became effective June 1979; sections have since been amended. The EPA adopted GLPs in August 1989. Other countries have also adopted GLPs. While there may be some slight variations, they all have the same central focus. Most countries have regular inspections and data audits to monitor laboratory compliance with the GLP requirements.

Sponsors of FDA-regulated products are required by the Federal Food, Drug, and Cosmetic Act (FFDCA) and Public Health Service Act to submit evidence of their product's safety in research and/or marketing applications. These products include food and color additives, animal drugs, human drugs and biological products, human medical devices, diagnostic products, and electronic products. These data are then used to answer questions regarding the toxicity profile of the article, the observed noadverse-effect dose level in the test system, the risks associated with clinical studies involving humans or animals, the potential teratogenic, carcinogenic, or other adverse effects of the article, and the level of use that can be approved.

Sponsors of EPA-regulated products are required by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA) to submit evidence that assures the quality and integrity of test data submitted to EPA. These data are used by EPA to regulate pesticides and industrial chemicals.

The importance of nonclinical laboratory studies demand that they be conducted according to scientifically sound protocols and with meticulous attention to quality. GLPs provide that guidance. GLP regulations cover a large part of the aspects of nonclinical research. They include:

- inspection of a testing facility;
- personnel;
- testing facility management;
- study director;
- quality assurance unit;
- animal care facilities;
- facilities for handling test and control articles (EPA also has 'reference substances');
- lab operation areas;
- specimen and data storage;
- equipment design, maintenance, and calibration;
- standard operating procedures (SOPs);
- animal care;

- characterization and handling of articles (EPA also includes this for reference substances);
- protocol;
- reporting results;
- record retention; and
- disqualification of testing facilities.

Details of the GLPs and GLP inspections may be found on government-specific websites. Many are listed in the references below. Briefly, the FDA GLPs, define the scope of the regulation and have a detailed listing of definitions. If the FDA is to consider a nonclinical laboratory study in support of an application for a research or marketing permit, the testing facility, records and specimens must be available for inspection by an authorized employee of the FDA.

Every person who is responsible for any part of any GLP study must have the appropriate education, training, and experience, or combination thereof, to enable that person to perform the assigned functions. Each testing facility shall maintain up-to-date records of training, experience and job description for everyone involved in the conduct of a nonclinical laboratory study. There shall be adequate personnel to conduct the study according to the protocol who shall take appropriate precautions to avoid contamination of the test and control articles and the test systems.

The Study Director is responsible for the aspects of the study, but management also has responsibilities. For every nonclinical laboratory study, the management needs to assign a study director and assure that there is a quality assurance unit. Management must also assure that test and control articles or mixtures have been appropriately tested for identity, strength, purity, stability, and uniformity, as applicable. They need to assure that personnel understand the procedures they are to perform and are available in addition to availability of resources, facilities, equipment, materials, and methodologies. The Study Director has overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results, and represents the single point of study control. This point has been interpreted in many ways, but the intent is that the study director is responsible for all aspects of the study, including procedures that may take place at another facility. Examples may include analysis of analytical samples for confirmation of concentration or homogeneity of a test article mixture, or for analysis of biological samples (e.g., pharmacokinetic or histology).

The quality assurance unit is responsible for monitoring each study to assure management that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with the regulations in this part. The quality assurance unit shall be entirely separate from and independent of the personnel engaged in the direction and conduct of that study.

The GLPs specify certain requirements for the facilities. In particular, there needs to be dedicated areas for separation of species or test systems, isolation of individual projects, quarantine of animals, routine or specialized housing of animals, safe sanitary storage of waste before removal from the testing facility, feed, bedding, supplies, and equipment. Storage areas for feed and bedding shall be separated from areas housing the test systems and shall be protected against infestation or contamination. There also need to be separate areas for receipt and storage of the test and control articles, mixing of the test and control articles with a carrier, and storage of the test and control article mixtures. In addition, space needs to be provided for limitedaccess archives.

Requirements for equipment also exist. The equipment must be tested, inspected, maintained, and calibrated on a regular basis, according to written standard operating procedures (SOPs). These procedures need to be documented. Written records also need to be maintained for nonroutine repairs performed on equipment as a result of failure and malfunction.

The testing facility shall have SOPs in writing setting forth nonclinical laboratory study methods that management is satisfied are adequate to insure the quality and integrity of the data generated in the course of a study. All deviations in a study from SOPs shall be authorized by the Study Director and shall be documented in the raw data. Significant changes in established SOPs shall be properly authorized in writing by management. The facility needs to maintain an historical file of SOPs, and all revisions. These SOPs and any appropriate laboratory manuals must be immediately available to the laboratory procedures being performed.

There are many requirements for animal care. Details could be found in the GLPs and also in the (US) Department of Agriculture Guide for the Care and Use of Laboratory Animals. Besides proper husbandry practices, animals need to be identified under certain circumstances.

All of the reagents and solutions used in the laboratory areas need to be labeled to indicate identity, titer or concentration, storage requirements, and expiration date. These must be discarded if the reagents or solutions are deteriorated or outdated. The GLPs have strict guidelines for the test and control articles (EPA discusses test substances, control substances, and reference substances).

According to the FDA GLPs, a test article is "any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any other article subject to regulation under the act or under Sections 351 and 354-360F of the Public Health Service Act." A control article means "any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any article other than a test article, feed, or water that is administered to the test system in the course of a nonclinical laboratory study for the purpose of establishing a basis for comparison with the test article."

The EPA GLPs for both the TSCA and the FIFRA have basically the same definitions for control substances and reference substances. A control substance means "any chemical substance or mixture, or any other material other than a test substance, feed, or water, that is administered to the test system in the course of a study for the purpose of establishing a basis for comparison with the test substance for chemical or biological measurements." A reference substance means "any chemical substance or mixture, or analytical standard, or material other than a test substance, feed, or water, that is administered to or used in analyzing the test system in the course of a study for the purposes of establishing a basis for comparison with the test substance for known chemical or biological measurements."

Test substances are defined differently. The TSCA GLPs state that a test substance means "a substance or mixture administered or added to a test system in a study, which substance or mixture is used to develop data to meet the requirements of a TSCA Section 4(a) test rule and/or is developed under a TSCA Section 4 testing consent agreement or Section 5 rule or order to the extent the agreement, rule or order references this part." The GLPs for FIFRA state that a test substance means "a substance or mixture administered or added to a test system in a study, which substance or mixture: (1) Is the subject of an application for a research or marketing permit supported by the study, or is the contemplated subject of such an application; or (2) Is an ingredient, impurity, degradation product, metabolite, or radioactive isotope of a substance described by paragraph (1) of this definition, or some other substance related to a substance described by that paragraph, which is used in the study to assist in characterizing the toxicity, metabolism, or other characteristics of a substance described by that paragraph."

The FDA GLPs state that the identity, strength, purity, and composition or any other characteristics

that appropriately define the test or control article shall be determined for each batch and shall be documented. In addition, the methods of synthesis of the test and control articles need to be documented. The stability of each test or control article needs to be determined by the testing facility or by the sponsor either before study initiation, or concomitantly. Specific requirements for each storage container for a test or control article shall be labeled by name, chemical abstract number or code number, batch number, expiration date if any, and, where appropriate, storage conditions. Retention samples are needed for any study longer than 4 weeks in duration.

Test and control article handling procedures are addressed. Procedures need to be established for a system for the handling of the test and control articles to ensure that proper handling and storage are utilized; the materials are allocated so that there is no possibility of contamination, deterioration, or damage. At all times, identification of the contents must be maintained throughout the distribution process and all actions are documented. Identification of the contents of a container helps to ensure that the material will be used properly. It is also prudent to label temporary or transport containers to avoid mix-ups.

Documentation and the same type of procedures are important for mixtures of articles with carriers. In addition, procedures for each test or control article that is mixed with a carrier, appropriate analytical methods shall be conducted to determine the uniformity and stability of the mixture and the concentration of the test or control article in the mixture. In GLP studies, these assays should incorporate validated methods. SOPs need to define general ranges for standard parameters used for analytical acceptability.

The protocol for nonclinical studies must address specific concerns. In addition, it must be approved and written so that it clearly indicates the objectives and all methods for the conduct of the study. The nonclinical GLP studies have to be conducted in accordance with the protocol. This includes documentation of all aspects of the study. Over time, personnel leave laboratories; therefore, the only way to reproduce a study is to have original documentation that is adequate and legible. Data need to be signed and dated by the person making the observations. Any change in entries shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed or identified at the time of the change. With computerized systems that incorporate automated data collection, the individual responsible for direct data input shall be identified at the time of data input. Any change in automated data entries shall be dated and made so as not to obscure the original entry, the reason for the change needs to be indicated, and the responsible individual needs to be identified.

A final report for each nonclinical laboratory study shall be prepared. Details are provided in the GLPs. The final report summarizes most of the experimental details of the study. The final report needs to include the name and address of the facility(s), objectives and procedures as stated in the approved protocol, including any changes in the original protocol, test, and control article information, information on the preparations used to administer the material, including dosage, dosage regimen, and route of administration. Also needed are duration of the study, a description of the methods, and the test system used. Any circumstances that may have affected the quality or integrity of the data must be addressed. In addition, the locations where all specimens, raw data, and the final report are to be stored, are needed. The quality assurance unit prepares and signs a statement of GLP compliance, and the final report is signed and dated by the study director. If there is any question regarding the GLP compliance to a study, the person(s) responsible should declare this information in the report, as ignoring any issues may lead to legal concerns. The study director addresses any corrections, as in the case of changes to protocols, as an amendment.

The final report and any amendments, all raw data, documentation, protocols and any amendments, and specimens (with the exception of specimens subject to degradation) generated as a result of a nonclinical laboratory study shall be retained in an archive. The archive facility needs to be set up for orderly storage and expedient retrieval. Conditions of storage shall minimize deterioration of the documents or specimens. The archives do not necessarily have to be an in-house facility; the laboratory may contract with commercial archives to store materials in a GLP fashion.

Any off-site data storage locations need to be indexed and documented so that this information is easily obtainable. In either case, the FDA requires that documentation records, raw data, and specimens pertaining to a nonclinical laboratory study shall be retained in the archive(s) for a period of at least 5 years following the date on which the results of the nonclinical laboratory study are submitted in support of an application for a research or marketing permit. With the exception of investigational new drug applications or applications for investigational device exemptions, if an application is approved for a research or marketing permit, for which the results of the nonclinical laboratory study were submitted, data needs to be held for a period of at least 2 years following the date of approval. Other situations (e.g., where the nonclinical laboratory study does not result in the submission of the study in support of an application for a research or marketing permit), data is to be kept for a period of at least 2 years following the date on which the study ends. Appropriate wet specimens, samples of test or control articles, and materials that may be subjected to degradation even under proper storage conditions, shall be retained only as long as the quality of the preparation affords evaluation.

Other documentation targeted for storage in archives include the master schedule sheet, copies of protocols, and records of quality assurance inspections, summaries of training and experience, job descriptions, records and reports of the maintenance and calibration and inspection of equipment. In any case, ensure that the protocol or SOPs address the proper archiving of appropriate materials.

The FDA may find that it needs to disqualify testing facilities if the facility has not complied with the requirements of the GLP regulations. All studies completed after the date of disqualification can be excluded from consideration. It is prudent to ensure that all laboratories used are GLP compliant, or studies and entire projects may be compromised.

See also: Food and Drug Administration, US; Good Clinical Practices (GCP); International Conference on Harmonization; Redbook.

Further Reading

Gad SC (ed.) (2001) *Regulatory Toxicology*, 2nd edn. New York: Taylor and Francis.

Relevant Websites

- http://www.mca.gov.uk Inspection: The United Kingdom Good Laboratory Practice Monitoring Authority.
- http://www.epa.gov US Environmental Protection Agency, Good Laboratory Practice and 40 CFR.
- http://www.fda.gov US Food and Drug Administration, Good Laboratory Practice.
- http://www.accessdata.fda.gov US Food and Drug Administration, Good Laboratory Practice, 21 CFR. and 21CFR
- http://www.oecd.org Organisation for Economic Co-operation and Development (OECD), Good Laboratory Practice.

Grain Incidents and Other Mercury Tragedies: Forms, Fate, and Effects

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Mercury is one of hundreds of toxic substances that people are being exposed to at ever-increasing rates. Incidents such as the outbreaks in Minamata Japan, Iraq, New Mexico, and the Great Lakes should serve as a strong warning of what unchecked industrial pollution and careless handling of toxic substances can do to humans, wildlife, and their surroundings. Strong regulations, education and enforcement are needed to prevent the suffering and tragedies that are commonplace today.

Incidents

Potential sources of human exposure to mercury include food contaminated with mercury, inhalation of mercury vapors in ambient air, and exposure to mercury through water, soil and sediment. Dietary intake is by far the most important source of exposure to mercury for the general population. Fish and other seafood products are the main source of methylmercury in the diet; studies have shown that methylmercury concentrations in fish and shellfish are \sim 10–100 times greater than in other foods, including cereals, potatoes, vegetables, fruits, meats, poultry, eggs, and milk. As of December 1998, mercury was the chemical contaminant responsible, at least in part, for the issuance of 1931 fish consumption advisories by 40 states, including the US territory of American Samoa. Almost 68% of all advisories issued in the United States are a result of mercury contamination in fish and shellfish. Advisories for mercury have increased steadily by 115% from 899 advisories in 1993 to 1931 advisories in 1998. The number of states that have issued mercury advisories also has risen steadily from 27 states in 1993 to 40 states in 1997, and remains at 40 states for 1998. Advisories for mercury increased nearly 8% from 1997 (1782 advisories) to 1998 (1931 advisories).

In Alamagordo, New Mexico, a farmer worked in a seed store, which supplied local farmers, and he maintained a few pigs at home. He noticed a significant amount of wastage in the form of spilled seed grain (treated with methyl mercuric dicyandiamide) at the store, and he began sweeping it up to feed to his pigs. Within a short time his pigs became ill. Of 18 pigs, 14 developed a neurologic illness and 12 died. Fearful of the loss of his investment, the farmer had rest of the pigs butchered, and froze the meat for the use of his family. Within 2 weeks of eating the poisoned pork, three out of the family of 10 were stricken with brain and spinal cord damage. One girl lay unconscious for 8 months in the hospital before waking totally blind and unable to speak. Twentytwo years after this incident all surviving members of the family were carefully examined and tested. In this interim the two youngest children had died, and autopsy and toxicological findings were available from one of these. Both were left in a vegetative state until their deaths. Some recovery did occur in the older children, but the visual defects, including blindness in one and constricted visual fields in the other, did not improve. Neither parent showed signs of poisoning, although both were exposed.

In 1971–72, a major epidemic occurred in Iraq in which 6530 persons were hospitalized and almost 500 died. In a well-intentioned humane response to famine, several nations shipped wheat grain intended for planting to Iraq. The seeds had been treated with a methylmercury-containing fungicide to hold down mold growth and preserve the viability of the seeds. The seeds were also dyed red to serve as a warning, and attempts were made to inform the natives of the hazards of eating the seeds directly. Unfortunately, the dye washed off readily and the fungicide did not; also, the warnings on the bags were in Spanish, because some of the grain had originated in Mexico. Though the bags were also marked with the skull and crossbones, as meaning poison, in the face of starvation many families milled the seeds directly into flour, and made and consumed the contaminated bread. Average intake was three loaves of bread per day, 80- $250 \,\mu g \, kg^{-1} \, da y^{-1}$. Neurologic syndrome of paraesthesia (peripheral nervous, sensory dysfunction) ataxia, dysarthria, and deafness were observed.

Thermometers contain the less toxic elemental form of mercury and have almost never been a safety issue in peoples' homes. However, in the 1970s and 1980s, workers at the Staco thermometer plant in Poultney, Vermont, began to notice a common series of health problems – headaches, bleeding or sore gums, upset digestive systems, and coordination problems. Upon investigation, mercury was detected in the air of workers' homes, on their clothing and furniture, and most tragically, in the bodies of many workers and their children. This was the first time in which the children of mercury-handling workers were proven to have been affected. The plant closed in 1984. Several plant workers have since settled lawsuits with the company for undisclosed sums.

Two major epidemics of methylmercury poisoning have occurred in Japan (in Minamata Bay and the Agano River in Niigata) between 1953 and 1960. In both cases, mercury was discharged as mercuric chloride, a catalyst for production of vinyl chloride and acetaldehyde. Bacteria methylated the inorganic mercury and the methylmercury bioaccumulated in fish and shellfish. In both cases there was an association between fish consumption and incidence and severity of disease. Mercury was found in concentrations of $\sim 10 \,\mathrm{mg \, kg^{-1}}$ of fish. In Minamata Bay, cats were first noted to fall ill, become ataxic, and die. Subsequently, a neurologic syndrome developed in adults and children. 'Fetal Minamata Disease' was the name given to the observed epidemic of 'cerebral palsy' (CP) in Minamata (6% of births in Minamata with CP compared to 0.5% of births elsewhere in Japan): 121 cases and 46 deaths were reported in Minamata from a neurologic disease manifested as paraesthesia, constricted visual fields, ataxia, and deafness (frequently tremor). The clinical presentation varied with the age group. Mortality was 50% in adults, 33% in children, and 12% in fetal exposure. Fetal exposure resulted in CP, involuntary movement, difficulty in chewing, abnormal speech, abnormal deep tendon reflexes, but no deafness or visual deficits.

In 1983, Pomo Indians in California had to stop eating local fish because of high mercury in the fillets. Chippewa Indians in Wisconsin in 1990 were found to have blood levels of mercury high enough to cause developmental problems in fetuses. The Chippewas had a fondness for the walleye that swam in local lakes.

Mercury caused another tragic incident in Hanover, New Hampshire. The story of Dartmouth College Chemistry Professor Karen E Wetterhahn made national headlines when mercury poisoning claimed her life at the age of 48. In August of 1996, Wetterhahn, a specialist in toxic metals, was working under a \$7 million federal grant to study toxic metals. She was poisoned in her lab by a drop of an experimental mercury compound dimethylmercury, which accidentally penetrated her latex glove and seeped through to her skin. Symptoms began gradually like a stomach flu, but then she began bumping into doors and suddenly falling down. Words became difficult, her hands tingled, and 5 months after the spill she was taken to the emergency room. Symptoms then progressed rapidly, by the weekend she could not walk, her speech was slurred, and her hands trembled. Diagnosed as mercury poisoning, treatment was started, but little was known about the rare man-made chemical dimethylmercury, a colorless liquid that looks like water but is three times heavier and far more toxic than other forms of mercury. Wetterhahn became ill in January of 1997 and was hospitalized. She rapidly went into a coma and died that June. As a result of her tragedy, safety standards for gloves and other protective equipment were revised, and a movement began to eliminate production and use of this most deadly form of mercury. There was only one other documented case of dimethylmercury poisoning: a Czech chemist in 1972 had suffered the same symptoms as Wetterhahn and died.

Sources of Mercury

Mercury is found in the environment in the metallic form and in different inorganic and organic forms. Most of the mercury in the atmosphere is elemental mercury vapor; most of the mercury in water, soil, sediment, plants, and animals is inorganic and organic mercury (primarily methylmercury). Mercury occurs naturally and is distributed throughout the environment by both natural processes and human activities. Solid waste incineration and fossil fuel combustion processes and human activities contribute $\sim 87\%$ of the emissions of mercury in the United States. Other sources of mercury releases to the air include mining and smelting, which are industrial processes involving the use of mercury such as chloralkali production facilities and production of cement. Mercury is released to surface waters from naturally occurring mercury in rocks and soils and from industrial activities, including pulp and paper mills, leather tanning, electroplating, and chemical manufacturing. An indirect source of mercury to surface waters is mercury in the air; it is deposited from rain and other processes directly to water surfaces and to soils. Mercury also may be mobilized from sediments if disturbed (e.g., flooding, dredging). Sources of mercury in soil include direct application of fertilizers and fungicides and disposal of solid waste, including batteries and thermometers, to landfills. The disposal of municipal incinerator ash in landfills and the application of sewage sludge to cropland result in increased levels of mercury in soil. Mercury in air also may be deposited in soil and sediments.

Chemical Speciation of Mercury

The divalent state of mercury (Hg^{2+}) dominates in most natural water; however, elemental Hg^0 is the most stable form in a broad pE/pH range. Hg^{2+} forms very strong complexes with oxygen and sulfur ligands, as well as with chloride. A significant hydrolysis starts at pH>1 and dominates at pH>2, in the absence of other complexing agents. The chloride complexes HgCl⁺ and HgCl₂ dominate over hydroxide species at pH < 5 and chloride concentrations > 1–3 mgl⁻¹, and HgCl₂ dominates in solution up to pH 6 in most groundwater systems under oxic or mildly reducing conditions. A significant fraction of the anionic HgCl₃⁻ can be expected at high chloride levels and pH < 5–6, as well as of HgOHCl at pH 5–7. The uncharged Hg(OH)₂ is the main species at pH > 6 in nonsulfidic water, unless the chloride concentration is very high.

Mercury forms strong complexes with humic and fulvic acids, and these complexes may dominate in humic rich water at pH>5–6. The strong complexes with humic substances expected to stabilize Hg²⁺. However, a reduction of the Hg⁰ can take place through the reversible quinine–hydroquinone redox couple in the humic substance. Dominating species, governed by the sulfur and organic degradation, would be Hg(SH)₂ at pH<6, HgS₂H⁻ at pH 6–9, and HgS₂²⁻ at pH>9.

The three major kinds of organic mercury compounds are phenylmercury (e.g., phenylmercuric acetate), methoxymercury (e.g., methoxymethylmercury acetate), and alkylmercury (e.g., methylmercuric acetate). Of these compounds, by far the most common as well as most dangerous are certain members of the alkylmercury group. Formation of alkylmercury compounds, monomethylmercury (CH_3Hg^+) and dimethylmercury $((CH_3)_2Hg)$, is achieved through microbial processes in soils and lake sediments. The transformation reaction pathways are indicated in Figure 1. The precursor Hg^{2+} can be methylated by both aerobic and anaerobic bacteria and subsequently demethylated by other bacteria. Monomethylmercury behaves as a cation capable of forming strong complexes with ligands containing O, S, Cl, etc., and this ion is kinetically inert toward breaking of the C-Hg bond. The formation of organomercury species is affected by parameters such as pH, redox conditions, mercury concentration, microbial population, and temperature.

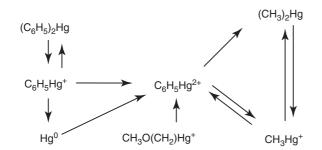


Figure 1 Transformations of mercury leading to alkylmercury.

Fate and Transport of Mercury

The global cycling of mercury is a complex process (Figure 2). Mercury evaporates from soils and surface waters to the atmosphere, is redeposited on land and surface water, and then is absorbed by soil or sediments. After redeposition on land and water, mercury is commonly volatilized back to the atmosphere as a gas or as adherents to particulates. Mercury exists in a number of inorganic and organic forms in water. Methylmercury, the most common organic form of mercury, quickly enters the aquatic food chain. In most adult fish, 90-100% of the mercury is methylmercury. Methylmercury is found primarily in the fish muscle (fillets) bound to proteins. Skinning and trimming the fish does not significantly reduce the mercury concentration in the fillet, nor is it removed by cooking processes. Because moisture is lost during cooking, the concentration of mercury after cooking is actually higher than it is in the fresh uncooked fish. Concentrations of total mercury in fish at the top of the food chain, such as pike, shark, and swordfish, are $\sim 10\,000-100\,000$ times higher than the concentrations of inorganic mercury found in the surrounding waters. Bioconcentration factors (BCFs) are simple ratios between the concentration of mercury in an organism and the concentration in the medium to which the organism was exposed. The BCF of methylmercury in fish is of the order of 3 million. Methylmercury levels in predator fish are, on average, ~ 7 million times higher than the concentrations of dissolved methylmercury found in the surrounding waters.

Mercury's pathway into wildlife primarily begins in the skies, with mercury-loaded rainfall. Sulfatereducing bacteria, mainly living in sediments and in mats of floating algae, absorb rainwater mercury and turn it into its organic form, methylmercury (CH_3Hg^+) . Organisms, which eat such bacteria, feed successive populations of larger organisms in the food web. At each step, methylmercury levels get concentrated. For wetland-dependent animals such as wading birds, raccoons and some panthers, concentrations can reach dangerously high levels. The use of methylmercury as a fungicide has been suspended in the United States, and since this was the only commercial use for the chemical, it is no longer manufactured in this country. It is, however, still found in the environment as a result of bacterial methylation of inorganic mercury.

Toxicology

Depending on the chemical form and the dose received, mercury can be toxic to both humans and

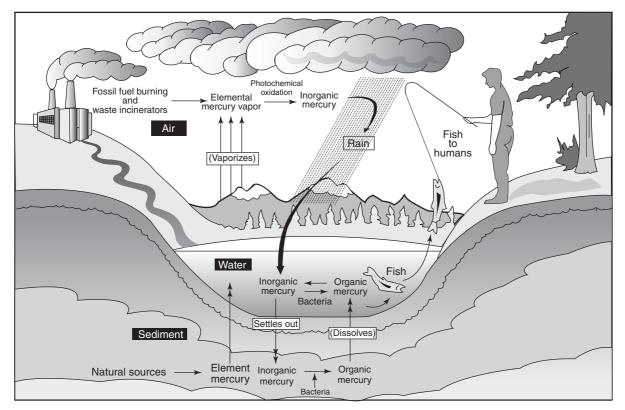


Figure 2 Fate and transport of mercury in water, soil, and sediments.

wildlife. Some chemical forms of mercury are absorbed more effectively by the body than others, and once inside the body, some forms are more likely than others to produce serious damage to internal organs. If inorganic mercury is swallowed, over 98% is excreted rapidly in the urine and feces. Unless ingested repeatedly or in massive amounts, metallic mercury is relatively harmless. Ingestion of the more soluble salts of mercury, however, can cause serious problems. Mercuric chloride is corrosive to the intestinal tract and, like other forms of inorganic mercury, can seriously damage the liver and especially the kidneys. Once absorbed by the body, inorganic mercury may be transported via blood to all parts of the body. Most cases of mercury poisoning from inhalation are chronic. If inhaled, inorganic mercury is initially deposited in the lungs, where in acute cases it may cause irritation and destruction of the lung tissue. The symptoms include inflammation of the gums, metallic taste, diarrhea, mental instability, and tremors. Although ingestion of methylmercury may cause serious damage to organs such as the liver, kidneys, and pancreas, the most serious consequences of methylmercury poisoning cause irreversible damage by attacking the central nervous system. Once absorbed, methylmercury is transported throughout the body by the circulatory system, primarily in the red blood cells. Any effects of mercury on the brain are likely to be permanent, since cells of the central nervous system, once damaged, do not recover.

Pharmacokinetics

Methylmercury is rapidly and nearly completely absorbed from the gastrointestinal tract; 90-100% absorption is estimated. Methylmercury is somewhat lipophilic, allowing it to pass through lipid membranes of cells and facilitating its distribution to all tissues, and it binds readily to proteins. Methylmercury binds to amino acids in fish muscle tissue. The highest methylmercury levels in humans generally are found in the kidneys. Methylmercury in the body is considered to be relatively stable and is only slowly transformed to other forms of mercury. Methylmercury readily crosses the placental and blood/brain barriers. Its estimated half-life in the human body ranges from 44 to 80 days. Excretion of methylmercury is via the feces, urine, and breast milk. Methylmercury is also distributed to human hair and to the fur and feathers of wildlife; measurement of mercury in hair and these other tissues has served as a useful biomonitor of contamination levels.

Acute Toxicity

Acute high-level exposures to methylmercury may result in impaired central nervous system function, kidney damage and failure, gastrointestinal damage, cardiovascular collapse, shock, and death. The estimated lethal dose is $10-60 \text{ mg kg}^{-1}$.

Chronic Toxicity

Both elemental mercury and methylmercury produce a variety of health effects at relatively high exposures, neurotoxicity is the effect of greatest concern. This is true whether exposure occurs to the developing embryo or fetus during pregnancy or to adults and children. A reference dose (RfD) is defined as an estimate of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD for methylmercury has been determined by the US Environmental Protection Agency (EPA) to be 1×10^{-4} mg kg⁻¹ day⁻¹ (i.e., a person could consume 0.1 µg methylmercury for every kg of his/her body weight everyday for a lifetime without anticipation of risk of adverse effect).

Developmental Toxicity

Methylmercury causes subtle to severe neurologic effects depending on dose and individual susceptibility. EPA considers methylmercury to have sufficient human and animal data to be classified as a developmental toxicant. Methylmercury accumulates in body tissue; consequently, maternal exposure occurring prior to pregnancy can contribute to the overall maternal body burden and result in exposure to the developing fetus. In addition, infants may be exposed to methylmercury through breast milk.

Mutagenicity

Methylmercury appears to be clastogenic but not to be a point mutagen; that is, mercury causes chromosome damage but not small heritable changes in DNA. EPA has classified methylmercury as being of high concern for potential human germ cell mutagenicity.

Carcinogenicity

Experimental animal data suggest that methylmercury may be tumorigenic in animals. Chronic dietary exposures of mice to methylmercury resulted in significant increases in the incidences of kidney tumors in males but not in females. The tumors were seen only at toxic doses of methylmercury. All of the carcinogenic effects in animals were observed in the presence of profound damage to the kidneys. Tumors may be formed as a consequence of repair in the damaged organs. EPA has classified it as a possible human carcinogen, group C.

Interactive Effects

Potassium dichromate and atrazine may increase the toxicity of mercury, although these effects have been noted only with metallic and inorganic mercury. Ethanol increases the toxicity of methylmercury in experimental animals. Vitamins D and E, thiol compounds, selenium, copper, and possibly zinc are antagonistic to the toxic effects of mercury.

Regulations and Advisories

EPA regulations and advisories on mercury are indicated in Table 1.

Correctives and Prospects for the Future

Of $\sim 200\,000$ tons of mercury emitted to the atmosphere since 1890, $\sim 95\%$ reside in terrestrial soil and sediments, $\sim 3\%$ in ocean surface water, and 2% in the atmosphere. The global atmospheric burden of mercury is continuing to increase. Between 1990 and 1996, atmospheric mercury levels have risen between 5.5% and 17% in the upper Midwest, depending on the season, with an average annual increase of 8%. Even when mercury pollution is detected and halted, the problem of cleaning up the damage remains. Once released to the environment, mercury may continue to cycle between the sediments, water, and biota for tens, hundreds, or even thousands of years before finally being flushed from the system. Mercury in the form of vapor and/or inorganic salts may be transported great distances over several months in the atmosphere before falling out or being deposited by precipitations. It may be emitted back into the atmosphere as a gas or associated with dust particles to be redeposited elsewhere. Mercury in soils has a long retention time, possibly hundreds of years, and may continue to be released into the air and surface water for many years to come. Standfield and Lopez reported that if all mercury releases were stopped today, it could take at least 50 years for the methylmercury levels in fish to return to preindustrial levels. High levels of mercury have been found in fish from several parts of North America, primarily where chlor-alkali plants were discharging mercury-laden wastewater. Many fishes taken from Lake St. Clair (between Lake Huron and Lake Erie) in 1970 contained \sim 5–7 ppm of mercury. It has been estimated that ~ 5000 years will be required for the mercury,

Table 1 EPA regulations and advisories	
Maximum contaminant level in drinking water	0.002 mg l ⁻¹
Toxic criteria ^a	
Freshwater	2.10 µg l ⁻¹ (maximum)
	$0.012 \mu g l^{-1}$ (continuous)
Saltwater	1.80 μ g l ⁻¹ (maximum)
	$0.025 \mu g l^{-1}$ (continuous)
Human health consumption of	0.14 μg I ^{−1}
water and organisms	1
Human health consumption of	0.15µgl ⁻¹
organisms only	
Water quality guidance for the	
Great Lakes System	
(protection of aquatic life in ambient water)	
Acute water quality criteria for	$1.694 \mu g l^{-1}$ (maximum)
mercury total recoverable	1.00+μgτ (maximum)
Chronic water quality criteria	0.908 µg l ^{−1} (continuous)
for mercury total recoverable	·····,g· (········)
Water quality criteria for	$1.8 \times 10^{-3} \mu g l^{-1}$ (maximum)
protection of human health	
(drinking water and	
nondrinking water)	
Water quality criteria for	$1.3\times10^{-3}\mu gl^{-1}$ (maximum)
protection of human health	
(mercury including	
methylmercury)	
Emissions from mercury ore	2300 g per 24 h (maximum)
processing facilities and	
mercury chlor-alkali plants Emissions from sludge	3200 g per 24 h (maximum)
incineration plants, sludge	S200 g per 2411 (maximum)
drying plants, or a	
combination of these that	
process wastewater treatment	
plant sludge	
-	

 Table 1
 EPA regulations and advisories

^a For those States not complying with Clean Water Act Section 303(c) (2) (B) – criterion concentration for priority toxic pollutants.

stored presently in the Lake St. Clair ecosystem, to effectively flush out by natural processes.

The following approaches can be used for decontaminating mercury-contaminated sediments.

- Dredging of sediments.
- Increasing the pH of the sediments in order to favor demethylation and increased volatilization.

- Introducing oxygen-consuming materials so as to create anaerobic conditions in the sediments and hence reduce mercury methylation.
- Covering the sediments with fresh, finely divided, highly adsorptive materials as reactive cap.
- Covering the sediments with any inorganic, inert material as nonreactive cap (e.g., sand).

None of the above-mentioned methods is without drawbacks, either in terms of cost, performance, effectiveness, or side effects. For example, mercury concentrations in fishes were reported to be higher after dredging of sediments of polluted waterways on two occasions. The burrowing activities of benthic microorganisms can frustrate efforts to seal off contaminated sediments with an overlayer of inert, adsorptive material.

See also: Kidney; Mercury; Methylmercury; Neurotoxicity.

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Great Smog of London

Yvonne R Rodriguez

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It had been a cold winter in London of 1952. December 4th of that year seemed to be just as cold as the other days except that in the evening a light fog rolled in. The following day began with a foggy morning. People bundled up and went to work, smoke stacks belched dark smoke out into the air, and coal was being burned in homes and offices to keep warm. As the day progressed, the fog that began in the morning turned into a brown and yellowish smog with an acrid smell. The smog lasted until December 9th. In the end, the death toll caused by this fog was estimated to be in excess of 4000 people. In addition to the deaths, the smog left a large number of new respiratory illnesses and cardiovascular disease cases among the surviving population.

What Caused the Smog?

The winter of 1952 had been colder than usual. On December 4th the wind began to die down, the ground was cold, the air was moist, and a precipitation formed, more commonly referred to as fog. The cold moist air was trapped beneath a layer of warm air forming a temperature inversion. An anticyclone settled in around the city of London, preventing any wind circulation to occur beneath the temperature inversion.

By 1952, wood was scarce and an expensive commodity in England. Bituminous coal was the primary source of heat for all. The coal being burned for warmth in combination with the industrial smoke stacks pumped pollution into the stagnant air. These pollutants combined with the existing fog created the Great Smog of London. This type of air pollution is known as 'reducing-type' pollution. The burning of the coal gives off a sulfurous gas, plus the industrial particulate matter belched from the smoke stacks mixed with the fog trapped within the temperature inversion results in 'reducing-type' pollution. This type of pollution is capable of causing the corrosion of metals and masonry used in buildings. In 1952, daily recordings of pollutants were taken for only those that were thought to have been the main contributors to pollution, mainly sulfur dioxide (SO_2) and total suspended matter (smoke). On the worst day of the Great Smog in London, the highest pollution levels of SO2 and smoke reached levels of 1.34 ppm and 4.5 mg m⁻³, respectively. The average daily levels of exposure during the Great Smog were 0.57 ppm of SO₂ and 1.4 mg m⁻³ for smoke.

Health Effects

Exposure to the air pollution during the 1952 episode could have detrimental effects to the lungs. Sulfur dioxide is water soluble, if inhaled the upper linings of the lungs would absorb it. The inhalation of SO_2 would then cause the lungs to bronchoconstrict. This is a condition that narrows the airways, resulting in difficulty breathing from a lack of airflow. In addition to the sulfur dioxide exposure, particulate matter from the smoke would aggravate and enhance any symptom already appearing with those exposed. Those already suffering from respiratory problems exposed to the Great Smog would have had an extremely difficult time breathing.

The exact number of deaths resulting from this event was difficult to determine. For example, a person with a mild case of bronchitis aggravated by the smog to become a serious case might not have perished until January of 1953. This type of death would not have been included in the final death toll attributable to the smog event. It is estimated that over 4000 deaths resulted from the Great Smog of 1952. In the week ending December 6th of 1952, 945 deaths were recorded in London Administrative County. The following week, the death toll reached 2484. On December 8th and 9th alone the toll peaked ~ 900 deaths per day. Although the smog lasted 5 days, the death rate in London continued to remain higher than normal through Christmas. Many of the victims were those already suffering from a respiratory illness and the elderly.

The Great Smog of London in 1952 was not an isolated incident. Other such 'reducing-type' killer smogs have occurred in Meuse Valley, Belgium (1930), Donora, Pennsylvania (1948), and again in London (1962). The death toll blamed on the air pollution in these cases was not as high.

Legislation

Air pollution had long been recorded in the past as a nuisance. The Public Health (London) Act of 1891, addressed air pollution and the stacks that emitted it; however, the Act failed to define black smoke, allowing companies to avoid the law by claiming their smoke was not black but another color. Attempts were made to change the wording of the Act but failed due to large companies investing time and money into fighting any new laws. After the Great Smog of 1952, new legislation was enacted addressing both residential and industrial sources of pollution.

On July 5th, 1956, the London Clean Air Act was enacted. This legislation differed from all the other attempts to regulate air pollution by actually defining dark smoke. The law restricted smoke and prohibited the release of dark smoke, which was defined as "anything darker than lattice 2 on the Ringelmann chart." There are 5 different levels noted on the Ringelmann chart, 1 being the clearest and 5 the blackest. The tester would place the lattice chart a distance away so that the shade of the smoke could be compared to the grid. The Clean Air Act of 1956 allowed the government to designate areas free from smoke, by regulating and restricting the emissions from potential sources. From 1952 to 1987, air pollution legislation has worked to reduce 80% of the smoke once emitted over the City of London.

Since the events of December 1952, several advances in air pollution control, legislation, and factors contributing to air pollution have been made. Improvements in air pollution controls have helped lessen the amount of emissions. Combine this progress with an increase of restrictions on air emissions, and advances in understanding conditions that lead to air pollution reduces the likelihood that a situation as devastating as the Great Smog of London could occur again. Nevertheless, burning coal containing high amounts of sulfur in certain areas of the world subject to extreme temperature inversion conditions increases the risk of respiratory problems.

See also: Pollution, Air; Sulfur Dioxide.

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Green Chemistry

Richard E Engler

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Green chemistry (also called sustainable chemistry) is the use of chemistry to prevent pollution. More specifically, green chemistry is the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. By offering environmentally benign alternatives to the more hazardous chemicals and processes that are often used in consumer and industrial applications, green chemistry promotes pollution prevention at the molecular level:

$$Risk = f(hazard, exposure)$$
[1]

Risk is a function of hazard and exposure (equation [1]). Traditional environmental protection focuses on controlling exposure: minimizing human or environmental exposure to hazardous substances during chemical manufacture, processing, and use. Green chemistry changes the focus to the hazard component of the equation. Reducing hazard is a more fundamental, foolproof way to reduce risk. An exposure control can fail, whether it is a worker's respirator, the lining of a hazardous waste landfill, or the scrubber on an exhaust stack. On the other hand, the risk of a less hazardous chemical will remain low, even if the respirator fails, the filter leaks, or the vat spills. Reducing hazard also increases the safety of chemical manufacturing and may contribute to homeland security.

Green chemistry in the United States grew out of the Pollution Prevention Act (PPA) of 1990. The PPA established a hierarchy that emphasizes source reduction over other methods of dealing with hazardous materials. According to the PPA, source reduction is preferable to recycling, which in turn is preferable to treatment, which in turn is preferable to disposal.

Chemistry provides ways to reduce pollution at the source. The field of green chemistry is developing as chemists begin to use the science of chemistry to reduce the hazard of the chemical products or processes they design, thus minimizing the negative impact of chemicals on human health and the environment. The 12 Principles of Green Chemistry help define green chemistry.

Twelve Principles of Green Chemistry (Adapted from Anastas and Warner, Green Chemistry: Theory and Practice)

- 1. *Prevent waste*: Design chemical syntheses to prevent waste, leaving no waste to treat or clean up.
- 2. *Design safer chemicals and products*: Design chemical products that are fully effective yet have little or no toxicity.
- 3. Design less hazardous chemical syntheses: Design syntheses to use and generate substances with little or no toxicity to humans and the environment.
- 4. Use renewable feedstocks: Use raw materials and feedstocks that are renewable rather than depleting. Renewable feedstocks are often made from agricultural products or are the wastes of

other processes; depleting feedstocks are made from fossil fuels (petroleum, natural gas, or coal) or are mined.

- 5. Use catalysts, not stoichiometric reagents: Minimize waste by using catalytic reactions. Catalysts are used in small amounts and can carry out a single reaction many times. They are preferable to stoichiometric reagents, which are used in excess and work only once.
- 6. Avoid chemical derivatives: Avoid using blocking or protecting groups or any temporary modifications, if possible. Derivatives use additional reagents and generate waste.
- 7. *Maximize atom economy*: Design syntheses so that the final product contains the maximum proportion of the starting materials. There should be few, if any, wasted atoms.
- 8. Use safer solvents and reaction conditions: Avoid using solvents, separation agents, or other auxiliary chemicals. If these chemicals are necessary, use innocuous chemicals.
- 9. *Increase energy efficiency*: Run chemical reactions at ambient temperature and pressure, whenever possible.
- 10. Design chemicals and products to degrade after *use*: Design chemical products to break down to innocuous substances after use so that they do not accumulate in the environment.
- 11. Analyze in real time to prevent pollution: Include in-process real-time monitoring and control during syntheses to minimize or eliminate the formation of by-products.
- 12. *Minimize the potential for accidents*: Design chemicals and their forms (solid, liquid, or gas) to minimize the potential for chemical accidents such as explosions, fires, and releases to the environment.

Some consider green chemistry to be a separate field of chemistry, such as organic or physical chemistry. It is, however, an overarching concept that applies to any of the traditional fields of chemistry. It tends to be multidisciplinary, drawing on organic, inorganic, physical, polymer, and biochemistry, as well as biology, engineering, physics, and other related fields.

Green chemistry is good business. Implementing green chemistry technologies may reduce the costs of raw materials, improve efficiency, and reduce or eliminate waste, regulatory burden, and the need for personal protective equipment. Many companies, large and small, have discovered and implemented green chemistry technologies that not only benefit human health and the environment, but also benefit their corporate competitiveness and profitability. The commercial success of many winners of the US Presidential Green Chemistry Challenge Awards (described below) is perhaps the best example of green chemistry as good business.

In the United States, research, education, awards, and outreach opportunities are available through several organizations and in particular through the US Environmental Protection Agency's Green Chemistry Program and the American Chemical Society's Green Chemistry Institute. International coordinators include the International Union of Pure and Applied Chemistry (IUPAC), and the Organisation of Economic Cooperation and Development (OECD). In addition, many countries have implemented national programs including Australia, Italy, Japan, Spain, and the United Kingdom.

The US Green Chemistry Program recognizes and supports chemical technologies that reduce or eliminate the use or generation of hazardous substances during the design, manufacture, and use of chemical products and processes. The Green Chemistry Program supports fundamental research in environmentally benign chemistry as well as a variety of educational activities, international initiatives, conferences and meetings, and green chemistry tools. The program also administers the prestigious Presidential Green Chemistry Challenge Awards. Each year five technologies win Challenge Awards: one from an academic researcher, one from a small business, and three in specific areas of green chemistry.

See also: Pollution Prevention Act, US; Risk Assessment, Ecological.

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G-Series Nerve Agents

Harry Salem*

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The G-series and V-series are the two main classes of traditional nerve agents. The G-series consists of GA, GB, GD, GE, GF, and GV.

Although toxic organophosphates were investigated extensively as pesticides during the 1920s and 1930s, their potential as chemical warfare agents was not recognized until 1937. Tabun or GA was the first military nerve agent, and was first examined for use as an insecticide in 1936 by Dr. Gerhard Schrader at the Bayer facility in Germany. At the end of the Second World War, Germany began to produce a second nerve agent, Sarin (GB), and were investigating a third, Soman (GD).

The G-agents are the more volatile of the nerve agents and present respiratory and percutaneous threats. The syndrome of cholinergic effects can be remembered by the acronym SLUDGE representing salivation, lacrimation, urination, diarrhea, gastrointestinal cramping, and emesis, or by DUMBBELLS, representing diarrhea, urination, miosis, bronchorrea, bradycardia, emesis, lacrimation, and salivation.

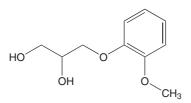
See also: GF; Nerve Agents; Sarin; Soman; Tabun; V-Series Nerve Agents: Other than VX.

Guaifenesin

Brenda Swanson-Biearman

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- REPRESENTATIVE CHEMICAL: 3-(2-Methoxyphenoxy)-1,2-propanediol
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 93-14-1
- Synonyms: Glyceryl guaiacolate; α-Glyceryl guaiacol ether; Guaianesin; Robitussin[®]
- CHEMICAL FORMULA: C₁₀H₁₄O₄
- CHEMICAL STRUCTURE:



Uses

Guaifenesin stimulates receptors in the gastric mucosa, reflexively increasing glandular secretion by the respiratory epithelium promoting lower respiratory tract drainage by thinning bronchial secretions, lubricating irritated respiratory tract membranes through increased mucous flow, and facilitating removal of viscous mucus. As a result, sinus and bronchial drainage is improved, and dry, nonproductive coughs become more productive and less frequent. However, clinical studies documenting the efficacy of this drug as an antitussive or expectorant for patients with upper respiratory infections or chronic bronchitis are lacking and the therapeutic efficacy of this agent is questionable.

Exposure Routes and Pathways

Guaifenesin is available in liquid or capsule form for oral dosing. Accidental or intentional exposure occurs most commonly by ingestion.

Toxicokinetics

Guaifenesin is readily absorbed from the gastrointestinal tract, specifically the intestine, and is rapidly metabolized and excreted in the urine. It is hydrolyzed 60% in the blood within 7h. Following hydrolysis, urinary metabolites include β -(4-hydroxy-2-methoxyphenoxy)lactic acid, β -(2-methoxyphenoxy)lactic acid, and guaiacol ether. Following oral administration, no unchanged drug is detected in the urine. Guaifenesin has a plasma half-life of 1 h.

Mechanism of Toxicity

Guaifenesin lacks specific toxicity. Toxicity associated with guaifenesin will generally result from the

^{*}The views of the author do not purport to reflect the postion of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

presence of antihistamines, decongestants, analgesics, cough suppressants, and/or alcohol in preparations containing a combination of ingredients.

Acute and Short-Term Toxicity (or Exposure)

Animal

Guaifenesin is used as an adjunct in anesthesia for horses. Thrombus formation has been noted in some horses given 10% guaifenesin intravenously.

Human

Guaifenesin is of low order of toxicity. Adverse effects are primarily minor gastrointestinal complaints. Doses larger than those required for expectorant action may produce vomiting, but gastrointestinal upset is rare at therapeutic dosages. Excessive use has been associated with urolithiasis.

Chronic Toxicity (or Exposure)

Animal

Large doses of guaifenesin in some animal models resulted in increased respiratory tract secretions.

Human

Abuse of guaifenesin-containing products has led to the development of urolithiasis secondary to guaifenesin's metabolite, β -(2-methoxyphenoxy)lactic acid.

Clinical Management

Basic and advanced life-support measures should be used as needed. In exposures where guaifenesin is the sole ingestant, treatment is rarely necessary. In exposures involving guaifenesin in multisymptom products containing antihistamines, analgesics, decongestants, and/or antitussives, treatment is directed toward the coingestant(s). Consideration should be given to the alcohol content of liquid preparations of guaifenesin and combination products.

See also: Ethanol; Gastrointestinal System.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Guaifenesin.

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Hair

Pertti J Hakkinen

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True hair is found only in mammals, and there is no such thing as a completely hairless mammal. Hair itself is dead, but is produced in hair follicles by specialized keratinocytes at the base of the hair. The outermost layer of hair is a cuticle, and most hairs have a cortex in which the dead keratinized cells are very densely packed, and an inner medulla in which they are not as densely packed. The pigmentation in hair, like that of skin, comes from melanocytes. Hair exposure to some chemicals may produce hair discoloration, for example, green hair from copper in water or cosmetics, or blue hair in cobalt workers.

The cycle of hair growth involves an active phase of production, anagen, during which the hair grows in length by addition of cells to the bottom end. When this stage ends, it is replaced by catagen, an inactive phase with no new hair cell production. The hair in catagen may detach itself from the underlying matrix that produced it, and be held in the follicle simply by friction. The follicle eventually enters into the transitional stage of telogen, renewing itself for activity, and then returns to the anagen phase. The anagen phase may last up to 2 years, and 86–95% of scalp hair follicles belong to this stage.

Hormones, especially testosterone, are well known as having effects on hair growth. 'Male pattern baldness' in humans is due to the suppressive effect of testosterone on the follicles of the scalp, and is not normally seen in women because of low testosterone levels. Interestingly, testosterone has the opposite effect on hair follicles of the face, for example, the onset of puberty in men and the elevated levels of testosterone stimulate the follicles of the face to make beard hairs. Any physical or chemical agent that affects rapidly dividing cells will affect hair growth, usually by stopping it, and hair loss (alopecia) is a very common side effect of radiation treatment and chemotherapy. Parasites, disease, and poor nutrition can also affect hair growth.

The advantages of analyzing hair samples include the easy and noninvasive collection of the samples, the small sample size required for analysis, and easy storage at room temperature. Scalp hair of a long enough length may be able to provide retrospective information of the previous 5-7 years, and axillary and pubic hair can be utilized if the scalp hair is cut too short to be used. Further, the basic chemical composition of the hair shaft is not influenced by changes in the blood chemistry, or by exposure to chemicals that occurred after hair and nail formation. However, hair analysis can be altered by dyeing, bleaching, and permanent waving, which can decrease the drug or toxic content in hair. In addition, the US Agency for Toxic Substances and Disease Registry (ATSDR) has noted that many questions about sampling and analytical procedures have yet to be answered and procedures have not been standardized. For example, do certain cutting tools introduce contaminants into hair, what part of the scalp should be used for hair sample collection, and what is the influence of washing techniques?

Hair has been used in the biomonitoring of various elements, for example, arsenic, thallium, and zinc, and has been used in the monitoring of drugs and biological substances. The level of mercury in hair is widely used as a biological indicator for exposure to methyl mercury (MeHg). In addition, hair samples have been utilized to evaluate environmental exposure to pollutants such as lead, and occupational exposures to metals such as nickel and chromium. However, the ATSDR has stated:

- That it "believes many scientific issues need to be resolved before hair analysis can become a useful tool to understand environmental exposures."
- "Although hair analysis may answer some questions about environmental exposure to a few substances, hair analysis often raises more questions than they answer."
- Health professionals "have no scientific basis for deciding whether a particular hair analysis result would be associated with adverse health effects. As the exception, scientists have studied how hair concentrations of methyl mercury in pregnant mothers relate to adverse developmental effects in their children."

- "Scientists have not been able to develop models that can use a hair analysis result to predict concentrations of contaminants in other biological media, e.g., blood."
- Health professionals "cannot use a hair analysis result to compute a body burden, an internal dose, or other parameter that would enable a mean-ingful toxicologic evaluation of a hair analysis result."

Nicotine has been measured in the hair of workers exposed to environmental tobacco smoke (ETS), and a significantly greater level in the level of nicotine in the hair of non-smokers exposed to ETS in the workplace has been observed. However, cotinine (the primary metabolite of nicotine) is a preferred marker of exposure to ETS, particularly as measured in blood, saliva or urine, because up to 80% of a dose of nicotine is metabolically converted to cotinine. Cotinine metabolite has a half-life of 15–17 h, while nicotine has a much shorter half-life and has different clearance rates in smokers and nonsmokers.

Hair samples may be very useful in cases of drug abuse since the detection of the drug together with its metabolites may confirm intake of the substance followed by metabolism, in contrast to exogenous exposure that would not find the metabolites in the hair. Some cases of drug abuse and poisoning can be suggested by examination of hair and/or nail, and can be confirmed by analysis of hair or nail clippings. Further, hair has been used for DNA typing in crime cases.

Hair analysis is also very useful in identifying 'doping' in amateur and professional athletes. Doping substances that can be detected in hair include clenbuterol, corticosteroids, ephedrine, methenolone, nandrolone metabolites, salbutamol, stanozolol, and testosterone. The storage of both nandrolone and its metabolites (norandrosterone and noretiocholanolone) in hair allows for detecting the difference between intake of doping agents and intake of other 19 norsteroids such as norandrostenedione and norandrostenediol, which are available in overthe-counter vitamin supplements.

Hair may also be an important tool for the diagnosis and monitoring of various disease states. For example, the concentrations of polyamines (e.g., putrescine, spermidine, and spermine) in the hair may be helpful in diagnosing and assessing disease activity in women with cervical or ovarian cancer. Assessing the level of polyamines in the hair shaft is preferred to measuring them in plasma and urine because the polyamine levels can vary during the day in plasma and urine. Increased levels of porphyrins in hair have been detected in patients with porphyria

cutanea tarda. Hair analysis is useful for monitoring treatment compliance in psychiatric, epileptic, and HIV patients. Further, the levels of androgens in terminal scalp hair may provide a basis for predicting baldness since the ratio of testosterone to epitestosterone is significantly greater in the hair of balding fathers and their sons than in the hair of nonbalding control subjects.

Hair follicle cells possess the enzyme system necessary for the metabolic activation of certain drugs and polycyclic aromatic hydrocarbons (PAHs), and thus can be suitable for use in estimating susceptibility to environmental cancer. Since these cells are readily available and easily obtained, they serve as a good resource for population monitoring studies. A plucked anagen phase hair follicle consists of a hair shaft, inner root sheath, a cuticle layer, and most of the outer root sheath and the upper half of the bulb (but not the dermal papilla). Mitotic cells are located at the periphery of the outer root sheath and can be used for biomonitoring. A nuclear aberration (NA) assay in hair follicles has been developed to assess human exposure to genotoxic agents, and has been used for a study group comprised of cyclophosphamide (CP)-treated multiple sclerosis patients to show a significant increase of NA index in the CP treatment groups compared to the placebo group. CP was also observed to cause a significant drop in the mitotic index of follicle cells at some time points in CP treatment groups. In addition, hair follicles can retain smoke-related DNA adducts, and their use as an alternative to blood lymphocytes have already been recognized in monitoring exposure to PAHs, for example, benzo[a]pyrene, which can be activated by follicle cells.

An *in vitro* method using hair follicle cells to investigate unscheduled DNA synthesis (UDS) has been developed. Plucked follicles were exposed to tritiated thymidine and the UDS activities were determined autoradiographically (the number of dark grains in the outer root sheath of nonperipheral cells were used to estimate UDS). Many, but not all chemicals that have been shown to induce UDS in rat liver cells also stimulated UDS in hair follicles.

See also: Biomarkers, Human Health; Biomonitoring; Nails (of the Fingers and Toes).

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Hallucinogens See LSD (Lysergic Acid Diethylamide); Belladonna Alkaloids.

Harmonization

Carolyn Vickers

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An approach described as 'harmonization' has been employed in a range of national, regional, and international programs aimed at improving the risk assessment and risk management of potentially toxic substances, including pesticides, biocides, food additives and contaminants, industrial chemicals, etc. It is generally used to describe a convergence of approaches which minimizes differences between them, but which may or may not achieve 'standardization' as the final endpoint.

Major areas of harmonization work arose from the United Nations (UN) Conference on Environment and Development (UNCED) held in 1992, and the 2002 World Summit on Sustainable Development. Agenda 21, Chapter 19, the 'blueprint' for the environmentally sound management of toxic chemicals under the principles of sustainable development, has guided most international and national chemical-related activities. Chapter 19 is the agreed upon, endorsed international program of action of governments for developing and implementing national programs for chemicals management within the principles of sustainable development.

One of the major harmonization activities under the UNCED umbrella is the International Programme on Chemical Safety (IPCS) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project). The Intergovernmental Forum on Chemical Safety (IFCS) Forum III in Salvador da Bahia, in October 2000, agreed on Priorities for Action Beyond 2000. Forum III declared that by 2004, IPCS and the Inter-Organization Programme for the Sound Management of Chemicals (IOMC, which comprises seven intergovernmental organizations), should have ensured that recommendations for harmonized approaches are available for terminology, cancer, and reproductive and developmental toxicology, and that common principles for the approach to other specific toxicological endpoints, such as immunotoxicology, endocrine disruptors, and ecotoxicology, should be adopted wherever possible.

The IPCS Harmonization Project, which is ongoing, states that 'harmonization', in the context of risk assessment of chemicals should not simply be equated with standardization. It is not a goal of the project to standardize risk assessments globally, as that is considered to be neither appropriate nor feasible. Instead, harmonization is thought of as an effort to strive for consistency among approaches and to enhance understanding of the various approaches to chemicals risk worldwide. Thus, harmonization is defined, in a stepwise fashion, as an understanding of the methods and practices used by various countries and organizations so as to develop confidence in, and acceptance of, assessments that use different approaches. It further involves a willingness to work toward convergence of these approaches or methods as a longer-term goal.

Achieving harmonization of approaches is considered to provide: a framework for comparing information on risk assessment; understanding of the basis for exposure standards for specific chemicals in different countries; saving of time and expense by sharing information and avoiding duplication of work; and credible science through better communication among organizations and peer review of assessments and assessment procedures. The stated project mission is to ensure better chemical risk assessment and hence management practices that promote the protection of human health and the environment within the framework of sustainable development.

Application of this approach to the targets set by the IFCS has resulted in a range of harmonized products for application in risk assessment and risk management that have been implemented in national and other risk assessment systems. These include:

- The IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis.
- IPCS Guidance Document for the Use of Chemical-Specific Adjustment Factors (CSAF) for Interspecies Differences and Human Variability in Dose/Concentration–Response Assessment.
- IPCS Harmonization of Methods for the Prediction and Quantification of Human Carcinogenic/ Mutagenic Hazard, and for Indicating the Probable Mechanism of Action of Carcinogens (This publication is also known as the *IPCS Qualitative Scheme for Mutagenicity*).
- IPCS Glossary of Key Exposure Assessment Terms.
- IPCS/OECD Descriptions of Selected Key Generic Terms Used in Chemical/Hazard Risk Assessment.

This ongoing project is overseen by a geographically representative Harmonization Steering Committee and a number of ad hoc Working Groups that manage the detailed work. Finalization of documents includes a rigorous process of international peer review and public comment.

Another major area of harmonization activity produced the Globally Harmonized System for the Classification and Labeling of Chemicals (GHS). The work on the GHS began with the premise that existing chemical toxicity classification and labeling systems should be harmonized in order to develop a single, globally harmonized system. This built upon the already harmonized system in place for physical hazards and acute toxicity in the transport sector, based on the work of the United Nations Economic and Social Council's Committee of Experts on the Transport of Dangerous Goods (UNCEDTG). Harmonization had not been achieved in the workplace or consumer sectors, however, and transport requirements in countries were often not harmonized with those of other sectors in that country.

As with the IPCS Harmonization Project, Chapter 19 of Agenda 21 provided the international mandate to complete this task. The work was coordinated and managed under the auspices of the IOMC Coordinating Group for the Harmonization of Chemical Classification Systems (CG/HCCS). The technical focal points for completing the work were: the International Labour Organization (ILO) for the hazard communication; the Organization for Economic Cooperation and Development (OECD) for the classification of health and environmental hazards; and the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods (UNSCETDG) and the ILO for the physical hazards.

In 1999, the United Nations Economic and Social Council decided to enlarge the mandate of the Committee of Experts on the Transport of Dangerous Goods by reconfiguring it into a Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labeling of Chemicals (CETDGGHS), and by creating, in addition to the Sub-Committee of Experts on the Transport of Dangerous Goods (TDG Sub-Committee), a new Sub-Committee of Experts on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS Sub-Committee). The GHS was adopted in December 2002 by the GHS Sub-Committee and endorsed by its parent Committee.

In its Plan of Implementation (para 22.(c)) adopted in Johannesburg on September 4, 2002, the World Summit on Sustainable Development encouraged countries to implement the new GHS as soon as possible with a view to having the system fully operational by 2008. See also: Inter-Organization Programme for the Sound Management of Chemical; Intergovernmental Forum on Chemical Safety (IFCS); International Labour Organization (ILO); International Programme on Chemical Safety; Organisation for Economic Cooperation and Development.

Relevant Websites

http://www.unece.org – Globally Harmonized System for the Classification and Labeling of Chemicals (GHS).

Hazard Identification

Michael A Kamrin

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Hazard identification is the first step in the risk assessment process and addresses two questions: (1) Does a given material present a potential hazard? (2) What type of adverse health effect(s) is it likely to cause? Is it, for example, a neurotoxicant, a developmental toxicant, or a potential carcinogen?

Hazard identification depends on a careful scientific evaluation of several different types of information:

- Physical characteristics (e.g., corrosivity, flammability, and reactivity with other substances).
- Results of *in vivo* animal testing studies.
- Results of *in vitro* laboratory tests (e.g., cell cultures or isolated tissues).
- Results of epidemiological studies with human populations.
- Structure–activity analysis (i.e., comparisons with the known toxicity of structurally similar chemicals).

In Vivo Animal Studies

The most commonly used information in hazard identification is obtained from animal bioassays. Although the use of animals in toxicity testing has become a highly controversial topic in recent years, responsible studies with a variety of species of laboratory animals (mainly rats, mice, guinea pigs, rabbits, dogs, and occasionally primates) frequently represent the only sources of information on which to judge the potential adverse effects of a chemical on humans. While always associated with a good deal of uncertainty, the use of animals in toxicology testing rests on the premise that the results observed in animals are applicable to humans.

- http://www.who.int See separate pages for Intergovernmental Forum on Chemical Safety (IFCS); International Programme on Chemical Safety (IPCS); and Inter-Organization Programme for the Sound Management of Chemicals (IOMC).
- http://www.un.org United Nations (UN), Conference on Environment and Development (UNCED) held in 1992, and the 2002 World Summit on Sustainable Development. Agenda 21.

Chemicals usually can cause multiple effects and the outcome of an exposure is likely to depend on the length of time over which an exposure occurs, the primary route of exposure, the sensitivity of the individual or species, and the resulting dose. Effects can occur as a result of exposure at the point of contact (e.g., skin, eyes, gastrointestinal tract, and respiratory tract) or any internal or systemic target. Animal bioassays are often placed in one of three major groups based primarily on the duration of exposure period; these groups are acute, subacute and subchronic, and chronic.

Acute Studies

Acute studies are designed to evaluate the possible adverse effects that may occur after short-term exposure (e.g., 24 h or less to \sim 1 week). Exposure may occur from one or possibly a few exposures to the test substance over the time period. In humans, such exposures might result from one-time accidents or other unusual incidents. Acute effects may occur in any organ system and can range from mild irritation of the eyes, skin, and respiratory tract to coma and death.

Subacute and Subchronic Studies

Subacute studies occur over time periods that range from greater than a week to several months. Subchronic studies address exposures that occur for a period of ~90 days. Both subacute and subchronic studies involve repeated, usually daily, exposures to the test substance. These studies have been used to reflect effects that might result from continual occupational exposures over a period of several weeks or months. Subchronic studies in rodents (lifetime of 2 years) have been used as the basis for exposure criteria that are intended to protect against the occurrence of adverse effects in humans resulting from exposures lasting from 1 to 7 years. Animals are observed daily throughout the course of the study and observations made with respect to clinical signs of toxicity (e.g., loss of body weight, incoordination, and loss of balance), general appearance, and/or unusual behavioral patterns. At the end of the study, all animals are sacrificed and a gross and microscopic pathological examination of selected tissues is conducted. Included with subchronic and subacute studies are special developmental toxicity studies in which pregnant animals are exposed daily to the test chemical throughout the critical stages (organogenesis) of fetal development. Some chemicals (e.g., thalidomide) exert their adverse effects at very specific points in fetal development so that even single acute exposures during that critical period can cause an effect.

Chronic Studies

Chronic studies are typically conducted for periods \geq 90 days and usually up to the length of the lifetime of the test animal. In some cases, as with bioassays for carcinogenesis, the animals are exposed daily for their lifetimes (usually 18 months in mice and 2 years in rats). Chronic studies focus on identifying effects (e.g., such as cancer or certain reproductive effects) that might occur following continuous exposure over several generations.

The results of animal bioassays for toxicity form the basis of risk assessment and risk management under most regulatory statutes at the federal, state, and international levels. To obtain consistency and to ensure that the studies are designed, conducted, and analyzed in a sound scientific manner, experimental protocols for animal studies are required to meet various US federal statutes. Requirements are all carefully described in the Code of Federal Regulations. The study guidelines consist of a series of Standard Evaluation Procedures that clearly specify experimental conditions such as the number of animals per test group, the number of treatment groups, methods of chemical administration, types of observations that must be made and detailed procedures, and end points for clinical, hematological, ophthalmoscopic, and histopathologic evaluation. A study that does not follow the appropriate guidelines may not be acceptable to the regulatory agency. Currently, attempts are being made to standardize (harmonize) the test guideline requirements in different countries (e.g., United States, Canada, and the European Union).

Depending on the physicochemical nature of the chemical and the route of exposure of particular concern or relevance to expected human exposures, the test materials may be administered orally by incorporation in food or water or by gavage (feeding tube) directly into the stomach, dermally (by application to the skin), or by inhalation in the form of a gas or aerosol. In most cases, the test chemical is administered in a substance that is believed to be a toxicologically inert vehicle (such as saline or corn oil) and the observations in the treated animals are compared with those in control animals receiving only the vehicle. The tests are usually conducted with groups of control animals and three or more groups of experimental animals, each receiving different doses of the test material; groups of each sex are employed. The number of animals in a treatment group varies depending on the nature of the test, ranging from about 6–20 animals for acute and subchronic studies to 50–60 animals in chronic cancer bioassays.

In Vitro Tests in Hazard Identification

In vitro tests are tests in which chemical interactions with any of a wide variety of organs, tissue preparations, cell cultures, enzymes, receptors, etc. are studied 'in the test tube'. Such studies have always been considered important tools in studying the effects of toxic chemicals and identifying the mechanisms through which toxicity occurs and usually complement *in vivo* studies with intact animals. Indeed, with growing concern over the use of animals in laboratory research, there has been a concerted effort in recent years to develop *in vitro* tests that might serve as alternatives for some *in vivo* tests; despite some success, there remains a strong consensus that both types of testing will continue to be required.

The intact animal in a toxicity study is equivalent to a black box. It is possible to make gross observations of toxic signs, symptomology, and clinical effects but these seldom provide the details required to understand the precise mechanisms of toxicity or the primary cause, at the subcellular level, of the adverse effects. The use of *in vitro* systems allows, for example, measurements of the ability of a neurotoxicant to inhibit cholinesterase, identifies potentially reactive metabolites formed in the liver, or demonstrates the ability of a compound to bind to a macromolecular receptor like DNA. In vitro studies are also extremely important in metabolic investigations and the identification of reactive intermediates. The use of specific intact organs or a variety of cell or tissue cultures of human, animal, plant, or microbial origin often provides useful laboratory models for studying the potential of a compound to penetrate skin, damage the cornea of the eye, or otherwise interact with living tissues. Adverse effects may be indicated through changes in cell turnover, membrane permeability, or damage to cell organelles (e.g., mitochondria).

The results of *in vitro* studies to determine genetic toxicity have become an important component of assessing the carcinogenic potential of a chemical. Thus, an integral part of the routine testing of all chemicals is a battery of *in vitro* tests to determine each chemical's potential mutagenicity or clastogenicity (potential for DNA damage). These tests include the well-known Ames mutagenicity test with various strains of *Salmonella*, cytogenetic, and unscheduled DNA synthesis assays employing various cell cultures including those of human cells. A positive result with these types of tests suggests genotoxic or mutagenic potential that is taken into account in the total weight of evidence evaluation of carcinogenicity.

Epidemiology in Hazard Identification

Since epidemiology relates directly to the incidence of illness or toxicity in humans, the existence of positive epidemiological data is most relevant to assessing potential human hazard or risk. The major advantages of epidemiological studies are that they are usually based on large numbers of humans exposed to 'real-world' levels of the chemical. Any effects observed are directly relevant to humans and do not require the type of extrapolations used to relate animal studies to humans.

Unfortunately, epidemiological data can only be obtained retrospectively after a chemical has been on the market for a number of years and, obviously, cannot play a role in the prospective hazard evaluation of a new chemical.

Structure–Activity Relationships

In the absence of any other information, the analysis of possible structure–activity relationships (SARs) is

Hazard Ranking

Andrew Maier and Charley Pittinger

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Chemical hazard ranking plays an important role in protecting public health and the environment. Understanding comparative risk profiles early in product development allows for directing resources to substances with better safety profiles. For existing chemicals or products, hazard ranking and screening tools allow for setting priorities in implementing risk management strategies. Hazard ranking is playing an often useful as a first attempt at hazard identification. SAR analysis involves a comparison of the structural, physical, and chemical properties of a chemical of unknown hazard with those of similar chemicals having known toxic effects. In some cases, SAR analysis may simply provide a qualitative indicator of a specific type of activity (i.e., if it is a polycyclic aromatic hydrocarbon it is likely to be treated as a suspected carcinogen in the absence of any toxicity data). In other cases, SAR relationships can actually be used to obtain a quantitative estimate of an effect (e.g., with homologous series of compounds or organophosphorus cholinesterase inhibitors). SAR analysis is frequently used by regulatory agencies to develop a series of triggers of concern. Thus, when faced with a new chemical that has never been tested, the presence of certain functional groups may be an indicator of possible toxicological concern.

It is important to recognize, however, that SAR analysis can be misleading and must be used cautiously. With some chemicals, the requirements for exerting an adverse effect are very specific, and small, seemingly insignificant changes in structure (e.g., the *ortho*, *meta*, or *para* position of an aromatic substituent) can have an enormous impact on biological activity.

See also: Ames Test; Analytical Toxicology; Animal Models; Biomarkers, Human Health; Epidemiology; Good Laboratory Practices (GLP); *In Vitro* Test; *In Vivo* Test; Risk Assessment, Human Health; Risk Characterization; Toxicity, Acute; Toxicity, Chronic; Toxicity, Subchronic.

Further Reading

Reese CD (2003) Occupational Health and Safety Management. Boca Raton, FL: CRC Press.

increasing role as a tool in meeting or exceeding regulatory initiatives and satisfying product stewardship goals (e.g., International Organization for Standardization (ISO) 14000, the chemical industry's Responsible Care[®] programs, 'green certification programs', socially responsible investments, etc.).

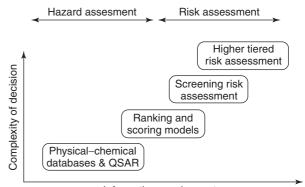
In addition, regulatory initiatives such as the European Union's Registration, Evaluation and Authorization of Chemicals (REACH) program, Canada's Domestic Substances List, and the High Production Volume (HPV) chemical programs in the United States and Europe are key drivers for the large-scale application of hazard ranking and screening tools for safety and risk assessment. Further, occupational and environmental hazard communication methods being standardized under the Global Harmonized System (GHS) for the classification and labeling of chemicals have long required effective assessment and ranking of hazards. Hazard ranking also provides a means for public education about relative risks of chemicals substances or activities. For example, the organization Environmental Defense uses comparative hazard ranking tools as part of its 'Scorecard' for providing information on the relative toxicity of chemicals.

Literally hundreds of hazard/risk databases, models, and algorithms have been developed to address this need for hazard ranking in risk and safety assessment. The available tools are very diverse, having different scientific, geographical, and chemical product focuses, as well as differing levels of sophistication and transparency. For example, some systems evaluate toxicity based solely on acute toxicity potential, while others rely on more comprehensive reviews of the toxicology database. Some systems include a detailed exposure assessment, while others include only minimal or no input for exposure potential. Some systems integrate ecological as well as human health toxicity considerations, while others have a single focus area. In addition, many published approaches include the consideration of toxicity or exposure information, but do not weigh other considerations such as public or regulatory agency risk perception that are critical factors in developing the overall risk profile for the chemical of interest. Because of this diversity in approaches, in many cases, the outcomes of such hazard assessments can vary considerably depending on the tool that is used.

Several authors have provided comparative summaries of hazard and risk ranking tools. One publication organized examples of related hazard ranking tools according to a hierarchy based on the complexity of hazard and risk assessment decisions being supported. This relationship is shown in **Figure 1**.

The diversity in some commonly used hazard ranking tools and approaches is represented in the examples below. The Further Reading and Relevant Website sections include a more comprehensive compilation of available approaches.

- Categorization base on acute toxicity potential *Hazardous Materials Identification System* (HMIS and HIMS III).
- Ranking based on qualitative exposure potential and longer-term toxicity assessment – *Chemical Hazard Evaluation for Management Strategies:*



Information requirements

Figure 1 Hierarchy of hazard ranking tools in risk decision making. (Reproduced from Pittinger *et al.* (2003) *Risk Analysis* 23: 529–535, with permission from Blackwell Publishing.)

A Method for Ranking and Scoring Chemicals by Potential Human Health and Environmental Impacts.

- Ranking based on regional and global exposure estimations and qualitative human health and ecological toxicity assessment *European Union Risk Ranking Method*.
- Ranking based primarily on estimated and modeled exposure and qualitative toxicity data – Use Clusters Scoring System (UCSS).
- Recommendations for control strategies based on qualitative exposure and toxicity information *Control of Substances Hazardous to Health* (COSHH).

See also: Exposure Assessment; Exposure Criteria; Hazard Identification; High Production Volume (HPV) Chemicals; Risk Assessment, Ecological; Risk Assessment, Human Health.

Further Reading

- Hansen BG, van Haelst AG, van Leeuwen K, and van der Zandt P (1999) Priority setting for existing chemicals: European Union risk ranking method. *Environmental Toxicology and Chemistry* 18: 772–779.
- Pittinger CA, Brennan TH, Badger DA, Hakkinen PJ, and Fehrenbacher MC (2003) Aligning chemical assessment tools across the hazard–risk continuum. *Risk Analysis* 23: 529–535.
- Swanson MB and Socha AC (1997) Chemical Ranking and Scoring: Guidelines for Relative Assessments of Chemicals. Pensacola, FL: Society for Environmental Toxicology and Chemistry (SETAC) Press.
- Swanson MB, Davis GA, Kincaid LE, Schultz TW, Bartmess JE, Jones SL, and George EL (1997) A screening method for ranking and scoring chemicals by potential human health and environmental impacts. *Environmental Toxicology and Chemistry* 16: 372–383.

Relevant Websites

- http://eerc.ra.utk.edu Davis GA, Swanson M, and Jones S (1994) Comparative Evaluation of Chemical Ranking and Scoring Methodologies. Prepared for US Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC.
- www.scorecard.org Environmental Defense (ED), 'Scorecard'.

Hazardous Chemicals. Import/Export of

Marjorie Collins

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Background

Chemicals, many potentially hazardous to health and the environment, are bought and sold in enormous quantities throughout the world. Similarly, hazardous waste often finds its way beyond the borders of the countries which generate it. Largely unregulated for many years, the transfer of chemicals between countries was addressed by voluntary measures beginning in the 1980s, when the procedure that has come to be known as Prior Informed Consent (PIC) took hold. This required exporters trading in hazardous substances to obtain the informed consent of importers before proceeding with any trade. This procedure was strengthened with the adoption of the Rotterdam Convention, making PIC legally binding. Importing countries are provided with the tools and information they need to identify potential hazards and to decline import of chemicals they cannot manage safely. If a country agrees to import chemicals, the Convention promotes their safe use through standards for labeling, technical assistance, and other forms of support. It also ensures that exporters comply with the requirements. The Rotterdam Convention came into force on February 24, 2004.

Related to chemical import and export, but somewhat separate from it, is the issue of the transboundary shipment of hazardous wastes. The international law dealing with it is the Basel Convention, which came into force in 1992. One of its guiding principles is that, in order to minimize the threat, hazardous wastes should be dealt with as close to where they are produced as possible. Movements of wastes must comply with a prior written notification by the exporting country to the importing country. Movement documents are required to track the waste from the beginning of its journey to the point of disposal.

- www.paint.org National Paint & Coatings Association (NPCA) (2002) Hazardous Materials Identification System (HMIS and HIMS III).
- http://www.hse.gov.uk United Kingdom HSE (Health and Safety Executive) (2004) COSHH Essentials: Easy Steps to Control Chemicals.
- http://www.epa.gov US Environmental Protection Agency (USEPA) (2004) Use Clusters Scoring System.

This entry will focus on the United States' interpretation of the regulation of the import and export of hazardous chemicals, specifically with regard to the Toxic Substance Control Act (TSCA). It will not generally address issues related to hazardous waste.

TSCA Basics

The Environmental Protection Agency (EPA) regulates the import and export of chemical substances from the United States under the TSCA. TSCA was first enacted in 1976 and has been amended significantly three times. TSCA gives the EPA broad authority to regulate the manufacture, use, distribution in commerce, and disposal of chemical substances. TSCA is a federally managed law and is not delegated to states.

One of the main objectives of TSCA is to characterize and evaluate the risks posed by a chemical to humans and the environment before the chemical is introduced into commerce. TSCA accomplishes this through the requirement that manufacturers perform various kinds of health and environmental testing, use quality control in their production processes, and notify EPA of information they gain on possible adverse health effects from use of their products. Under TSCA, 'manufacturing' is defined to include 'importing', and thus all requirements applicable to manufacturers apply to importers as well.

TSCA classifies chemicals as either 'existing' or 'new'. Existing chemicals are listed in the TSCA Chemical Substances Inventory (the 'Inventory'). Both existing and new chemicals can be covered by TSCA requirements.

The main regulatory requirements under TSCA which apply to importers and exporters of hazardous chemicals are:

- Premanufacture Notice (PMN)
- Testing Requirements
- Recordkeeping and Reporting Requirements
- TSCA Export Requirements
- TSCA Import Certification

Premanufacture Notice – TSCA Section 5 (40 CFR 700, 720–725, 747)

If a business is importing chemicals or chemicalcontaining items into the United States, that business must determine whether or not any chemical imported in bulk or as a part of a mixture, is a TSCA chemical substance and/or a 'new chemical substance' prior to its importation for a nonexempt commercial purpose. Under Section 5 of TSCA, persons who intend to manufacture or import a 'new chemical substance' into the United States must seek EPA approval by submitting a premanufacture notice (PMN) to EPA at least 90 days prior to importation to enable EPA to determine whether the new chemical may present an unreasonable risk to human health or the environment. A new chemical substance is one that is not already in commerce in the United States, as determined by inclusion in the TSCA Inventory of Chemical Substances maintained by EPA. New chemical substances include certain genetically modified microorganisms.

In addition, prior to importation of a chemical substance subject to TSCA into the United States, an importer of record must determine whether the substance is subject to a Significant New Use Rule issued under Section 5 of TSCA. Section 5 of TSCA authorizes EPA to designate use of a chemical substance as a 'significant new use', and require the submission of information to EPA prior to the chemical substance being manufactured (including imported) or processed for that use.

The PMN must include information on the manufacturing process, disposal method, and health and environmental effects of the substance. After its review of the PMN, EPA may approve the PMN and/ or may limit, restrict, or prohibit the manufacture, use, distribution, and/or disposal of the chemical substance.

If the PMN is approved and the substance is imported, a 'Notice of Commencement' (NOC) is required to be submitted to EPA within 30 days of first importation. After the EPA receives the NOC, the subject chemical substance will be added to EPA's TSCA Inventory of existing chemical substances for future importation and/or domestic production.

There are some exemptions to the PMN requirement, including chemicals such as drugs and pesticides that are regulated by other statutes, as well as chemicals developed under certain special circumstances.

In addition to the PMN requirement an importer of record must determine whether the substance is subject to a Significant New Use Rule issued under Section 5 of TSCA, prior to importation of a chemical substance subject to TSCA. Section 5 of TSCA authorizes EPA to designate use of a chemical substance as a 'significant new use', and require the submission of information to EPA prior to the chemical substance being manufactured (including imported) or processed for that use.

There are certain exemptions to the 90 days review of new chemicals. The TSCA compliance certification is still required to import these chemicals. Some examples of exemptions are as follows:

- Research and Development Exemption (40 CFR 720.36)
- Test Marketing Exemption (40 CFR 720.38)
- Low Volume/Low Release/Low Exposure Exemption (40 CFR 723.50)
- Polymer Exemption (40 CFR 723.250)

Testing Requirements – TSCA Section 4 (Regulations: 40 CFR 766, 790–799)

In addition to the PMN process, chemical manufacturers and importers may be required to perform other testing on health and environmental effects of their products under TSCA. A person who imports or intends to import a chemical substance or mixture subject to a test rule under Section 4 must comply with Section 4 requirements unless the importation qualifies for an exemption included in the regulations at 40 CFR Section 790.42, or under a specific test rule listed under Parts 766 or 799. Following promulgation of a test rule under Section 4 of TSCA, the responsibility to comply with the rule continues for a period of 5 years from the date the data from all required tests have been submitted or an amount of time equal to that which was required to develop the test data, whichever is longer. Importers therefore have a continuing responsibility to determine whether a chemical substance or mixture which they import or intend to import is subject to a test rule.

Recordkeeping and Reporting Requirements – TSCA Section 8 (40 CFR 710, 712, 717, 716)

Section 8 of TSCA authorizes EPA to require chemical manufacturers, importers, processors, and distributors of TSCA-covered chemical substances and mixtures to keep certain records and report certain information to EPA. Specific TSCA Section 8 rules (and implementing policy documents in the case of TSCA Section 8(e)) that apply are:

- Inventory Update Rule
- Preliminary Assessment Information Reporting Rule

- Chemical Specific Recordkeeping and Reporting
- Allegations of Significant Adverse Reactions Recordkeeping and Reporting Rule
- Unpublished Health and Safety Data Reporting
- Substantial Risk Information Reporting Requirement

These requirements are explained in more detail below.

Inventory Update Rule (IUR)

Companies that manufacture or import more than 10 000 lb of certain chemicals that are included on the TSCA Chemical Substance Inventory (primarily organics) are required to report current data on the production volume, plant site, and site-limited status of these chemicals. Reporting under the IUR takes place at 4 year intervals, which began in 1986. Certain small businesses as defined by 40 CFR 710.29 are excluded. The next round of reports will be due in 2006.

Preliminary Assessment Information Rule (PAIR)

Under PAIR, producers and importers of a listed chemical are required to report the following sitespecific information on a two page form:

- Quantity of chemical produced and/or imported.
- Amount of chemical lost to the environment during production or importation.
- Quantity of enclosed, controlled and open releases of the chemical.
- Per release, the number of workers exposed and the number of hours exposed.

Exemptions for such reporting are as follows:

- Production or importation for the sole purpose of research and development (R&D).
- Production or importation of less than 500 kilograms during the reporting period at single plant site.

Companies whose total annual sales from all sites owned by the domestic or foreign parent company are below \$30 million for the reporting period and who produced or imported less than 45 400 kg of the chemical

• Production or importation of the listed chemical solely as an impurity, a nonisolated intermediate, and under certain circumstances as a by-product.

Allegations of Significant Adverse Reactions Rule

This rule provides a mechanism to identify previously unknown chemical hazards by tracking patterns of adverse effects when they are noticed or detected by requiring companies to record, retain, and in some cases, report to the USEPA 'allegations of significant adverse reactions' for substances/mixtures that they produce, import, process, or distribute.

An 'allegation' is defined as "a statement, made without formal proof or regard for evidence, that a chemical substance or mixture has caused a significant adverse reaction to health or the environment."

'Significant adverse reactions' are defined as "reactions that may indicate a substantial impairment of normal activities, or long lasting or irreversible damage to health or the environment."

TSCA Section 8(c) records must be kept at a company's headquarters or at a site central to the chemical operations and must be retrievable by the alleged cause of the reaction (i.e., specific chemical identity, mixture, article, company process or operation or site emission, effluent, or discharge). The records must be maintained by the company for 30 years (for allegations made by employees) or 5 years for allegations made by plant site neighbors or customers.

The records must contain the original allegation as received, an abstract of the allegation, the results of any self-initiated investigation, and copies of any additional information regarding the allegations (e.g., copies of any reports required to be made to the US Occupational Safety and Health Administration Division).

Verbal allegations must be transcribed either by the company or the individual making the allegation (if transcribed by the individual, they must be signed). To be recordable, allegations must implicate a substance that caused the reaction by naming either the specific substance, a mixture, or article containing the substance; a company process in which the substances are involved; or identifying a discharge from a site of manufacture, processing, or distribution of the substance.

Certain allegations of human health and environmental adverse effects are 'exempt' from the requirements of the TSCA Section 8(c) rule including those that: (1) are made anonymously; (2) identify 'known human effects'; and (3) those that are directly attributable to an incident of environmental contamination that has already been reported to the US Government under any applicable authority.

Unpublished Health and Safety Studies Rule

Businesses may be required to submit to EPA a list and/or copies of unpublished studies that address the health or safety issues of certain listed chemicals. Businesses that must report under the TSCA Section 8(d) rule include:

- Current as well as prospective producers, importers, and (if specified) processors of the subject chemical(s); and
- Businesses that, in the 10 years preceding the effective date that a substance or mixture is added to the rule, either had proposed to produce, import, or (if specified) process, or had produced, imported, or processed (if specified) the substance or listed mixture.

Once a chemical substance or mixture is added to the rule, reporting obligations terminate (i.e., sunset) no later than 2 years after the effective date of the listing of the substance or mixture, or on the removal of the substance or mixture from the rule.

Unpublished studies on listed substances or mixtures are potentially reportable (i.e., studies may be subject to either copy submission requirements or listing requirements). Generally, copies of studies possessed at the time a person becomes subject to the rule must be submitted, and the following categories of studies must be listed:

- Studies ongoing as of the date a person becomes subject to the rule (copies must be submitted when completed).
- Studies initiated after the date a person becomes subject to the rule (copies must be submitted when completed).
- Studies which are known as of the date a person becomes subject to the TSCA Section 8(d) rule, but not possessed.
- Studies previously sent to US Government Agencies without confidentiality claims.

The term 'health and safety study' is intended to be interpreted broadly and means "any study of any effect of a chemical substance or mixture on health or the environment or on both," including but not limited to:

- epidemiological or clinical studies,
- studies of occupational exposure,
- in vivo and in vitro toxicological studies, and
- ecotoxicological studies.

Substantial Risk Information Requirement

Businesses are under a duty to report to EPA within 15 days any new information which reasonably supports the conclusions that a substance or mixture the business manufactures, imports, processes, or distributes presents a substantial risk of injury to health or the environment.

The term 'substantial risk' information refers to that information which reasonably supports a conclusion that the subject chemical or mixture presents a substantial risk of injury to health or the environment; however, such information need not and most typically does not establish conclusively that a substantial risk exists.

In deciding whether information is 'substantial risk' information, one must consider (1) the seriousness of the adverse effect, and (2) the fact or probability of the effect's occurrence. In determining TSCA Section 8(e)-applicability/reportability, these two criteria should be weighted differently depending upon the seriousness of the effect or the extent of the exposure, that is, the more serious the effect, the less heavily one should weigh actual or potential exposure, and vice versa. For example, in cases where serious effects such as birth defects or cancer (as evidenced by benign and/or malignant tumors) are observed, the mere fact that the implicated chemical is in commerce (including chemicals at the research and development stage) constitutes sufficient evidence of exposure to submit the newfound toxicity data.

EPA has also received numerous Section 8(e) submissions alerting the Agency that chemical substances already known to be capable of causing serious health and/or environmental effects were detected in significant amounts in environmental media (e.g., soil, surface waters, groundwater, air (including workplace air)) or in products not known previously by the Agency to contain such chemicals. In such cases, the discovery of previously unknown and significant human and/or environmental exposure, when combined with knowledge that the subject chemical is already recognized or suspected as being capable of causing serious adverse health effects (e.g., cancer, birth defects, neurotoxicity) or serious environmental effects (e.g., nontrivial aquatic species toxicity), can provide a sufficient basis to report the new-found exposure data to EPA under Section 8(e) of TSCA.

The decision-making process for Section 8(e)reportability should focus primarily on whether the toxicity or exposure information offers reasonable support for a conclusion of substantial risk under the criteria described above, but should not focus at all on whether the information is conclusive regarding the risk. A decision to report information to the Agency under Section 8(e) should not involve exhaustive health and/or environmental risk assessments of the subject chemical(s). Further, determining reasonable support for a conclusion of substantial risk should not include any evaluation of either the economic or social benefits of the use(s) of the subject chemical substance(s). Finally, determining whether reasonable support exists for 'substantial risk' is not synonymous with the determination of an 'unreasonable risk' as that term is used elsewhere in TSCA.

TSCA Export Requirements – TSCA Section 12(b) (40 CFR 707)

Chemical exporters are potentially subject to Section 12(b) of TSCA. EPA's TSCA Section 12(b) export notification requirements apply to chemical substances or mixtures for which data are required under TSCA Section 5(b), an order has been issued under TSCA Section 5, a proposed or final rule has been issued under TSCA Sections 5 or 6, or an action is pending relief has been granted under TSCA or Sections 5 or 7. With regard to Section 4 of TSCA, only those chemical substances or mixtures listed in final TSCA Section 4 test rules and TSCA Section 4 Enforceable Consent Agreements are subject to the export notice requirements under TSCA Section 12(b). Notification of export is generally not required for articles, as provided by 40 CFR section 707.60(b).

Exporters of chemicals that are subject to final or proposed rules and orders under Sections 5, 6, and 7 of TSCA must notify EPA of the country of destination the first time a chemical is shipped to the country during a calendar year. In addition, exporters of chemicals subject to final rules or orders under Section 4 of TSCA must notify EPA of the country of destination the first time a chemical is shipped to the country.

TSCA Import Certification – TSCA Section 13 (40 CFR 707 and 19 CFR 12.118–12.128)

Under EPA and Customs regulations, the importer of a chemical substance or mixture must certify at the port of entry that each shipment is either subject to and in compliance with TSCA (a positive certification); or the shipment is not subject to TSCA

Hazardous Waste

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There are two definitions of hazardous waste. The first definition was written into legislation by the US Congress, and the second, which has its basis in the first, was written into regulation by the US Environ(a negative certification). The following series of questions need to be asked when making a determination.

- 1. Is the material in the shipment to be imported an 'article', or tobacco or tobacco product? If yes, an import certification is not required (positive or negative). If no, continue to the next question.
- 2. Is the material in the shipment to be imported a pesticide; a source or special nuclear material or by-product; a firearm or ammunition; or a food, food additive, drug, cosmetic, or device? If yes, the material is not subject to TSCA, but a 'negative' TSCA import certification is required unless the shipment is clearly identified as being a pesticide or other chemical not subject to TSCA (e.g., the shipment is accompanied by FDA Form FD701 or EPA (FIFRA) Form 3540-1). If no, continue to the next question.
- 3. Does the shipment contain any chemical substances or mixtures regulated under TSCA Section 5 (including new chemical substances), TSCA Section 6, or TSCA Section 7? If yes, continue to the next question. If no, a positive TSCA import certification can be made.
- 4. Are the chemical substances and/or mixture in the shipment compliant with TSCA Sections 5, 6? If yes, a positive TSCA import certification can be made. If no, import certification cannot be provided and the shipment cannot be imported until the chemical substance and/or mixtures is compliant with all applicable requirements under TSCA Sections 5, 6, and 7.

See also: Environmental Protection Agency, US

Relevant Websites

http://www.basel.int - Basel Convention.

http://www.pic.int - Rotterdam Convention.

http://www.epa.gov – Import/Export Requirements (under TSCA, from the US EPA).

mental Protection Agency (EPA). In 1976, US Congress defined the term 'hazardous waste' in the Resource Conservation and Recovery Act (RCRA), an amendment to the Solid Waste Disposal Act of 1965, as

A solid waste, or combination of solid waste, which because of its quantity, concentration, or physical,

chemical, or infectious characteristics may – (A) cause, or significantly contribute to an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness; or (B) pose a substantial present or potential hazard to human health or the environment when improperly treated, stored, transported, or disposed of, or otherwise managed.

42 USC 6903(5)

In Subtitle C of RCRA, Congress created the framework for the 'cradle-to-grave' management of hazardous waste. The broad, subjective statutory definition of hazardous waste, however, did not specify which wastes would be subject to Subtitle C and its accompanying regulatory scheme. A more precise definition of hazardous waste was needed to determine exactly which wastes would be subject to RCRA Subtitle C regulation. Therefore, the US Congress directed the US EPA to promulgate regulations identifying the characteristics of hazardous waste and listing particular hazardous wastes, "taking into account toxicity, persistence, and degradability in nature, potential for accumulation in tissue, and other related factors such as flammability, corrosiveness, and other hazardous characteristics" (42 USC 6921(a)). In 1980, the US EPA fulfilled this statutory mandate by promulgating 40 CFR Part 261, which is titled *Identification and Listing of* Hazardous Waste. Since then, the US EPA has modified Part 261 numerous times to reflect Congressional amendments and trends in waste generation.

In the regulatory sense, hazardous waste identification relies not so much on a definition as on a series of steps that involve checking against lists of waste exclusions and inclusions. The three steps of the hazardous waste identification process are codified in 40 CFR 262.11.

The first step of hazardous waste identification is determining whether a material is a solid waste. A solid waste is any material that is discarded. (The modifier 'solid' is not indicative of the physical state of the material. That is, a solid waste may be in the liquid or gaseous phases as well as the solid phase.) A material is considered discarded when it is abandoned, recycled, or inherently waste-like. Abandonment occurs when a material is disposed of; burned/ incinerated; or accumulated, stored, or treated before, or in lieu of, abandonment. Recycling occurs when a material is used in a manner that resembles disposal (e.g., placed on the ground); burned to recover its energy; reclaimed to recover a usable product; or accumulated speculatively. In addition, a few specific materials have been designated inherently waste-like (e.g., certain dioxin-containing wastes) and are considered solid wastes when recycled in

any manner. However, a number of materials do not meet the definition of solid waste when discarded because the materials qualify for one of several exclusions from the definition of solid waste that are codified in 40 CFR 261.4(a). For example, in order to avoid duplicative regulations, materials regulated and managed under the Clean Water Act or the Atomic Energy Act are exempt from the definition of solid waste (40 CFR 261.4(a) (2) and 261.4(a) (4), respectively).

The second step in the hazardous waste identification process is determining whether a solid waste qualifies for one of the statutory or regulatory exclusions from the definition of hazardous waste. Several exclusions from the definition of hazardous waste appearing in the regulations have their origins in the statute. For instance, the US EPA incorporated Congress' Subtitle C exclusion for cement kiln dust into the regulations at 40 CFR 261.4(b) (8). In addition, the US EPA has promulgated a number of exclusions independently of Congress, all of which are codified in 40 CFR 261.4(b). For example, the US EPA exempted all solid wastes routinely generated in residences from the definition of hazardous waste (40 CFR 261.4(b) (1)). Waste-specific exclusions may also be obtained on a site-by-site basis by petitioning the US EPA.

The third step of hazardous waste identification is determining whether a solid waste that is not specifically excluded is a hazardous waste. There are two broad categories of hazardous waste: listed and characteristic. The hazardous waste determination process in 40 CFR 262.11 states that the listings are reviewed first. If a solid waste does not meet any of the listing descriptions, then the characteristics are checked. The listings are intended to regulate common hazardous waste streams by specifically listing them by name or by description. The characteristics, on the other hand, are written as broad descriptions, each of which may capture an unspecified number of solid wastes within hazardous waste regulation. All hazardous wastes are assigned a waste code consisting of an initial letter and a number. Listed waste codes begin with an 'F', 'K', 'P', or 'U', while all characteristic waste codes begin with the letter 'D'.

A waste is listed by the US EPA if it meets one of the three criteria codified in 40 CFR 261.11. First, a waste can be promulgated as a listed waste if it exhibits any of the four characteristics (a detailed discussion of the characteristics follows). Although a waste may be listed because it exhibits a characteristic, it is not defined in the regulations in terms of its characteristic(s). Rather, the US EPA defines such wastes in terms of the process by which it is generated or by its chemical name. For example, K044 is listed because it exhibits a characteristic, but its listing description reads, 'wastewater treatment sludges from the manufacturing and processing of explosives', without reference to the characteristic.

Second, a waste can be listed because it contains any of the toxic constituents listed in 40 CFR Part 261 Appendix VIII, provided the concentration of the constituent in the waste, the persistence of the constituent, any toxic degradation products of the constituent, as well as other factors are taken into account. Hazardous constituents are listed in Appendix VIII if the constituents have been shown in scientific studies to have toxic, carcinogenic, mutagenic, or teratogenic effects on humans or other life forms. The majority of listed wastes have been listed in this manner.

Finally, a waste can be listed if it has been found to be fatal to humans in low doses or, in the absence of data on human toxicity, it has been shown in studies to:

- Have an oral LD₅₀ toxicity (rat) $\leq 50 \text{ mg kg}^{-1}$, or
- Have an inhalation LC₅₀ toxicity (rat) ≤2 mg l⁻¹, or
- Have a dermal LD_{50} toxicity (rabbit) ≤ 200 mg kg⁻¹, or
- Be otherwise capable of causing or significantly contributing to an increase in serious, irreversible, or incapacitating reversible illness. Wastes listed in this manner are classified as acute hazardous wastes that become subject to full hazardous waste regulation in smaller quantities than those for other hazardous wastes. An example of an acute listed hazardous waste is P015, beryllium powder.

Based on the first three criteria, the US EPA has promulgated several hundred listed wastes, dividing the listed wastes into three groupings: wastes from nonspecific sources ('F wastes'), wastes from specific sources ('K wastes'), and (two types of) commercial chemical products ('P or U wastes'). All wastes on the 'P list' are acute hazardous wastes.

Before a characteristic of hazardous waste can be promulgated it must be assessed against two criteria that are codified in 40 CFR 261.10. The first criterion is that a waste exhibiting the characteristic in question must meet the broad statutory definition of hazardous waste. The second criterion is that the characteristic must be able to be measured using standardized test methods or detected through knowledge of the waste. Based on these criteria, the US EPA has identified four characteristics of hazardous waste: ignitibility, corrosivity, reactivity, and toxicity. For each characteristic, the US EPA has promulgated at least two distinct properties. A solid waste need only exhibit one property of one characteristic to be subject to regulation as a characteristic hazardous waste.

The US EPA has identified four properties of ignitibility. One of the four properties pertains to liquids that are not aqueous solutions containing less than 24% alcohol by volume. A liquid meeting this description that has a flash point $\leq 60^{\circ}$ C (140°F), as determined by a specified closed cup test, is one example of an ignitible hazardous waste that carries the waste code D001.

Two properties of corrosivity have been identified by the US EPA, either of which can render a solid waste a hazardous waste, identified by the waste code D002. An aqueous solid waste that has a pH ≤ 2 or ≥ 12.5 , as measured by a specified test, is a corrosive hazardous waste. Likewise, a liquid solid waste that corrodes steel at a rate ≥ 6.35 mm year⁻¹ at 55°C, as measured by a specified test, is also a corrosive hazardous waste.

A solid waste is hazardous for reactivity if it displays any one of eight separate properties that the US EPA has specified. Unlike the other three characteristics of hazardous waste, several of the properties of reactivity rely on subjective criteria rather than scientifically measured properties. In such cases, a generator of a solid waste must make a judgment about the regulatory status of the waste based on his/her knowledge of the nature of the waste. For example, a solid waste that forms potentially explosive mixtures with water, or a waste that is normally unstable and readily undergoes violent change without detonating, is a D003 reactive waste.

The final characteristic, toxicity, is defined by the concentration levels of 40 hazardous constituents -26 organics, 8 metals, and 6 pesticides – in an extract of a representative sample of waste. The extract is obtained by subjecting a sample to the Toxicity Characteristic Leaching Procedure, a test designed to estimate the ability of the contaminant to leach if the waste containing it were placed in a landfill. The concentration levels of hazardous constituents measured in a waste extract are compared to maximum allowable concentrations limits established in the regulations. If any of the regulatory concentration limits are equaled or exceeded, the waste stream (not just the extract) is a characteristic hazardous waste for toxicity. Each of the 40 toxicity characteristic (TC) waste codes, D004-D043, corresponds to a different hazardous constituent. For example, if the TCLP leachate of a waste has $\geq 5.0 \text{ mg l}^{-1}$ lead, the waste is TC hazardous for lead and carries the waste code D008. If the leachate has $\ge 0.2 \text{ mg l}^{-1}$ mercury, the waste is TC hazardous for mercury and carries the waste code D009.

It is important to note that the same chemical or compound can have different wastecodes based on the manner in which it is generated. For example, American Petroleum Institute (API) separator sludge from the petroleum refining industry is identified by the waste code K051. Separator sludge generated in a unit other than an API separator would not be K051 because it does not meet the listing description that, as a K listing, specifies the source of the sludge. The same waste generated in another type of separator would most likely be captured by either F037 or F038, neither of which, as F-listed wastes, specifies the source of the waste stream. In addition, if neither listing applies, the sludge could be regulated as a characteristic hazardous waste with the waste code D018 if the TCLP leachate meets or exceeds 0.5 mgl^{-1} benzene, the regulatory limit of the toxicity characteristic for benzene.

According to 40 CFR 262.11, the person(s) who produces a waste (generator) is responsible for carrying out the hazardous waste identification process. First, the generator must review all hazardous waste listings. Only if no listings apply is the generator required to check for characteristics, using testing and/or process knowledge. Although it is not necessary for hazardous waste identification purposes to determine whether a listed waste is also characteristic, it may be necessary to take the added step of identifying characteristics to determine the appropriate

treatment for the waste. If a solid waste does not meet a listing description and it does not exhibit any of the four characteristics, it is not a hazardous waste as defined by the federal regulations.

Hazardous waste identification is the first step in determining how a waste must be managed. Wastes that meet the definition of hazardous waste are subject to comprehensive federal regulations (40 CFR 262–279) that govern the generation, transportation, storage, treatment, disposal, and recycling of hazardous waste. The level of regulation varies based on criteria such as the amount of waste generated at a site on a monthly basis, the nature of the waste, and, in some cases, whether the waste is recycled. All hazardous waste regulations are similar in that they are intended to ensure the safe management of hazardous waste from cradle to grave.

See also: Clean Water Act (CWA), US; Environmental Toxicology; Resource Conservation and Recovery Act, US

Relevant Websites

http://www.dtsc.ca.gov – Managing Hazardous Waste; US California Department of Toxic Substances Control.

http://www.osha.gov – Safety and Health Topics: Hazardous Waste; US Occupational Safety and Health Administration.

Health Assessments

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Introduction

The 1986 Superfund Amendments and Reauthorization Act directs the Agency for Toxic Substances and Disease Registry (ATSDR) to perform specific public health activities associated with exposure to hazardous substances released into the environment. Among these activities, ATSDR was mandated to perform a health assessment for each facility listed or proposed to be listed on the National Priorities List. The health assessment has to be conducted within 1 year of being listed (or proposed for listing). In addition, ATSDR may conduct a health assessment for a particular facility or release when petitioned by the public.

A health assessment is the evaluation of data and information on the release of hazardous substances into the environment in order to assess any current or future impact on public health, develop health advisories or other recommendations, and identify studies or actions needed to evaluate and mitigate or prevent human health effects (55 Federal Register 5136, February 13, 1990, as codified at 42 Code of Federal Regulations Part 90).

The health assessment is a mechanism to respond to community health concerns associated with human exposure to hazardous substances at a site. It has three major purposes: (1) evaluating the public health implications of the site; (2) addressing those implications by developing health advisories or making recommendations, including further health or environmental studies; and (3) identifying populations where actions are necessary to mitigate or prevent adverse health effects.

When complete health or environmental data are lacking, it may be necessary to conduct further assessments for a site or facility as the data become available. A major reason for preparing a health assessment is to determine the need for health effects studies at a site to further assess any current or future risks to public health. The health assessment is an evaluation of relevant environmental data, health outcome data, and community concerns associated with a site where hazardous substances have been released. The health assessment identifies populations living or working on or near hazardous waste sites for which public health actions are needed, such as health studies, health education, or chemicalspecific research.

Health assessments are based on factors such as the nature, concentration, toxicity, and extent of contamination at a site; the existence of potential pathways for human exposure; community health concerns; the size and nature of the community likely to be exposed; relevant community-specific, past and current, health outcome data; and any other information available to ATSDR that is relevant to a determination of potential risks to public health.

Thus, health assessments are designed not only to evaluate health effects, but also to identify populations for which additional studies or public health actions are required. Hence, the health assessment is designed to identify: (1) knowledge gaps concerning the toxicity of substances identified at the facility or release under review; (2) communities near facilities or releases where biologic measurements of human exposure or medical investigations (e.g., communitybased health outcome parameters) are needed; and (3) the need for additional health information (e.g., pilot studies, epidemiological studies and registries, and site-specific surveillance). A variety of health studies based on the review of the health assessment may then be initiated, including: pilot health effects studies (disease- and symptom-prevalence studies, cluster investigations, exposure studies), epidemiological studies, or disease registries.

Three sources of information build the foundation of the health assessment: (1) environmental characterization data, (2) community health concerns, and (3) health outcome data.

Environmental characterization data for a hazardous waste site includes information on environmental contamination and environmental pathways. Such information is provided in site-specific reports obtained from the Environmental Protection Agency (EPA) and pertinent state and local environmental departments. Site visits are also an important source of environmental characterization data.

Community health concerns associated with a site constitute a key data component of the health assessment. The community associated with a hazardous waste site includes the population living around the site, local public health officials, other local officials, and the local media. In order to acquire information on community health concerns, the health assessor must become an investigator; obtaining that information provides the health assessor with an opportunity to involve the public in the health assessment process. In addition, community health concerns can serve as a guide in evaluating health outcome data.

Health outcome data and parameters are the third major source of data for health assessments. The identification, review, and evaluation of health outcome parameters are interactive processes involving ATSDR, data source generators, and the community involved. Health outcome data are communityspecific and may include databases at the local, state, and national level, as well as data from private health care organizations and professional institutions and associations. Databases to be considered include medical records, morbidity and mortality data, tumor and disease registries, birth statistics, and surveillance data. Relevant health outcome data play an important role in assessing the public health implications associated with a hazardous waste site and in determining which follow-up health activities are needed.

Health Assessment Process

To evaluate the public health implications posed by contamination at a site, the assessor must obtain and evaluate data and information on the site's history, the types and levels of contamination, environmental transport mechanisms, routes of human exposure, community health concerns, relevant health outcome parameters, and medical and toxicological implications of the site's contaminants. This evaluation is a dynamic process that considers available data from varying perspectives.

Every health assessment includes six basic steps for acquiring the data and information necessary to evaluate the site's health risks:

 Evaluating information on the site's physical, geographical, historical, and operational setting. Information should be evaluated to provide a historical perspective on the site, and describe its operations, current status, and the surrounding community. Once the important background details have been presented, the assessor should describe characteristics of populations on or near the site and the use of local land and resources. The text should state the total number of exposed and potentially exposed persons and indicate the characteristics of this population. Population estimates should include persons exposed in the past and present, as well as those at risk for future exposures. Demographic information may include population discussions of specific groups surrounding the site (e.g., residential, commercial, and occupational populations). If warranted, details about the size, exact location, age distribution, socioeconomic, genetic, and ethnic makeup of populations on and near the site should be discussed based on available information. Ethnic and socioeconomic background information is essential for a full understanding of the health threat a site poses to specific subpopulations. Populations that may be at special risk from exposure to the site, such as children, pregnant women, and the elderly, should receive special note.

- 2. Identifying health concerns of the affected community. The nature and degree of the residents' health concerns will vary from site to site. However, addressing the health concerns of the community is crucial if the health assessment is to satisfy its purpose of helping the public and health professionals understand the risks posed by a site.
- 3. Determining contaminants of concern associated with the site. This is the foundation of the health assessment and, therefore, should be composed carefully so that the significant hazards of concern are clearly and concisely presented. In this step, the assessor describes the contaminants that might pose a threat to public health and physical hazards at the site. To determine whether a contaminant is a contaminant of concern based on noncancer end points, the maximum media concentration should be compared to an appropriate health assessment comparison value. Health assessors should use ATSDR's Environmental Media Evaluation Guides (EMEGs). If no EMEG is available, the assessor should use other health guidelines, such as EPA's reference dose (RfD), to back-calculate a medium concentration. The assessor should also evaluate the potential carcinogenicity of contaminants. For carcinogens, comparison values based on a 10^{-6} cancer risk level for exposure to the contaminated media can be calculated from values such as EPA's cancer slope factors. If the maximum medium concentration exceeds a comparison value, the contaminant should be selected for further evaluation.
- 4. Identifying and evaluating exposure pathways (environmental transport mechanisms and human exposure pathways). In this step, the health assessor evaluates exposure pathways at the site. There are five elements in an exposure pathway: (1) source of contamination (source of contaminant release into the environment), (2) environmental media (this includes groundwater, surface water, air, surface soil, subsurface soil, sediment, and

biota. Transport mechanisms serve to move contaminants from the source to points where human exposure can occur), (3) point of exposure (a location of potential or actual human contact with a contaminated medium, for example, residence, business, residential yard, playground, campground, waterway or water body, contaminated spring or hand-drawn well, food services, etc.), (4) route of human exposure (means by which the contaminant actually enters or contacts the body, such as ingestion, inhalation, dermal contact, and dermal absorption), and (5) receptor population (persons who are exposed or potentially exposed to the contaminants of concern at a point of exposure). Completed exposure pathways exist when the five elements of a pathway link the contaminant source to an exposed population. Potential exposure pathways exist when information on one or more of the five elements is missing.

- 5. Determining public health implications based on available community-specific health outcome databases and other medical and toxicological information. In this step, the health assessor discusses the health effects of site contaminants, evaluates health outcome data, and addresses all questions raised by the community. Accordingly, there are three parts to this step: (1) toxicological evaluation, (2) health outcome data evaluation, and (3) community health concerns evaluation. The health assessment must demonstrate how information in each step of the health assessment relates to the public health discussion.
- 6. Determining conclusions and recommendations concerning the health threat posed by the site. The final step of the health assessment should address conclusions about the site and the health threat it poses. The first conclusion should be a statement about the site's level of public health hazard. The assessor should assign one of the five public health categories: urgent public health hazard, public health hazard, indeterminate public health hazard, no apparent public health hazard, or no public health hazard. For the categories 'urgent public health hazard' and 'public health hazard', the text should identify the contaminant(s), the completed exposure pathway(s), the health effect(s), and the exposed population(s). The text should also summarize conclusions about the following issues: health effects from exposure to site contaminants, response to community health concerns, results of health outcome data evaluation, and the effect that missing or insufficient information has on analyses and conclusions. Furthermore, every conclusion of the health assessment should have one or more recommendations associated with it.

Information reviewed for each step in the health assessment process is evaluated for adequacy of data and potential health impacts at a hazardous waste site. Consideration is given to known past or expected future contamination and exposures.

Categorizing Hazards

The final section of the health assessment should address conclusions about the site and the health threat it poses. The first conclusion should be a statement about the site's level of public health hazard. The assessor should assign one of the five public health categories:

- *Category A* Urgent Public Health Hazard: This category is used for sites that pose an urgent public health hazard as the result of short-term exposures to hazardous substances.
- *Category B* Public Health Hazard: This category is used for sites that pose a public health hazard as the result of long-term exposures to hazardous substances.
- *Category* C Indeterminate Public Health Hazard: This category is used for sites with incomplete information.
- *Category D* No Apparent Public Health Hazard: This category is used for sites where human exposure to contaminated media is occurring or has occurred in the past, but the exposure is below a level of health hazard.
- *Category E* No Public Health Hazard: This category is used for sites that do not pose a public health hazard.

Health Assessments versus Risk Assessments

Deliberate differences exist between ATSDR's health assessments and the EPA's risk assessments. The two agencies have distinct purposes that necessitate different goals for their assessments.

A risk assessment is defined as a qualitative and quantitative process conducted by EPA to characterize the nature and magnitude of risks to public health from exposure to hazardous substances, pollutants, or contaminants released from specific sites. Risk assessments include the following components: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Statistical and biological models are used in quantitative risk assessments to calculate numeric estimates of risk to health by using data from epidemiological investigations and animal toxicity studies. The product of quantitative risk assessment is a numeric estimate of the public health consequences of exposure to a chemical. In preparing a risk assessment for a site, a risk assessor also attempts to include all adverse health effects, characterizing the risk to sensitive populations when the information is available. EPA risk assessments are used in risk management decisions to establish cleanup levels; to set permit levels for discharge, storage, or transport of hazardous waste; determine allowable and to levels of contamination.

ATSDR health assessments are based on environmental characterization information, community health concerns, and health outcome data. Because of the nature of these databases, health assessments use quantitative as well as qualitative data, focusing on medical public health and toxicological perspectives associated with exposure to a site. The health assessment specifically addresses community health concerns (e.g., sensitive populations, possible disease outcomes) and evaluates relevant, communityspecific health outcome data. Combined with environmental data, information obtained from those two data sources are used to determine the public health implications of the site guiding the initiation of follow-up health activities when indicated.

Contact Details

The ATSDR Information Center Mailstop E-29, 1600 Clifton Road Atlanta, GA 30333, USA Tel.: +1-404-498-0110

See also: Chemical Hazard Communication and Material Safety Data Sheets; Hazard Identification; Hazard Ranking; Risk Assessment, Human Health.

Further Reading

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Heat Shock Proteins

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Heat shock proteins (HSPs) are a family of proteins expressed in almost all organisms from prokaryotes to humans. HSPs were originally described about four decades ago as proteins that were induced in the *Drosophila melanogaster* in response to a heat stress and hence derive the name HSP. However, research over the years has uncovered these proteins to have a multitude of functions. Primarily, all HSPs act as molecular chaperons and assist in proper folding of naïve proteins. Furthermore, HSPs have important roles in cellular processes including cell survival, inflammation, immunity, ion channel repair, and others. HSPs are also induced by a variety of stressors. Reactive oxygen species, cytotoxic injury, necrosis, ultraviolet radiation, metals, and many others are some examples.

HSPs are named according to their molecular mass in kilodaltons. Major classes include small HSPs, HSP40, HSP60, HSP70, HSP90, and HSP110 families. Most HSPs have both a constitutive and inducible form. Members of the small HSPs family include HSP32 or heme oxygenase-1, HSP27, HSP22, and HSP20. Of these HSP32 is most relevant in toxicology as it is induced by diverse physiological stressors, including hypoxia, ischemia-reperfusion, hydrogen peroxide, heavy metals (selenium, arsenite, cadmium), and other toxicants (acetaminophen, carbon tetrachloride). Upregulation of HSP32 has been suggested to be protective against several insults although the mechanisms thereof remain unclear. HSP70 and HSP90 are the two main chaperon systems. HSP70 in the cytosol binds to nascent proteins before they are released from the ribosomes. Through several different complex steps involving other chaperone interacting or organizing protein folding, assembly or disassembly of target proteins is accomplished. HSP40 for example assists in loading the target substrate molecules on to the HSP70 chaperone complex. HSP70 also has a well-studied role in ischemiareperfusion organ damage and overexpression of

HSP70 negatively correlates with infarct size. HSP90 is believed to act as a component of the cycle involving chaperone HSP70. In addition, HSP90, along with HSP70 and 56, bind some nuclear receptor (estrogen receptor) in an inactive complex. Binding of the nuclear receptor ligand cleaves of HSP90 hence allowing nuclear translocation and estrogen responsive gene expression to occur. Elevated levels of HSP90 can turn off estrogen mediated gene expression by destabilizing the receptor-ligand complex.

Cellular control of HSP expression: All HSPs are regulated by a small family of transcription factors called heat shock factor (HSF1–4). During a stress condition, HSF1 and 2 are hyperphosphorylated in a *ras*-dependent manner by MAP kinases. Binding of these active HSF1 factors to DNA sequences called heat shock elements in the promoters of all stressinducible genes occurs. This leads to increased transcription of HSP genes and induction of HSP proteins.

HSPs as cellular markers of stress: HSPs are involved in various aspects of cellular function and a lot is being learnt about its role in normal and pathological states. Recent studies from lower animals, especially fish, have revealed the potential use of induced fish HSPs as a biomarker of exposure to environmental stressors. Industrial effluents, polycyclic aromatic hydrocarbons, metals such as copper, zinc, mercury, pesticides, etc. have shown to induce HSP in fish. Further, the HSP response may vary with the stressor, tissue, species of fish, and the family of HSP studied. Hence it appears that a more extensive and probably a high-throughput profiling (using genomic and proteomic) approaches may be necessary to identify patterns of HSP modulation by various stressors.

See also: Mechanisms of Toxicity; Oxidative Stress.

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Helium

Mary Lee Hultin

Published by Elsevier Inc.

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7440-59-7
- SYNONYMS: Helium, compressed; Helium, refrigerated liquid (cryogenic liquid)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Nonflammable gas; Simple asphyxiant
- CHEMICAL FORMULA: He

Uses

Liquid helium is used to produce low temperatures. The inert, nonflammable gas is used in balloons and in scientific studies (e.g., meteorological). It is also used in inert gas shielding for arc welding, in filling light bulbs, as a carrier gas in chromatography, and as a substitute for nitrogen in air supplies for deep diving.

Exposure Routes and Pathways

Exposure is possible through inhalation of the gas or dermal contact with liquid helium.

Toxicokinetics

Helium is an inert gas that acts in the lungs as an asphyxiant by keeping oxygen from reaching the blood.

Mechanism of Toxicity

Helium may displace oxygen, leading to oxygen deficiency.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies in animals indicate that helium acts as a simple asphyxiant.

Human

Helium is nontoxic at normal temperature and pressure. The primary concern is its ability to displace oxygen in the air. Oxygen content must remain above 19% by volume in order to prevent symptoms of oxygen deficiency. At extremely low temperatures, a clinical case of quick freeze injury to both hands due to helium was reported. The exposed individual was wearing protective gloves which, upon rapid removal after exposure, reduced the depth and severity of the injury. Skin contact with liquid helium can cause frostbite.

Clinical Management

Rescue workers must wear a self-contained breathing apparatus before entering areas of oxygen deficiency.

If a victim is unconscious or does not respond, the victim should be moved to fresh air. If breathing has stopped, trained personnel should begin artificial respiration or, if the heart has stopped, cardiopulmonary resuscitation. Oxygen may be administered by a person trained in its use.

Exposure Standards and Guidelines

The Temporary Emergency Exposure Limits (TEEL) are: TEEL-0 = 60 ppb; TEEL-1 = 145 ppb; TEEL-2 = 280 ppb; TEEL-3 = 500 ppb.

The US Department of Energy classifications for TEELs are:

- TEEL-0 is the threshold concentration below which most people will experience no appreciable risk of health effects.
- TEEL-1 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing other than mild transient adverse health effects or perceiving a clearly defined, objectionable odor.
- TEEL-2 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action.
- TEEL-3 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing life-threatening health effects.

See also: Respiratory Tract.

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Hemlock, Poison

Michael Wahl

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• SYNONYMS: Conium maculatum, Umbelliferae family; Spotted hemlock; Deadly hemlock; Poison parsley; Poison stinkweed

Exposure Routes and Pathways

Ingestion of plant parts is the route of exposure. Toxicity may be experienced by those who ingest animals that have fed extensively on poison hemlock (e.g., quail, robins, skylarks, etc.).

Mechanism of Toxicity

Coniine (piperidine alkaloid), N-methyl coniine, conhydrine, λ -coniceine, and pseudconhydrine are the toxins identified. Coniine has a number of pharmacological activities resembling nicotine. It is capable of producing stimulation followed by depression of autonomic ganglia.

Acute and Short-Term Toxicity (or Exposure)

Animal

All animal species (except certain small birds) appear to display toxicity similar to that seen in humans, such as muscle tremors, salivation, dyspnea, vomiting, polyuria, central nervous system (CNS) depression, and death.

Human

Poison hemlock toxicity has effects similar to those of nicotine. The alkaloid content varies significantly between species, plant parts, and geographic location. The alkaloid concentration increases in all parts as the plant matures but remains the highest in the roots. Initial CNS stimulation, nausea, vomiting, and sore throat are followed by cardiorespiratory depression and ascending paralysis.

In Vitro Toxicity Data

Binding studies of congeners of cicutoxin have demonstrated a strong correlation between blocking of a noncompetitive GABA agonist to chloride gated GABA channels and acute toxic effects in a mouse cortex model.

Clinical Management

There is no antidote to hemlock poisoning. Death is generally due to paralysis of respiratory muscles. After assessment of airway, breathing, and circulation with necessary supportive care, decontamination of the gastrointestinal tract should be undertaken for substantial recent ingestions. Oxygen and benzodiazepines should be administered as needed for patients experiencing seizures.

See also: Coniine; Nicotine; Toxicology, History of.

Further Reading

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Hemlock, Water

Michael Wahl

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• SYNONYMS: *Cicuta maculata*, Apiaceae (carrot) family, *Cicuta* species; Cowbane; Snakeweed; Wild carrot; Poison parsnip; Spotted hemlock; Masquash root; Beaver poison; False parsley; Fever root; Wild parsnip

Exposure Routes and Pathways

Exposure occurs via ingestion of any part of the plant (especially the root). The roots of this plant are sometimes mistaken for wild carrots or wild turnips. These exposures result in large ingestions and can produce profound clinical effects and death.

Mechanism of Toxicity

The major toxicity results from the central nervous system (CNS) stimulant properties of cicutoxin.

Cicutoxin is concentrated in the roots but also may be found in aboveground parts. A mouthful of the root may be sufficient to kill an adult. Death results from status epilepticus, possibly caused by excessive stimulation of cholinergic receptors in the basal ganglia or brain stem.

Acute and Short-Term Toxicity (or Exposure)

Animal

All species of animals are at potential risk of poisoning with symptoms similar to those found in humans.

Human

All parts of the plant are considered toxic, with the root being the most toxic portion. In a typical case of water hemlock poisoning, severe nausea, vomiting, and abdominal pain begin within 5–90 min postngestion. These symptoms are rapidly followed by seizures and profound CNS depression. Excess salivation, diaphoresis, flushing, and dizziness are also commonly seen. The major toxicity is related to

Hemocompatibility

Kathleen Rodgers

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Introduction

During the use of medial devices, there are situations, such as open heart surgery or vascular grafts, in which the device comes into direct contact with the blood. Blood contacting devices include needles, cannulae, blood containers, extracorporeal circuits, and dialysis components. Each of these uses will have differing concern with regards to hemocompatibility. For example, during open heart procedures, patients' blood must be continuously processed using extracorporeal circuits fitted with pumps and suitable active components (e.g., specific filters, oxygenators). This would involve prolonged interaction with patients' blood, with the additional component of flow rate. On the other hand, a needle would reside in the blood strain only transiently, whereas cannulae would be implanted longer. The primary hemocompatibility parameter of concern with a needle would be hemolysis, the destruction of red blood cells as a result of interactions between the needle and the blood. However, with prolonged implantation with CNS stimulation. Death usually results from status epilepticus and respiratory failure.

Clinical Management

A patient exposed to water hemlock may convulse suddenly and without warning; therefore, it is important to establish intravenous access immediately. For recent ingestions activated charcoal and a cathartic should be considered. Seizures generally respond to benzodiazepines. Care should be symptomatic and supportive.

Survivors may experience long-term effects including changes in sensorium with impaired intellectual function and acute anxiety reactions.

See also: Benzodiazepines; Charcoal; Toxicology, History of.

Further Reading

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the cannulae, there may arise the additional concern of thrombogenicity or clotting.

As a result of the contact of blood with nonendothelial surfaces, several humoral and cellular systems can be activated. Exposure of blood proteins and cells to blood contacting medical devices can activate plasma proteolytic systems (coagulation (blood clotting system), fibrinolysis (process by which clot is broken down), complement cascade (a system of soluble proteins involved in microbiocidal activity and the release of inflammatory components), Kallekrein-kinin and contact systems) and at least three cellular elements (leukocytes, endothelial cells, and platelets). Contrary to the normal situations whereby these mechanisms are localized and intended to promote wound healing, activation of these systems by medical devices can result in nonlocalized systemic reactions. The preclinical and clinical assessments of hemocompatibility are designed to minimize modification of these systems.

Definitions

The general term 'hemocompatibility' refers to those properties that allow medical devices to maintain contact with flowing blood without causing adverse reactions, without releasing leachable components and without suffering adverse reactions. The properties that define hemocompatibility include inability to initiate thrombogenic phenomena, to cause any hemolysis and to activate the complement system. The standard tests, as will be outlined below, address broad groups of devices and are not device specific. The exception to this is the addition of pump flow rates to the assessment of extracorporeal devices. The hemocompatibility depends not only on the surface characteristics of the device, but also on extrinsic conditions such as site of placement, duration of contact with blood and local hemodynamic status. Blood is a non-Newtonian fluid, composed of a suspension of cells as a significant fraction of the This characteristic results in a nontotal. homogenous fluid that will be modified by blood flow and shear. Blood velocity will determine shear rate, the main parameter of the stress to which blood cells are exposed. A reduction in the response of humoral and cellular components to the introduction of the device into the blood flow is paramount to improving hemocompatibility and assuring the safety of the device.

Inflammatory Response

As a result of exposure to a blood contacting medical device, blood may initiate the production of proteolytic substances. This will result in the production of thrombin, plasmin, and proinflammatory complement cleavage products, C3a and C5a. Although the production of proteolytic substances is initiated by proteins present in plasma (humoral phase), its amplification results mainly from the contribution of cellular elements. As mentioned, the cellular reaction of blood to biomaterials is not limited to platelet activation and aggregation. Products of the activation of the humoral phase, for example fibrin degradation products and C3a/C5a, are chemotactic for polymorphonuclear cells and monocytes. This will lead to more cells to be available for interaction when surfaces are wounded by the introduction of the device. These cellular elements will bind to surfaces (both endothelial and non-endothelial) and adhesion of leukocytes will be followed by degranulation and release of factors that further contribute to reduced hemocompatibility. Endothelial cells will also respond to humoral signals to increase leukocyte adhesion (through expression of integrins and selections) and procoagulant activity. These alterations in cellular activity, if modified by the introduction of the device, will result in an inflammatory response that may have consequences beyond those tested by in vitro hemocompatibility tests. For example, the assessment of complement activation has

been included in guidelines to lessen the potential for dialysis-induced chronic lung disease.

Testing of Hemocompatibility

Testing of hemocompatibility involves the assessment of hemolysis, cell depletion, and generation of thromboemboli. The conditions under which the assessment is to be done can vary with the medical device to be assessed (e.g., extracorporeal blood devices, and vascular grafts). However, the requirement for the test to reflect the proposed use of the device is not extensive. In one example, guidance for extracorporeal blood devices indicates that hemolysis and cell depletion should be evaluated over a 6 h circulation period. In this testing, blood compatibility parameters at both the maximum and low flow rates should be assessed. To evaluate the ability of a surface to affect clotting mechanisms, this test should also include a visual inspection for thromoemboli. The ability of the materials to cause platelet activation should also be assessed. This is in addition to the standard hemocompatibility/ hemolysis testing recommended under the US Food and Drug Administration tripartite guidance on ISO 10993. In this guidance, assessment of hemocompatibility is required for many types of medical devices including external communication devices that are indirectly in contact with the blood path or directly in contact with circulating blood. Further, this testing is required for implantable devices in contact with blood. Standard practice for the performance of these tests is available from the American Society for Testing and Materials (ASTM) and ISO Standards.

In ASTM F78-98 'Standard Practice for Selecting Generic Biological Tests Methods for Materials and Devices', the selection test methods to evaluate medical devices is described. Regarding hemocompatibility tests for blood compatibility, hemolysis, and complement activation are described. Under blood compatibility, hemolysis and thrombosis are described as the most obvious examples of incompatibility with blood. It is suggested that thrombogenicity (formation of thromboemboli or platelet activation) be tested under dynamic conditions that simulate in the use procedures for the device. Complement activation is of concern in some cases and should be tested in vitro by assessing the status of various complement components. However, complement activation will probably not represent the only portion of the inflammatory response stimulated by medical devices.

Hemolysis procedures are described in ASTM F756-93 'Standard Practice for Assessment of Hemolytic Properties of Materials' and ASTM F1841-97 'Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps'. The presence of

hemolytic material in contact with blood may produce increased levels of free hemoglobin leading to anemia and stress on kidneys and other organs. In F756-93, the hemolytic properties of the material in contact with blood are assessed. In this test, anticoagulated rabbit blood is collected and exposed under static (countertop) or dynamic (rocker plate) conditions, to the medical device. After a proscribed time, the cellular component of the blood is removed and the amount of free hemoglobin determined. In F1841-97, the integrity of red blood cells in human, cow, or pig blood passed through continuous flow blood pumps is assessed. Again, the blood is exposed to standardized blood flow conditions for a proscribed time and the level of hemolysis then determined.

Within the ISO guidelines, there are guidelines that vary with blood contacting devices with specific uses. For example, blood collection sets are covered under ISO 1135-3, 'Blood Taking Set' and ISO 3826-4, 'Plastic Collapsible Containers for Human Blood and Blood Components'. Both documents list requirements for testing of biocompatibility, including cell culture cytotoxicity, short-term intramuscular implantation, hemolysis in vitro, delayed contact sensitization, intracutaneous irritation, pyrogenicity, and sterility. Within this, customary measurements for whole blood containers are total hemoglobin, hematocrit, and cell counts. The preferred hemolysis test is a static assay under the conditions of use (21 days of storage at 4-8°C with citrate phosphate dextrose or 42 days with citrate phosphate dextrose adenine solution). Common measurements on containers for red cell concentration are erythrocyte adenosine triphosphate, lactate, and glucose. Red cells may also be assessed microscopically for changes in morphology.

The need for further definition that evaluates medical devices under conditions that reflect the intended use is evident. For example, under the broad grouping of devices, termed externally communicating devices with the same testing recommendations are percutaneous circulatory support devices, extracorporeal oxygenators, and apheresis equipment. However, in clinical use, the potential of these devices to affect blood parameters varies significantly as they are exposed to blood for differing lengths of time, present different risks for air emboli at blood air interfaces and protein denaturation due to foaming. These differences should be assessed during the assessment of the hemocompatibility of blood contacting medical devices.

New Directions in Development of Hemocompatible Materials

Medical devices that can contact blood during their use utilize a broad spectrum of synthetic materials including: polyethylene, polystyrene, polyurethane, silicone, polysulfone, polyamide, polypropylene, polyvinyl chloride, polyester, and polytetrfluoroethylene, etc.

A great deal of research has been conducted into the increase of biocompatibility and hemocompatibility of various polymers, especially those used in blood purification (hemodialysis), blood circulation and implant materials. Several strategies have been attempted to increase hemocompatibility including modification of material surface properties, structure and addition of drugs to the surface of the device. Structure influences the surface area and level of trauma that the blood encounters. Compact materials with smooth surfaces are typically preferred as both the surface area and possible trauma to cellular elements are minimized. On the other hand, smooth surfaces are not practical for all blood-conducting medical devices, for example, vascular grafts. Polymer based artificial grafts are often used in situations where large caliber vascular grafts (internal diameter of 7 mm or greater). These grafts have controlled surface patterns and porosity. Polyester yarn is knitted or woven into various porous patterns. Polytetrafluorethylene tubes are expanded into porous conduits. This porosity is considered to be critical for proper healing and overall graft patency, but causes the blood to leak through the graft wall and is a serious drawback. Currently, collagen, gelatin and albumin are used as sealants. Hydrogels are being considered and will be modified to maximize sealant properties while minimizing complement- and cellactivation by varying other surface properties by degrees and types of substituents.

Surface tension, the residual binding capability of the exposed surface, can affect the hemocompatibility of a material. Blood cells and vessels are negatively charged an isoelectric point between pH 4.8 and 5. The vessel wall being negatively charged causes platelets to be repelled and helps reduce thrombogenic potential. Distribution of charged sites and surface polarity will affect plasma protein absorption to the material. Modification in one area may not solve all issues with regards to hemocompatibility. For example, hydrophilic substituents have a stimulatory effect on the complement cascade, but simultaneously have negligible effect on platelet activation. However, hydrophobic substituents show a reduced complement activation, but stimulate platelet adhesion. Often, blending or mosaics are used to balance hydrophilic/hydrophobic properties to minimize hemocompatibility.

Modification of the surface of a material with a therapeutic can also improve blood compatibility. For example, to reduce thrombogenesis, blood contacting materials might be heparinized. Numerous clinical studies have compared heparin-coated versus noncoated medical devices. The coating is thought to improve patient safety by reducing the adherence of blood components and by inhibiting blood clotting. Heparinbonded devices showed lessened humoral and cellular activation, in particular a reduced complement activation and enhanced platelet protection. Clinical trials demonstrated shortened hospital stays, less drainage bleeding and reduced cerebral complications with heparin-coated oxygenated devices. The failure of the oxygenator due to a significantly reduced pressure gradient was also observed when heparin-coated devices were utilized. This surface modification has led to a decrease in healthcare costs and an increase in patient safety due to increased hemocompatibility of this blood-coating device. As this area progresses, further benefit to healthcare will be evident.

Pharmacological inhibition of the key enzymes responsible for the consecutive activation of cascade of reactions, including aprotinin, tranexamic acid, aminocapron acid, C1-esterase inhibitor, antioxidants, and free radical scavengers are also under evaluation to improve hemocompatibility. Research into the modification of surfaces with integrin (receptors that link cellular components to extracellular matrix proteins) fragments, such as peptides with RGD amino acid sequence, has been conducted to increase hemocompatibility and biocompatibility. Further research into the improvement of device function by the local modification of the device surface is ongoing and may prove to be one of the most exciting areas of research in this field.

Conclusions

Contact of blood with medical devices can result in not only hemolysis, but activation of proteolytic, inflammatory, and thrombogenic responses. Assessment and improvement of hemocompatibility are essential to the formation of an ideal blood-contacting medical device. Standard methods are available to measure hemocompatibility at a gross level and more sensitive/thorough techniques are being developed/validated. As these are available, better materials can be developed through direction given by the results from the conduct of these more sensitive assay methods.

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Heparin

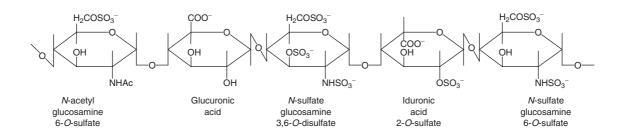
David Eldridge and Christopher P Holstege

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- SYNONYMS: Heparin sodium (CAS 9041-084); Heparin calcium (CAS 37270-89-6)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Anticoagulant
- CHEMICAL STRUCTURE:

Uses

Heparin is a heterogeneous mixture of anionic sulfated glycosaminoglycans of 5000–30 000 Da molecular weight commercially derived from bovine lung or porcine intestinal mucosa. Low molecular weight heparins (LMWHs) are specific heparin preparations prepared by chemical or enzymatic cleavage that produce a mixture of products with lower weights of 4000–6000 Da. These mixtures have distinctly different properties from 'unfractionated' heparin.



Heparin is used as an anticoagulant for prophylaxis and treatment of various thromboembolic disease processes. It is used to maintain relatively anticoagulated states in patients on extracorporeal circulation or hemodialysis and to help maintain patency of indwelling vascular catheters.

Exposure Routes and Pathways

Heparin is administered both parenterally and subcutaneously. Oral, rectal, sublingual absorption is poor due to heparin's large size. Intramuscular administration leads to irregular absorption and hematoma development at the site of injection.

Toxicokinetics

Subcutaneous administration leads to a peak heparin level 2–4 h after injection and an onset of anticoagulant effect within 1–2 h. Intravenous administration leads to an immediate peak heparin level with anticoagulant activity within 20–30 min. Heparin binds extensively to a number of plasma proteins. Its volume of distribution is 0.071kg⁻¹ in adults. The pharmacokinetics of heparin is complex and incompletely understood. Heparin metabolism occurs primarily in the reticuloendothelial system by desulfation. The LMWH agents have longer half-lives than standard heparin. Heparin's elimination halflife increases disproportionately with increasing dose, indicating saturable kinetics.

Mechanism of Toxicity

Heparin acts as a catalyst for antithrombin III (AT III), increasing its activity by approximately a thousand times. Antithrombin III is a plasma enzyme that inactivates certain activated serine proteases of the coagulation cascade, most importantly activated factors II (thrombin) and X. The larger heparin species (found in unfractionated heparin) catalyzes the inactivation of activated factors II and X. In contrast, LMWH chiefly inactivates activated factor X. The final effect of both is systemic anticoagulation. Heparin also possesses inherent platelet-aggregating properties and may also induce the production of platelet-aggregating antibodies. Heparin can inhibit aldosterone synthesis.

Acute and Short-Term Toxicity (or Exposure)

Human

Bleeding is the most common complication of heparin therapy and can occur even when dosing is

thought to be in therapeutic range. Hemorrhages can occur virtually anywhere and vary in severity from minor to life threatening.

Two forms of heparin-induced thrombocytopenia (HIT) have been observed. The first (HIT I) is a transient, mild, and benign thrombocytopenia seen soon after initiation of heparin therapy (normally within 2 days) and is felt to be due to inherent plateletaggregating properties of heparin. A second, more severe form of HIT (HIT II) is typically seen later and is immune-mediated. The incidence of HIT II is estimated at 3-5%. The onset is generally 3-14 days after initiation of heparin therapy but may occur sooner with repeat exposure. HIT II may occur with any dose and type of heparin, but the frequency is highest with continuous intravenous infusions of unfractionated heparin. HIT with subsequent thrombosis is a feared complication. These thrombi can form in the venous or arterial circulation. Thrombotic complications include necrotic skin lesions, myocardial infarction, stroke, and gangrene. Hyperkalemia may be seen with heparin therapy due to aldosterone synthesis inhibition.

Chronic Toxicity (or Exposure)

Animal

Heparin is used in veterinary medicine for the management of thromboembolic problems postvascular surgery. It is uncommon for animals to be maintained on heparin for these long term purposes. Heparin is used in a scientific setting in animal dialysis models as well as in models of graft rejection.

Human

Osteoporosis with subsequent rib and vertebral fractures has been reported with long-term use. The mechanism for these abnormalities is not completely understood. Heparin does not cross the placenta and generally is thought to be safe to use in pregnancy.

Clinical Management

The anticoagulant effect of heparin is best monitored by the activated partial thromboplastin time. If an excessive dose of heparin has been administered, careful monitoring for signs of bleeding and hemodynamic instability is indicated. If there is no clinical evidence of bleeding, discontinuation of heparin is normally sufficient. With potentially life-threatening hemorrhage, use of protamine sulfate, a specific, rapidly acting heparin antidote, should be considered. Protamine is given intravenously at a dose of $\sim 1 \text{ mg}$ per 100 units heparin. Considerable variation can exist between patients, and dosing should be individualized and monitored. Potential complications of protamine include hypotension, anaphylaxis, and pulmonary vasoconstriction.

Platelet counts should be carefully monitored for any decline. If thrombocytopenia develops, the time course and severity should help differentiate which type of HIT exists. If HIT I is suspected, heparin may be continued with caution. If HIT II is suspected, heparin therapy should be discontinued and an alternate form of anticoagulation therapy begun. If a low platelet count is encountered with a thrombotic complication, heparin should be discontinued immediately. Thrombolytic therapy or embolectomy may be necessary. Lepirudin (recombinant hirudin) is approved by the US Food and Drug Authority for use as an anticoagulant for HIT thromboembolism.

See also: Poisoning Emergencies in Humans; Warfarin.

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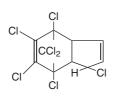
Hepatotoxicology See Liver.

Heptachlor

Benny L Blaylock

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 76-44-8
- SYNONYMS: 1,4,5,6,7,8,8-Heptachloro-3a,4,7,7atetrahydro-4,7-methanoindane; Biarbinex; Cupincida; Drinox; E 3314; Fennotox; Heptagran; Heptamul; Heptox; Termide; Velsicol 104
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organochlorine cyclodiene insecticide
- CHEMICAL FORMULA: C₁₀H₅Cl₇
- CHEMICAL STRUCTURE:



Uses

Heptachlor is used as an insecticide. Its use is banned for most applications.

Exposure Routes and Pathways

The main route of exposure is via ingestion. However, inhalation and dermal contact are also potential exposure routes.

Toxicokinetics

Heptachlor is readily absorbed from the gastrointestinal tract, respiratory tract, and skin. As is the case with other organochlorine insecticides, the rate of absorption from the gastrointestinal tract is affected by fiber and fat content in the diet, with a lack of these favoring increased absorption.

The primary metabolism of heptachlor is in the liver where microsomal enzymes convert the parent compound to the more toxic heptachlor epoxide as well as to the less toxic metabolites 1-chloro-3-hydroxychlordene, 1-hydroxychlordene, and 1-hydroxy-2,3-epoxychlordene. These latter metabolites are also more easily excreted. Both heptachlor and heptachlor epoxide are stored in adipose tissue and in the liver, kidney, and muscle tissues. The epoxide is the primary storage form. Heptachlor is able to cross the placenta and has been found in human milk. Metabolites of heptachlor are excreted in urine and feces.

Mechanism of Toxicity

As with other cyclodiene insecticides, heptachlor blocks the neuronal uptake of chloride ions by blocking the activity of γ -aminobutyric acid. This results in only a partial repolarization of activated neurons leading to an uncontrolled excited condition. Additionally, chlordane inhibits Ca²⁺,Mg²⁺-adenosine triphosphatase (ATPase) and Na⁺,K⁺-ATPase functions, leading to increased concentrations of intracellular free calcium in neurons and the release of neurotransmitters. This neurotransmitter release potentiates depolarization of adjacent neurons in a chain reaction manner, propagating stimuli through the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD₅₀ values for rats are reported at 100–220 mg kg⁻¹ while those for mice are 30–68 mg kg⁻¹. The toxic effects of heptachlor in animals are the same as those of chlordane. Chlordane toxicity in animals is similar to that of other organochlorine insecticides except tremor is absent. CNS involvement produces hyperexcitability and convulsions.

Human

The toxic effects of heptachlor are the same as those for chlordane. A case report of oral exposure to technical-grade chlordane reported neurological effects including irritability, salivation, dizziness, muscle tremors, and convulsions. However, exposure measurements were not provided in the report, and technical-grade chlordane contains varying amounts of heptachlor. The effects cannot be said to have resulted from exposure to heptachlor only.

Chronic Toxicity (or Exposure)

Animal

Daily oral administrations of 2 or 5 mg kg^{-1} body weight heptachlor for 78–86 days to pigs, sheep, and rats induced hepatic necrosis. Results of animal tests show that chronic exposure to heptachlor or its epoxide metabolite adversely affects the liver, kidney, and red blood cells. There is evidence that heptachlor and heptachlor epoxide are associated with infertility and improper development of offspring. Animal studies have shown that females were less likely to become pregnant when both males and females were fed heptachlor. The incidence of liver carcinomas increased in rats receiving doses of approximately $1.2 \text{ mg kg}^{-1} \text{ day}^{-1}$ of either heptachlor or heptachlor epoxide.

Human

Due to the inconclusive nature of the data, the potential for reproductive effects in humans due to heptachlor is not possible to predict. Also, based on animal data, there is no suggestion that heptachlor is teratogenic in humans. International Agency for Research on Cancer classifies heptachlor as 2B (possibly carcinogenic for humans). There is inadequate evidence in humans for the carcinogenicity of heptachlor but sufficient evidence in experimental animals for heptachlor carcinogenicity.

Clinical Management

Treatment is symptomatic. Anticonvulsive treatment with diazepam or phenobarbital is usually effective for control of convulsions. Cholestyramine treatment has been shown to increase elimination of heptachlor. Activated charcoal administered as a slurry is recommended. Gastric lavage may be useful if performed quickly after ingestion (within 1 h). Emesis is not recommended due to potential CNS depression or seizures.

Environmental Fate

As with most organochlorine insecticides, heptachlor and its epoxide are highly persistent in soils, with a reported representative field half-life of 250 days. Heptachlor and its epoxide are moderately bound to soils. This should significantly limit their mobility. Due to their persistence, even low mobility may result in appreciable movement. Therefore, heptachlor and heptachlor epoxide may pose a risk of groundwater contamination over time. Heptachlor epoxide exhibits a low susceptibility to biodegradation, photolysis, oxidation, or hydrolysis in the environment.

Due to its insolubility in water, heptachlor enters surface water primarily through run-off and drift. In water, microorganisms readily metabolize heptachlor to the epoxide. The epoxide then undergoes volatilization, adsorption to sediments, and photodegradation. These may be significant routes for disappearance of heptachlor from aquatic environments.

Ecotoxicology

Both heptachlor and the epoxide are very highly toxic to most fish species tested. The reported 96 h LC_{50} values are: $5.3-13 \,\mu g \, l^{-1}$ in bluegill sunfish; $7.4-20 \,\mu g \, l^{-1}$ in rainbow trout; $6.2 \,\mu g \, l^{-1}$ in northern pike; $23 \,\mu g \, l^{-1}$ in fathead minnow; and $10 \,\mu g \, l^{-1}$ in largemouth bass. Heptachlor is also very highly toxic to freshwater aquatic invertebrates including snails, worms, and crayfish. The toxicity of heptachlor varies significantly from species to species in marine aquatic organisms. Both heptachlor and heptachlor epoxide have been shown to bioconcentrate in fish, mollusks, insects, plankton, and algae.

Heptachlor has been shown to be moderately to highly toxic to several bird species including quail, pheasant, and mallard ducks. Studies have shown that heptachlor decreases the survivability of chicken eggs. Both the parent compound and the epoxide have also been found in the liver, brain, muscle, and eggs of birds.

Other Hazards

Heptachlor is not combustible, but may be dissolved in flammable liquids. Hydrogen chloride fumes gas may form in fire. Heptachlor can react with iron and rust to form hydrogen chloride gas.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.0001 \text{ mg kg}^{-1} \text{ day}^{-1}$.
- Maximum contaminant level is $0.0004 \text{ mg} \text{ l}^{-1}$.

- Reference dose is 0.005 mg kg⁻¹ day⁻¹.
 Permissible exposure limit is 0.5 mg m⁻³ (8 h).

See also: Carcinogen Classification Schemes; Charcoal; Chlordane; Diazepam; LD₅₀/LC₅₀ (Lethal Dosage 50/ Lethal Concentration 50); Organochlorine Insecticides.

Relevant Websites

http://www.atsdr.cdc.gov - Agency for Toxic Substances and Disease Registry. Toxicological Profile for Heptachlor.

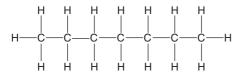
- http://extoxnet.orst.edu Extension Toxicology Network, a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho.
- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Heptachlor.
- http://www.osha-slc.gov US Department of Labor, Occupational Safety and Health Administration.

Heptane

Stephen R Clough

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 142-82-5
- SYNONYMS: *n*-Heptane; Dipropyl methane; Gettysolve-C; Heptyl hydride; Heptan (Polish); Eptani (Italian); Heptanen (Dutch); UN1206 (DOT)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aliphatic hydrocarbon
- CHEMICAL FORMULA: C₇H₁₆
- CHEMICAL STRUCTURE:



Uses

Heptane is used as the knock-testing 'standard' for octane rating measurements. An isomer, triptane (2,2,3trimethyl butane), is used in aviation fuel. All isomers are used in organic syntheses and are ingredients of gasoline, rubber solvent naptha, and other petroleum mixtures that are utilized as fuels or solvents.

Exposure Routes and Pathways

Although heptane exists as a liquid at room temperature, most adverse effects observed in people or animals exposed to this solvent occur via inhalation.

The most common occupational exposure routes, in order of decreasing importance, are inhalation, dermal contact, and ingestion.

Toxicokinetics

Heptane is converted to hydroxy derivatives (e.g., alcohol) by the cytochrome P450 mixed function oxidase system before being converted to keto forms. It may then be conjugated to the glucuronide and subsequently excreted.

Mechanism of Toxicity

Most of the current toxicological information suggests that heptane is, physiologically speaking, more neurotoxic than other aliphatic hydrocarbons such as pentane, hexane, and octane. However, debilitating peripheral neuropathy, such as that seen on chronic exposure to *n*-hexane, has not been observed in animals or humans. Some cases of polyneuritis, observed in the absence of hexane exposure, might be attributed to the presence of heptane in a solvent mixture. No one to date has discerned a true toxic mechanism for heptane.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats exposed to heptane showed neurologic signs that are very similar to those seen following exposure to technical-grade hexane. Righting reflexes in mice are affected at a concentration of 40 mgl^{-1} (~0.96%) while 70 mgl^{-1} (~1.7%) is lethal (isoheptane has the same effect at 50 mgl^{-1}). Narcosis has also been shown in mice exposed to air concentrations of 1–1.5% for 3–50 min. Other permutations of concentration and exposure period caused convulsions, tetany, respiratory arrest, and death.

Human

Heptane is toxic to the human nervous system (neurotoxic). Acute exposure symptoms include distorted perception and mild hallucinations. Humans exposed to 0.1% (1000 ppm) heptane exhibited dizziness in 6 min; higher concentrations caused marked vertigo and incoordination. Humans accidentally exposed to high concentrations showed similar symptoms, as well as mucous membrane irritation, nausea, and lassitude. All these symptoms pass quickly upon recovery in fresh air, but the recovery period is longer than that for pentane or hexane. A gasoline aftertaste has been experienced by people who have been experimentally exposed to heptane.

Chronic Toxicity (or Exposure)

Human

Several authors have noted signs of polyneuropathy or polyneuritis in groups of people exposed to mixtures of solvents that contain significant quantities of heptane.

Clinical Management

People who are exposed to high concentrations should vacate or be removed from the source of the vapor and seek fresh air.

Ecotoxicology

The lowest 24 h LC₅₀ reported for hexane was 10 mgl^{-1} using the water flea (*Daphnia magna*) as the test species (US Environmental Protection Agency ECOTOX database). An unpublished 48 h EC₅₀ of 1.5 mgl^{-1} was also observed (immobilization) in the same invertebrate species. A 24 h LC₅₀ of 4 mgl⁻¹

has been reported for goldfish, and a 96 h LC_{50} of $100 \text{ mg} \text{l}^{-1}$ was reported for Coho salmon.

Other Hazards

Heptane is very flammable and is therefore an explosion and/or fire hazard (lower and upper explosive limits are 1.05% and 6.7%, respectively, by volume). Care should be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity, and explosion-proof equipment should be used.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit values for heptane are 400 ppm as a time-weighted average (TWA) and 500 ppm as a short-term exposure limit. The National Institute for Occupational Safety and Health recommends a workplace environmental limit (TWA) of 85 ppm for heptane, and a 15 min ceiling limit of 440 ppm. On a weight/volume scale, this is the same limit imposed for pentane, hexane, and octane and is most likely designated to prevent polyneuropathy found following heptane exposure.

Miscellaneous

Heptane is a colorless, flammable liquid that is lighter than, but insoluble in, water. It has a definite petroleum odor that is easily detected at air concentrations of 200 ppm or greater (in air, $1 \text{ ppm} = 4.10 \text{ mg m}^{-3}$). Naturally occurring heptane is isolated from natural gas, crude oil, or pine extracts.

See also: Neurotoxicity.

Further Reading

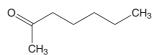
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- Verschueren K (1996) Handbook of Environmental Data on Organic Chemicals. 3rd edn. New York: Van Nostrand Reinhold.

Heptanone

Murali Badanthadka and Harihara M Mehendale

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- CHEMICAL NAME: 2-Heptanone
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 110-43-0
- SYNONYMS: Methyl *n*-amyl ketone; *n*-Pentyl methyl ketone; Methyl pentyl ketone
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Ketone
- CHEMICAL FORMULA: C₇H₁₄O
- CHEMICAL STRUCTURE:



Uses

2-Heptanone is used as an industrial solvent; as a solvent for synthetic resin finishes; as an inert reaction medium; as a flavor ingredient in foods; and as a fragrance ingredient in creams, lotions, perfumes, soaps, and detergents.

Background Information

2-Heptanone is a naturally occurring compound present in foods and essential oils. It is also used commercially as a solvent in a wide number of industrial applications. 2-Heptanone may be released to the environment as a fugitive emission during its production, formulation, use, or transport, and in the effluent of industrial processes.

Exposure Routes and Pathways

Occupational exposure to 2-heptanone may occur by inhalation or dermal contact during its production, formulation, or transport. Exposure to the general population may occur by ingestion of food in which it occurs naturally, by inhalation during the use of commercial products in which it is used as a solvent or by the ingestion of contaminated drinking water. The principal route of occupational exposure is by inhalation. Skin and eye contact may also occur.

Toxicokinetics

2-Heptanone is absorbed into the bloodstream after ingestion, inhalation, or dermal exposure. Results of tissue distribution studies of ¹⁴C-methyl *n*-amyl ketone in rats comparing intraperitoneal and inhalation

routes of exposure were similar. Liver tissue had the highest level of radioactivity regardless of the route of administration. However, no liver pathology was evident. Urinary excretion accounted for 25% of the administered dose after 12 h.

When 2-heptanone (950 mg kg^{-1}) was administered orally to rabbits, ~40% of administered dose was excreted as heptyl-2-glucuronide, and traces of the unchanged ketone were also found in the urine. Compound undergoes carbonyl reduction to a secondary alcohol and ω -1 oxidation to a hydroxy-ketone which is further oxidized to 2,6-heptadione.

A subchronic inhalation study was conducted in which male rats and monkeys were exposed to 0, 131, or 1025 ppm of 2-heptanone for 10 months $(6 \text{ h day}^{-1}, 5 \text{ days week}^{-1})$. Both parent compound and its metabolite methyl *n*-amyl alcohol were detected in the urine and serum of monkeys.

Mechanism of Toxicity

2-Heptanone is known to potentiate the nephrotoxic and hepatotoxic effects of halogenated hydrocarbons.

Acute and Short-Term Toxicity (or Exposure)

Animal

Guinea pigs exposed to 2-heptanone at 2000 ppm for 890 min caused light to moderate congestion of lungs leading to death. Exposure at 1500 ppm caused irritation to mucous membranes. At 4800 ppm concentration, central nervous system (CNS) depression occurred, followed by death in 4–8 h.

2-Heptanone in the undiluted form, ranging in quantities from 5 to 20 ml kg^{-1} , caused slight to moderate skin irritation in guinea pigs after 24 h exposure. No evidence of percutaneous absorption and death.

Male rats and monkeys exposed to subchronic inhalation of 0, 131, or 1025 ppm 2-heptanone for 10 months (6 h day⁻¹, 5 days week⁻¹) did not show any significant alterations in pulmonary function, electrocardiogram, or biochemical parameters.

Oral administration LD_{50} value is 1670 and 730 mg kg⁻¹ for rat and mice, respectively.

Human

2-Heptanone may cause mild skin irritation after a single exposure. At concentration of 4% in petrolatum it did not produce any positive reactions. Inhalation at higher concentration causes CNS depression. Vapor/liquid contact will irritate eyes, nose, throat, and skin. Ketones may potentiate the hepatotoxicity of halogenated hydrocarbons and inhibit aromatic hydrocarbon metabolism.

Chronic Toxicity (or Exposure)

Animal

Oral administration $(20 \text{ mg kg}^{-1} \text{ day}^{-1})$ for 13 weeks showed ketone bodies in urine of only male rats. Administration at $500 \text{ mg kg}^{-1} \text{ day}^{-1}$ caused increase of liver weight in both genders and kidney weight only in males, indicating a gender difference in 2-heptanone toxicity.

In Vitro Toxicity Data

A study reported that 2-heptanone binds to DNA spontaneously *in vitro*, to the extent of 400 pmol mg^{-1} DNA.

Clinical Management

Exposed individuals should be removed immediately to fresh air after inhalation. Copious dilution is appropriate after ingestion, dermal exposures, or eye exposures. Patients should be treated symptomatically.

Environmental Fate

Terrestrial fate: If released to soil, calculated soil adsorption coefficients ranging from 44 to 285 indicates that 2-heptanone may display moderate to high mobility and it has the potential to leach into groundwater. 2-Heptanone has the potential to biodegrade in soil. The vapor pressure of 2-heptanone is 3.86 mmHg at 25°C.

Aquatic fate: If released to water, 2-heptanone is expected to rapidly volatalize to the atmosphere. The half-life for volatilization from a model river 1 m deep, flowing at 1 m s^{-1} with a wind speed of 3 m s^{-1} is 8.4 h. The calculated bioconcentration factors ranging from 5.5 to 19 indicate that 2-heptanone is not expected to bioconcentrate in fish and aquatic organisms. The calculated soil adsorption coefficients ranging from 44 to 285 indicate that adsorption to sediment and suspended organic matter is not an environmentally important process. Screening studies indicate that 2-heptanone is likely to biodegrade in aquatic systems under aerobic conditions.

Atmospheric fate: If released to the atmosphere, 2-heptanone is expected to undergo a gas-phase reaction with photochemically produced hydroxyl radicals; the estimated half-life for this process is 1.9 days. 2-Heptanone has relatively high water solubility (4300 mg l⁻¹ at 25°C), which indicates that it may undergo atmospheric removal by wet deposition processes. Although 2-heptanone has the potential of being removed from the atmosphere by direct photochemical degradation, the rate of this process is not expected to be able to compete with atmospheric removal by the reaction with hydroxyl radicals.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 100 ppm (465 mg m⁻³) for 8 h time-weighted average (TWA). The threshold limit value is 50 ppm for 8 h TWA. The National Institute for Occupational Safety and Health recommended exposure limit is 100 ppm (465 mg m⁻³) for 10 h TWA.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Heptanone.

hERG (Human Ether-a-Go-Go Related Gene)

Jill Steidl

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Introduction

Cardiac muscle contraction is an electrical event initiated at the sinoatrial node. Each cardiac muscle cell fires an action potential as a result of excitation propagated from the sinoatrial node, which produces muscle cell contraction. A wave of action potentials spreads across the organ to produce coordinated contraction of the heart and efficient ejection of blood to the body. Excitation and the subsequent return of a cardiac muscle cell to rest (repolarization) during the action potential is dictated by the flow of ions across the cell membrane. Membrane repolarization is produced by the flow of potassium ions through various types of potassium channels.

bERG (human ether-a-go-go related gene; KCNH2) encodes for the ion channel that underlies the rapidly activating delayed rectifier potassium current, I_{Kr} . The *bERG* current (I_{Kr}) is critical for ventricular repolarization in humans. Inhibition of *bERG* currents can induce QT prolongation, which is associated with induction of the potentially fatal ventricular arrhythmia Torsade de Pointes. A wide range of pharmaceutical agents from a variety of chemical classes have been found to inhibit *bERG* currents and produce QT prolongation and/or Torsade de Pointes, resulting in labeling revisions or withdrawal from the market.

Expression of hERG Channels

bERG is primarily expressed in human heart, and to a minor extent in hippocampus. In human heart, *bERG* expression levels are highest in the ventricle. *bERG* cardiac expression varies with species, for example, ERG protein levels are higher in rat atria than in the ventricle.

Structure of hERG Channels

Ion channels are proteins that span the plasma membrane to allow passage of charged ions into and out of the cell. Four hERG subunits coassemble to form an ion channel selective for potassium. Each subunit has six membrane spanning regions (S1–S6) and an intracellular amino and carboxy terminus (Figure 1a). An additional hydrophobic region between S5 and S6 dips into the plane of the membrane to contribute to the formation of a central ion channel pore. The S4 α -helix of each subunit is characterized by the presence of positively charged amino acids (arginine or lysine) at every third or fourth position, which are thought to act as voltage sensors and modulate ion channel state.

Function of hERG Channels

hERG channels are modulated by membrane potential, and can exist in the closed (C), open (O), or inactivated (I) state (Figure 1b). hERG channels conduct potassium ions when they are in the open state, but not in the closed or inactivated states. When the membrane potential is hyperpolarized (the interior of the cell is negative in comparison to the outside of the cell), *hERG* channels primarily exist in the closed state. Upon depolarization of the membrane potential (less negative inside the cell), hERG channels transition to the open state and then undergo inactivation. Activation $(C \rightarrow O)$ and deactivation $(O \rightarrow C)$ are much slower than inactivation $(O \rightarrow I)$ and recovery from inactivation $(I \rightarrow O)$. These unique kinetics facilitate late phase cardiac action potential repolarization by *hERG* currents, and suppress premature cardiac beats.

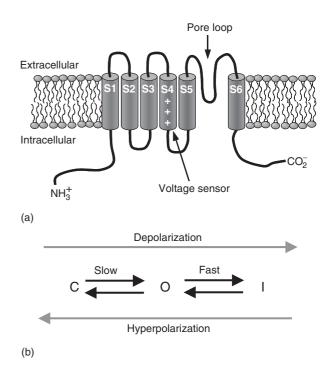


Figure 1 (a) The transmembrane topology of a *hERG* potassium channel subunit is depicted. (b) *hERG* potassium channel state is dependent on membrane potential. Depolarization favors the open (O) and inactivated (I) states, while hyperpolarization induces channel closing (C).

Role of *hERG* Ion Currents in the Cardiac Action Potential

The morphology of an action potential is dictated by the flow of ions across the cell membrane (Figure 2a). An inward flow of sodium and calcium ions has a depolarizing influence on the membrane potential, while an outward flow of potassium has a repolarizing effect. The upstroke of the cardiac action potential (phase 0) is due to an inward flux of sodium ions and the plateau phase (phase 2) is maintained

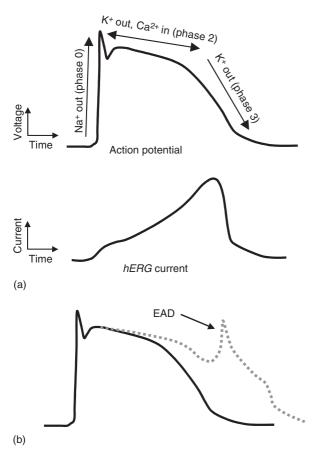


Figure 2 (a) A schematic depiction of a ventricular action potential (top) and relative profile of *hERG* current during such an action potential (bottom). The amplitude of the *hERG* current is small during the early phases of the action potential because channels open followed by rapid transition into a nonconducting inactivated state. In phase 3, *hERG* channels rapidly shift from the inactivated to the open state to facilitate repolarization of the action potential. (b) Inhibition of the *hERG* current can result in prolongation of the action potential duration and may initiate early after depolarizations (EADs).

by inward calcium currents and outward potassium currents. During the initial phases of the action potential (0 through 2), *bERG* channels open slowly followed by rapid inactivation, resulting in an accumulation of nonconducting inactivated channels by the end of phase 2. As calcium channels inactivate and the membrane potential begins to repolarize in early phase 3, inactivated *bERG* channels rapidly transition from the inactivated to the open state, creating a large outward potassium current that facilitates action potential repolarization. Slow transition of *bERG* channels from the open to the closed state suppresses the propagation of premature beats that may be encountered by the myocyte. Inhibition of *hERG* currents by pharmaceutical agents can delay repolarization of the cardiac action potential, which appears as a prolongation of the QT interval on an electrocardiogram. Delayed repolarization may result in the formation of calcium dependent early after depolarizations (EADs; Figure 2b), waveforms believed to trigger initiation of Torsade de Pointes.

Assessment of hERG Risk

Identification of risk for *hERG* inhibition is an important factor in the development of drugs. Potential for *hERG* inhibition can be assessed using a number of *in vitro* techniques, but the gold standard is whole cell patch clamp. Suitable cell types include isolated animal or human cardiomyocytes, cultured cardiac cell lines, or a heterologous expression system in which *hERG* is expressed in a noncardiac cell line. Other high throughput techniques such as radioligand binding, rubidium flux or fluorescence may be used for early assessment of *hERG* activity; however, data produced by these techniques is typically not accurate enough for establishing safety margins.

See also: Cardiovascular System.

Further Reading

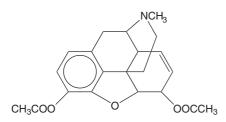
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Heroin

Michael Hiotis

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- REPRESENTATIVE CHEMICAL: Morphine
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 561-27-3
- SYNONYMS: Acetomorphine; Diacetylmorphine hydrochloride; Diamorphine hydrochloride; Heroin hydrochloride
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Opioid analgesic
- CHEMICAL FORMULA: C₂₁H₂₃NO₅
- CHEMICAL STRUCTURE:



Uses

Heroin is a semisynthetic narcotic that was first synthesized in 1874. It has been used as an analgesic for moderate to severe pain. In the United States, it is a schedule I substance and, therefore, does not have a medicinal use. It is a drug of abuse.

Exposure Routes and Pathways

Administration can be parenteral, sublinqual, oral, rectal, or by nasal insufflation. As a common drug of abuse, heroin is usually present in the street product at concentrations of 2–7%; purer forms of up to 90% are occasionally available.

Toxicokinetics

Heroin is rapidly absorbed from all sites of administration. It has high lipid membrane solubility, thus leading to rapid absorption from the blood and the blood-brain barrier. Heroin undergoes complete presystemic metabolism to morphine following oral administration. Peak morphine serum levels have occurred within 30 min after ingestion. With parenteral administration, peak levels have occurred in 10– 15 min. Heroin is rapidly hydrolyzed in whole blood to 6-monoacetylmorphine (6-MAM). The liver then converts most of the 6-MAM to morphine. These two metabolites are the primary contributors to pain relief. It is widely distributed in tissues. Protein binding is 40%. The volume of distribution approximates 251 kg^{-1} . The half-life of heroin in blood is less than 20 min, ~3 min after parenteral administration. The elimination half-life is 60–90 min. Urine yields primarily morphine in the free or conjugated form.

Mechanism of Toxicity

Heroin's primary toxic principle is its profound ability to depress the central nervous system (CNS). Opioid analgesics bind with stereospecific receptors at many sites within the CNS. Heroin, similar to other opioids, exerts its pharmacologic effect by acting at mu, kappa, and delta receptors in the brain. Although the precise sites and mechanisms of action have not been fully determined, alterations in the release of various neurotransmitters from afferent nerves sensitive to painful stimuli may be partially responsible for the analgesic effect. Activities associated with the stimulation of opiate receptors are analgesia, euphoria, respiratory depression, miosis, and reduced gastrointestinal motility.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs respond similarly to humans exposed to heroin. Symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at 0.02 mg kg^{-1} if needed.

Human

Any amount of heroin can be potentially toxic, especially when the purity of this illicit drug is not known. Heroin depresses the CNS, thereby producing coma and respiratory depression. Pulmonary edema has been described following heroin overdose. Respiratory arrest may occur. Miosis is often present but may be absent in the presence of hypoxia or mixed drug over doses. With depression of the CNS, there is also a decrease in sympathetic tone and an increase in parasympathetic tone. This yields bradycardia and hypotension. Hypothermia may also occur as a result of peripheral vasodilation. Urine can be screened for heroin metabolic products. Blood heroin levels are not clinically useful.

Chronic Toxicity (or Exposure)

Animal

Rats administered heroin chronically demonstrated decreased germinal epithelial thickening. It has also been reported that chronic heroin dosing of rodents disrupts estrous cycles.

Human

Chronic users of heroin may develop tolerance to some of its effects, thereby necessitating larger doses to develop the characteristic 'high'. Cessation of use can result in withdrawal. Classic symptoms are restlessness, insomnia, agitation, hypertension, tachypnea, tachycardia, piloerection, vomiting, and diarrhea.

In Vitro Toxicity Data

Recent studies in rat brains have investigated the role of chronic heroin and other narcotics on apoptosis. Chronic heroin and morphine treatment (as well as heroin/morphine withdrawal) resulted in changes in upregulation of Fas receptors as well as increased levels of dynamin.

Clinical Management

Basic life-support measures should be instituted as necessary. Intensive support therapy may be required to correct respiratory failure and shock. Patients with mild to moderate toxicity may present with lethargy, miosis, decreased blood pressure, heart rate, temperature, and skeletal muscle tone. In patients experiencing severe toxicity, coma, respiratory depression, noncardiogenic pulmonary edema, apnea, and sudden death may occur. If taken orally, administration of activated charcoal is recommended to minimize absorption of heroin. Emesis is contraindicated due to potential significant CNS and respiratory depression. Heroin is often smuggled via 'body packing', whereby an individual swallows receptacles (often condoms) containing heroin to evade customs officials. Most, but not all package types can be visualized on X-ray and flat plate X-ray should be performed to establish diagnosis and location. Whole bowel irrigation (WBI) may be a useful way to facilitate their removal from the gastrointestinal tract. WBI should be continued until rectal effluent is clear and no packets are detected on a contrast study of the bowel. The specific antagonist naloxone hydrochloride is used to counteract respiratory depression and coma. A dose of 0.4-2.0 mg is given intravenously and can be repeated at intervals of 2 or 3 min if necessary. The therapeutic effect of naloxone may be of shorter duration than that of the opiate activity; therefore, a naloxone continuous infusion may be of benefit. Arterial blood gases, vital signs, and level of consciousness should be monitored continuously until cessation of symptoms. Adulteration of street drugs can lead to other toxic effects and should be considered in the overall management.

See also: Drugs of Abuse; Morphine.

Further Reading

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Hexachlorobenzene

Elmar Udarbe Zamora

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- CHEMICAL ABSTRACT SERVICE REGISTRY NUMBER: CAS 118-74-1
- SYNONYMS: HCB; Perchlorobenzene; Pentachlorophenyl chloride; Benzene, hexachloro-; Esaclorobenzene (Italian); Hexachlorobenzol (German); Julin's carbon chloride; Phenyl perchloryl
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organochlorine
- Chemical Formula: C_6Cl_6

Uses

Hexachlorobenzene is a white crystalline solid that is poorly soluble in water. It does not occur naturally in the environment. It was widely used until 1965 to protect seeds of sorghum, onions, wheat, and other grains. It was also used to make fireworks, ammunition, and synthetic rubber. Currently, hexachlorobenzene is not used commercially in the United States. However, hexachlorobenzene is still produced as a by-product in the manufacture of chlorinated compounds and other chemicals, and in the waste streams of chloralkali and wood preserving plants. Burning of municipal waste also produces hexachlorobenzene.

Background Information

Hexachlorobenzene has been found in at least 84 of the 1430 National Priorities List sites identified by the US Environmental Protection Agency (EPA). The long-range atmospheric transport of hexachlorobenzene to the Arctic and other areas is a well-recognized phenomenon. The substance has been detected in Arctic air, snow, seawater, and flora and fauna. It had also been observed in other remote areas such as the North Pacific Ocean and in the rainfall of two remote islands on Lake Superior. Approximately 4000 persons were poisoned by hexachlorobenzene in Turkey from 1955 to 1959.

Exposure Routes and Pathways

Exposure to hexachlorobenzene occurs primarily from eating low levels in contaminated food, water, fish, and vegetables. Unborn children could also be exposed *in utero* and nursing babies may be exposed from mothers. Direct contact with contaminated soil is also another possible route of exposure. Workers involved in the manufacture or application of hexachlorobenzene also run the risk of exposure through inhalation.

Toxicokinetics

Hexachlorobenzene is slightly absorbed in the gastrointestinal tract but readily distributed in the body, preferentially to fatty tissues. It also readily passes the placenta. Hexachlorobenzene is concentrated in milk. It is metabolized by microsomal enzymes in the liver, kidney, lung, and intestine. Hexachlorobenzene is metabolized slowly by the liver to pentachlorophenol, pentachlorobenzene, tetrachlorobenzene, and some unidentified compounds. In humans, hexachlorobenzene is mainly excreted in the urine as its metabolites, pentachlorophenol and pentachlorobenzene is excreted, mostly unchanged, in the feces.

Mechanism of Toxicity

Hexachlorobenzene affects porphyrin synthesis and consequently the proteins involved in the metabolism and transport of oxygen. Its main target organ is the liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hexachlorobenzene has little acute toxicity. Oral LD_{50} values range from 1.7 to $4 g k g^{-1}$ in various species. The primary effect from eating highly contaminated food is hepatotoxicity. Animals exposed to hexachlorobenzene also exhibited acute neurologic toxicity with signs including tremors, paralysis, incoordination, weakness, and convulsions. The ovarian primordial germ cells of nonhuman primates were affected with associated systemic toxicity when exposed to hexachlorobenzene.

Human

Hexachlorobenzene is a skin irritant. In contrast to rodents, humans do not exhibit neurological signs with acute exposure to hexachlorobenzene.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure to hexachlorobenzene caused damage to the liver, thyroid, nervous system, bones, kidneys, blood, and immune and endocrine systems in animals.

Human

The people in Turkey who ate bread contaminated with hexachlorobenzene suffered from a liver disease called porphyria cutanea tarda. This disease can cause red-colored urine, skin sores, changes in skin color, arthritis, and problems of the liver, nervous system, and stomach.

There is no strong evidence that it causes cancer. Babies born from mothers exposed to hexachlorobenzene during pregnancy showed acute illnesses and rashes. Babies nursing from exposed mothers showed porphyria cutanea tarda, poor growth, arthritis, and enlarged thyroids.

In Vitro Toxicity Data

The metabolites of hexachlorobenzene, penta chlorophenol (PCP) and tetrachlorohydroquinone (TCHQ), appear to be capable of altering porphyrin metabolism in *in vitro* systems containing D-ALA

(aminolevulinic acid). In another study, PCP displaced the thyroid hormone, thyroxine (T4), from its receptor by direct competition. This suggests the mechanism involved in hexachlorobenzene induced hypothyroidism in rats. In rat ovary perfused with hexachlorobenzene, increased oxygen consumption suggests a disorder in the respiratory metabolism of ovarian cells after hexachlorobenzene exposure. Hexachlorobenzene was negative in the Ames mutagenicity assay.

Clinical Management

The airway, breathing, and circulation should be monitored and vital functions restored if necessary. All contaminated clothes should be removed. The chemical should be washed out of the eyes with clear water for at least 15 min, and off the skin with soap and water. If hexachlorobenzene has been ingested, milk, fat, oil, or lipid should not be given by mouth. If a very large amount of hexachlorobenzene has been ingested, gastric lavage should be performed. Activated charcoal can be administered. Convulsions should be controlled and treated like any other symptoms.

Environmental Fate

Hexachlorobenzene is degraded slowly and can therefore persist in the environment for long periods of time. It strongly adheres to soil and poorly dissolves in water, so residues can remain in sediments of lakes and rivers.

Ecotoxicology

There is high potential that it can bioaccumulate in bodies of fish, marine mammals, birds, lichens, and other animals and can enter the food chain. It can also accumulate in wheat, grasses, some vegetables, and other plants.

Other Hazards

Hexachlorobenzene could burn when exposed to extreme heat and toxic fumes may be produced. It should not be stored near sources of ignition. In cases of spills, hexachlorobenzene should be taken up with sand or other noncombustible material and then disposed off properly. For large-scale spills, it should be covered with sand/soil taking care to avoid dust formation. Protective clothing, gloves, and eyewear must be worn in handling spill situations.

Exposure Standards and Guidelines

The US EPA has set a maximum contaminant level of 1 ppb.

See also: Organochlorine Insecticides.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexachlorobenzene.

Hexachlorobutadiene

David Janz

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 87-68-3
- SYNONYMS: Hexachloro-1,3-butadiene; Hexachlorobuta-1,3-diene (HCBD); Perchlorobutadiene
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Chlorinated hydrocarbon
- CHEMICAL STRUCTURE: $CCl_2 = CCl-CCl = CCl_2$

Uses

Hexachlorobutadiene is an industrial by-product of tetrachloroethylene, trichloroethylene, and perchloroethylene production and is used as a solvent for elastomers, heat transfer liquids, transformer fluids, and hydraulic fluids. Hexachlorobutadiene may still be used in certain countries as a fumigant. It is also released during refuse combustion and is found in fly ash.

Exposure Routes and Pathways

Hexachlorobutadiene may be toxic by inhalation, ingestion, and dermal exposures. Occupational

exposure may occur through inhalation or dermal contact. The general population may be exposed via inhalation of ambient air and ingestion of food or water-containing hexachlorobutadiene.

Toxicokinetics

In rabbits, hexachlorobutadiene is absorbed through the skin. In rats, hexachlorobutadiene was found in lungs, blood, liver, brain, kidneys (proximal section of the nephron), spleen, and mesentery after a single injection (unspecified). Glutathione conjugation is the main route of biotransformation in mammals, followed by biliary excretion. Following oral administration of a nephrotoxic dose (200 mg kg^{-1}) of hexachloro-1,3-butadiene to male rats, the principal route of excretion was biliary, with 17–20% of the dose being eliminated on each of the first 2 days. Fecal excretion was <5% of the dose per day, suggesting enterohepatic recirculation of biliary metabolites. Urinary excretion was small, not exceeding 3.5% of the dose during any 24 h period.

Mechanism of Toxicity

Hexachlorobutadiene specifically damages the pars recta portion of the proximal tubule with loss of the brush border. The mechanism involves nonoxidative formation of the glutathione conjugate in liver with subsequent transport to the kidney for mercapturic acid conjugate processing. The resulting cysteine conjugates are substrates for cysteine-conjugate β -lyase, which removes ammonia and pyruvate from the cysteine conjugate to produce thionylacyl halides and thioketenes. These toxic thiol compounds can then bind covalently to proteins and DNA in proximal tubular cells to produce nephrotoxicity. Mitochondrial dysfunction is reported to be the ultimate subcellular toxic lesion. Enterohepatic recirculation of hexachlorobutadiene-glutathione conjugates is believed to play a major role in this mechanism, since cannulation of the bile duct of rats prevents nephrotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD₅₀ (single dose) is 90 mg kg^{-1} in guinea pigs, $87-116 \text{ mg kg}^{-1}$ in mice, and 200– 350 mg kg^{-1} in rats. The dermal LD₅₀ at 7 h was 126 mg kg^{-1} in rabbits.

Hexachlorobutadiene causes lung, liver, and renal injury (renal proximal tubular dysfunction) in animals. Hexachlorobutadiene and its metabolites were reported to be approximately four times more nephrotoxic to female rats than to males. Eye and nose irritation have been reported in animals exposed to 250 ppm for 4 h and 110 ppm for 6 h.

At dosages high enough to cause maternal toxicity (decreased weight gain) and slight fetal toxicity (decreased fetal weight) hexachlorobutadiene was not teratogenic.

Unscheduled DNA synthesis has occurred in experimental animals. Mutations have been produced in *Salmonella typhimurium* and sister chromatid exchange has occurred in the hamster ovary cell.

Human

Little information is available on the acute toxicity of hexachlorobutadiene in humans. Recent physiologically based pharmacokinetic models suggest an order of magnitude lower activation of reactive nephrotoxic metabolics in humans compared to rats.

Chronic Toxicity (or Exposure)

Animal

In a 30 day study in rats, 30, 65, and 100 mg kg⁻¹ day⁻¹ resulted in renal toxicity, increased kidney-body weight ratio, and renal tubular degeneration, necrosis, and regeneration; 100 mg kg^{-1} day⁻¹ resulted in decreased food consumption and body weight and minimal hepatocellular swelling at 100 mg kg⁻¹; and 10, 30, 65, and 100 mg kg⁻¹ day⁻¹ resulted in hemoconcentration. A statistically significant increase of kidney tumors was observed in male and female rats fed diets containing hexachlorobutadiene (99% pure) at 20 mg kg⁻¹ body weight per day for 22 months.

Human

Hexachlorobutadiene is on the National Institute for Occupational Safety and Health list of suspected carcinogens because it has the potential to cause kidney and lung cancer. A group of 205 vineyard workers who were exposed seasonally to hexachlorobutadiene $(0.8-30 \text{ mg m}^{-3} \text{ in air over the fumigated zones})$ showed multiple toxic effects contributing to the development of hypotension, cardiac disease, chronic bronchitis, disturbances of nervous function, and chronic hepatitis.

The following combination of tests could be useful for detecting renal dysfunction in occupationally exposed workers: examination of urine with reagent strips for the presence of glucosuria and proteinuria and quantitative determination of at least two proteins, one of the high molecular weight for glomerular function and one of the low molecular weight for tubular function. Some value has also come from the determination of the lysosomal enzyme N-acetyl- β -D-glucosaminidase in urine.

Clinical Management

No specific treatment is available. Patients acutely and chronically exposed should be monitored for renal, hepatic, and pulmonary damages. At least some of the renal toxicity appears to be reversible, so supportive care is indicated. Emesis may be indicated and is most effective if initiated within 30 min of ingestion. Charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered. A saline cathartic should be administered, unless sorbitol-charcoal slurry is used.

In cases of inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develops, the victim should be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Supplemental oxygen (100% humidified) should be administered with assisted ventilation as required.

Exposed eyes should be irrigated with generous amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should see a doctor. Exposed areas should be washed extremely thoroughly with soap and water. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

If released into air, hexachlorobutadiene will exist solely as a vapor. In soil, hexachlorobutadiene is expected to have low to no mobility, and volatilization is expected to be a significant fate process. If released into water, hexachlorobutadiene is expected to adsorb to particulates and sediment. Volatilization from water may be significant but will depend on the extent of adsorption to particulates and sediment. Disappearance half-lives of hexachlorobutadiene have been estimated to be 3–30 days in river water and 30–300 days in lake and groundwater. Bioconcentration factors of between 5800 and 17000 indicate a very high potential for accumulation in aquatic organisms.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value is 0.02 ppm.

See also: Glutathione; Pesticides; Sister Chromatid Exchanges.

Further Reading

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Hexachlorocyclohexanes

Guangping Chen

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• CHEMICAL NAMES:

1,2,3,4,5,6-Hexachlorocyclohexane (mixed isomers), CAS 608-73-1

 $1\alpha, 2\alpha, 3\beta, 4\alpha, 5\beta, 6\alpha$ -Hexachlorocyclohexane (α -isomer), CAS 319-84-6

 $1\alpha, 2\beta, 3\alpha, 4\beta, 5\alpha, 6\beta$ -Hexachlorocyclohexane (β -isomer), CAS 319-85-7

1 α ,2 α ,3 β ,4 α ,5 α ,6 β -Hexachlorocyclohexane (γ -isomer), Lindane, CAS 58-89-9

 $1\alpha, 2\alpha, 3\alpha, 4\beta, 5\alpha, 6\beta$ -Hexachlorocyclohexane (δ -isomer), CAS 319-86-8

 $1\alpha, 2\alpha, 3\alpha, 4\beta, 5\beta, 6\beta$ -Hexachlorocyclohexane (ε -isomer), CAS 6108-10-7

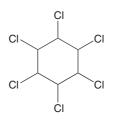
1 α ,2 α ,3 α ,4 α ,5 α ,6 α -Hexachlorocyclohexane (ζ -isomer), CAS 6108-11-8

 $1\alpha, 2\alpha, 3\alpha, 4\alpha, 5\beta, 6\beta$ -Hexachlorocyclohexane (η -isomer), CAS 6108-12-9

 $1\alpha, 2\alpha, 3\alpha, 4\alpha, 5\alpha, 6\beta$ -Hexachlorocyclohexane (θ -isomer), CAS 6108-13-0

• SYNONYMS: Benzene hexachloride; 1,2,3,4,5,6-Hexachlorocyclohexane; Lindane

- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organochlorine pesticides
- CHEMICAL FORMULA: C₆H₆Cl₆
- CHEMICAL STRUCTURE:



Uses

Hexachlorocyclohexanes (HCHs) are produced and used as insecticide on fruit, vegetables, and forest crops, and for direct application on animals and animal housing. It is also available as a prescription medicine to treat or control scabies and head lice in humans. It is a white solid substance that may evaporate under suitable conditions.

Background Information

HCHs are a group of manufactured chemicals. HCH has eight isomeric forms. The different isomers are named according to the position of the hydrogen atoms in the structure of the chemical. The four common isomers are α -, β -, γ -, and δ -HCHs. The most common of these is γ -HCH (also known as lindane).

Exposure Routes and Pathways

Oral exposure through diet is the primary pathway for the general population. HCHs can also be distributed in air and can be absorbed through inhalation.

Toxicokinetics

HCHs are readily absorbed through the gastrointestinal tract. Inhaling air contaminated with isomers of HCH can also lead to systemic absorption. HCHs can also be absorbed through the skin when used as a lotion, cream, or shampoo for the treatment or control of ectoparasites. In general, HCH isomers and their metabolites can be temporarily stored in body fat. Absorbed HCHs are mainly excreted via the urine. Lesser amounts are excreted in feces. In rats, the highest concentrations have been found in liver, kidneys, body fat, brain and muscles, with substantial deposition occurring in fatty tissue.

Mechanism of Toxicity

HCHs are highly lipophilic molecules exhibiting extended (years) biological half-lives. The γ isoform (γ -HCH; lindane) is a potent neurostimulant and

convulsant. y-HCH mediated neurotoxicity is primarily the result of blockade of Cl⁻ influx through ionotropic y-aminobutyric acid receptors, resulting in depolarization and hyperexcitation of the postsynaptic neuronal membrane. γ -HCH has been shown to enhance both spontaneous and evoked release of neurotransmitters from nerve terminals. These actions have been correlated with the ability of γ -HCH to elevate Ca^{2+} in brain synaptosomes. γ -HCH has also been shown to alter contractility in skeletal myocytes. δ -HCH is particularly potent toward disrupting Ca^{2+} homeostasis in a variety of excitable and nonexcitable cells and altering contractility of cardiac muscle. δ -HCH has been shown to stereoselectively mobilize Ca²⁺ from intracellular stores in cultured neural cells, which appears mediated by interaction with ryanodine receptors. γ -HCH and β -HCH have been reported to have estrogenic actions.

Clinical Management

HCH isomers can be measured in the blood, urine, and semen of exposed persons. HCH metabolites can also be measured to determine whether a person has been exposed to HCH. However, this method cannot be used to determine exposure to HCH alone, that is, other environmental contaminants may also produce the same metabolites.

Environmental Fate

HCHs persist in the environment. In air, the different forms of HCH can be present as a vapor or adsorbed to small particles. HCH can remain in the air for long periods of time and residues can travel great distances, depending on environmental conditions. HCH is degraded to less toxic substances by algae, fungi, and bacteria.

See also: Lindane; Organochlorine Insecticides.

Further Reading

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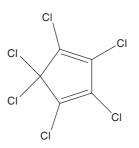
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- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexachlorocylcohexanes.
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Hexachlorocyclopentadiene

Murali Badanthadka and Harihara M Mehendale

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 77-47-4
- SYNONYMS: 1,2,3,4,5,5-Hexachloro-1,3-cyclopentadiene; 1,3-Cyclopentadiene, 1,2,3,4,5,5-hexachloropentadine; Perchlorocyclopentadiene; Perchloro-1,3-cyclopentadiene
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Chlorinated cyclic hydrocarbon
- CHEMICAL FORMULA: C₅Cl₆
- CHEMICAL STRUCTURE:



Uses

Hexachlorocyclopentadiene is used as a chemical intermediate for many insecticides, polymer resins, flame-retardant additives, resins, dyes, and in pharmaceuticals. It is also used to make shock-proof plastics, acids, esters, ketones, and fluorocarbons.

Background Information

Hexachlorocyclopentadiene is a light, lemon-yellow liquid with a sharp, musty odor. It is a manufactured

chemical that does not occur naturally in the environment. It is used to make a group of related pesticides: aldrin, chlordane, dieldrin, endosulfan, endrin, heptachlor, isodrin, mirex, and pentac. Only endosulfan and pentac are currently registered for use in the United States. Hexachlorocyclopentadiene is also used to make flame retardants, resins that will not burn, shock-proof and unbreakable plastics, acids, esters, ketones, fluorocarbons, and dyes.

Exposure Routes and Pathways

Although occupational exposure appears to be the main source of human contact, certain segments of the population may be exposed through ingestion of contaminated drinking water or contaminated fish. People living in the vicinity of hazardous waste disposal sites containing this compound may be exposed by inhalation of contaminated air. Workers involved in the manufacture or handling of this compound or treatment of wastes containing this compound could potentially be exposed by inhalation or dermal exposure.

Toxicokinetics

Hexachlorocyclopentadiene undergoes chemical alterations in water forming both lipophilic and hydrophilic products. The lipophilic products in fish were extremely volatile. Three days after the intraperitoneal injection of ¹⁴C-hexachlorocyclopentadiene, the ethyl acetate extractable radioactivity was ~47% while water-soluble and unextractable radioactivities were 11% and 20% of the injected radioactivity, respectively. Ethyl acetate extracts of fish included at least eight unidentified breakdown products. Watersoluble extract from fish included at least four unidentified products.

Rats given 6 mg kg⁻¹ hexachlorocyclopentadiene orally excreted 33% in urine and 10% in feces in 7 days. Most excretion occurred during the first 24 h after dosing. The kidney retained 0.5% and the liver >0.5%. Biliary excretion of only 16% with 66% still voided in the feces of bile duct cannulated rats suggested that the majority of orally consumed was not absorbed. Degradation apparently occurred in the gut since little of the fecal material was of an apolar nature. The kidney, liver, ovaries, and fat were the major sites of deposition of ¹⁴C-hexachlorocyclopentadiene equivalents. In rats, the kidney contained the highest levels of residues, whereas in mice the residues in the liver exceeded those in the kidney. Other than this difference, the fate of hexachlorocyclopentadiene in rats and mice, both male and female, was quite similar and in each case the tissue residues reached a plateau after about 2 weeks on the hexachlorocyclopentadiene-containing diets.

In another study, rats were either exposed to ¹⁴Chexachlorocyclopentadiene vapors for 1 h, or were dosed orally with ¹⁴C-hexachlorocyclopentadiene in corn oil. Tissue, urine, and feces samples were analyzed, as well as expired air, to assess the fate and retention time. Approximately 84% of the inhaled compound is retained. Inhaled ¹⁴C-hexachlorocyclopentadiene was excreted in the urine; orally administered ¹⁴C-hexachlorocyclopentadiene was eliminated in the feces. In rats exposed by inhalation, the trachea and lung had the highest residue accumulation. In animals receiving oral doses kidneys and liver were major sites of accumulation. These studies indicate that the route of exposure is critical to the pattern of retention and elimination.

Mechanism of Toxicity

Hexachlorocyclopentadiene's mechanism of toxicity is incompletely understood. Because of its characteristics as a chlorinated hydrocarbon, it would be expected to induce drug-metabolizing enzymes in the liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

Approximate lethal doses for rats and rabbits by single oral administration were between 420 and 620 mg kg^{-1} , respectively. The animals showed diarrhea, lethargy, and decreased respiration. Rabbits were reported to show diffuse degenerative changes in the brain, heart, liver, and adrenal glands; necrosis of the epithelium of renal tubules; and severe hyperemia and edema of the lungs by skin absorption.

Acute range-finding (14 days) and subchronic (90 days) inhalation studies were conducted with Sprague Dawley rats; and subchronic (90 days) inhalation studies were conducted with monkeys. The studies with rats showed steep dose-response curves with male rats being more sensitive than females. The threshold for toxic effects was <0.5 ppm hexachlorocyclopentadiene. Observation of lesions in the olfactory and bronchiolar epithelium as well as inflammatory exudate in the lumen of the respiratory tract was consistent with observed impaired respiratory function, confirming the lungs as the main target organ. Lacrimation, salivation, tremors, and degenerative changes in the brain, heart, liver, adrenal glands, and kidneys have been observed in animal inhalation studies. Hexachlorocyclopentadiene is not carcinogenic based on inhalation studies in rats and mice.

F344 rats and B6C3F1 mice were exposed to the hexachlorocyclopentadiene by gavage at 0-150 and $0-300 \text{ mg kg}^{-1}$, respectively. A dose-related decrease in mean body weight gain occurred in both sexes of rats and mice. Male rats in the $150 \,\mathrm{mg \, kg^{-1}}$ dose group and one in 75 mg kg^{-1} group died after exposure. All mice exposed to hexachlorocyclopentadiene at 300 mg kg^{-1} died. Liver:brain weight ratios were significantly increased in the 38, 75, and 150 mg kg⁻¹ exposed groups of female rats. Kidney:brain weight ratios in females significantly increased after 75 and 150 mg kg^{-1} exposure. No significant difference in relative weight of other organs was observed. In female mice, the kidney:brain weight ratios were significantly increased at all doses and the lung:brain weight increased after exposure at 300 mg kg^{-1} dose. Clinical signs of toxicity, cysts, and ulceration were seen after exposure. Histopathologically, lesions in the stomach, inflammation in the submucosa, edema, neovascularization and hyperplasia were noted. Toxic nephrosis of the kidney and acute tubular necrosis were also seen in both rats and mice.

Human

Eye and throat irritation has been reported in humans. Independent of the exposure route, the lung appears to be a major target organ resulting in cough, dyspnea, and chest pains. Headache and nausea are common after exposure. Exposed workers have developed reversible subclinical elevations of liver function tests and reversible proteinuria. Skin irritation and blistering may occur from direct contact with liquid hexachlorocyclopentadiene, and skin contact with vapor has been reported to result in skin irritation. Reported human cases have generally been mild.

Chronic Toxicity (or Exposure)

Animal

Pregnant mice and rabbits were administered up to $75 \text{ mg kg}^{-1} \text{ day}^{-1}$ of hexachlorocyclopentadiene by gavage during active oogenesis. Teratogenic effects have not been observed; however, nephrosis and acute tubular necrosis were seen in the dams.

Human

Hexachlorocyclopentadiene is not classifiable as a human carcinogen.

In Vitro Toxicity Data

Hexachlorocyclopentadiene is not mutagenic in the *Salmonella typhimurium* and *Escherichia coli* assay with and without metabolic activation.

Clinical Management

Oral Exposure

Because of potential central nervous system depression, emesis should not be induced. Significant esophageal or gastrointestinal tract irritation or burns may occur following ingestion. The possible benefit of early (within 1 h) removal of some ingested material by cautious gastric lavage must be weighed against potential complications of bleeding or perforation. The victim should be taken to a hospital immediately and should be treated symptomatically.

Inhalation Exposure

Patient should be moved to fresh air. Respiratory distress should be monitored and a healthcare person consulted.

Eye Exposure

Decontamination with copious amounts of room temperature water for at least 15 min should be done. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should consult healthcare facility.

Dermal Exposure

Decontamination should be done by removing contaminated cloth and washing the exposed area thoroughly with soap and water. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

Aquatic Fate

If released to water, hexachlorocyclopentadiene will degrade primarily by photolysis and chemical

hydrolysis to form both lipophilic and hydrophilic products. The water soluble substances included at least 11 unidentified breakdown products. Hydrolytic half-lives ranging from several hours to 2-3 weeks are predicted for waters with temperatures in the range of 20-30°C. 2,3,4,4,5-Pentachloro-2-cyclopentenone, hexachloro-2-cyclopentenone, and hexachloro-3cyclopentenone have been identified as primary photodegradation products of hexachlorocyclopentadiene. Hexachlorocyclopentadiene has the potential to adsorb suspended solids and sediments; nevertheless, adsorption does not significantly affect the rate of hydrolysis. Volatilization from water is expected to be a significant removal mechanism, although in highly turbid waters adsorption to suspended solids and sediments could substantially limit losses via volatilization. The volatilization half-lives from a model river and a model pond with and without adsorption have been estimated to be 5h, 37 days, and 58h, respectively. It appears as though hexachlorocyclopentadiene may also be susceptible to biodegradation. Potential bioaccumulation in some aquatic organisms depends upon the organism and the species.

Terrestrial Fate

If released to soil, hexachlorocyclopentadiene will get adsorbed to organic matter and degrades via photolysis on soil surfaces. Volatilization from soil surfaces is expected to be of minor importance. In moist soil, this compound would be subject to chemical hydrolysis (half-life of hours to weeks) and biodegradation under aerobic and anaerobic conditions. A study indicates that loss of hexachlorocyclopentadiene from soil is the result of abiotic and biotic degradation as well as partitioning within the media.

Atmospheric Fate

Organic compounds having a vapor pressure of greater than 1×10^{-4} mmHg at ambient temperature are expected to exist almost entirely in the vapor phase in the atmosphere. Hexachlorocyclopentadiene has a vapor pressure of 0.063 mmHg at 25°C; therefore, it is expected to exist predominantly in the vapor phase in the atmosphere. If released to the atmosphere, direct photolysis is expected to be the dominant removal mechanism. Reaction of hexachlorocyclopentadiene with photochemically generated hydroxyl radicals or ozone molecules is predicted to be too slow and environmentally insignificant.

Exposure Standards and Guidelines

The acceptable daily intake for hexachlorocyclopentadiene is $0.00462 \text{ mg day}^{-1}$. The Occupational Safety and Health Administration 8 h time-weighted average (TWA) is $0.01 \text{ ppm} (0.1 \text{ mg m}^{-3})$. The National Institute of Occupational Safety and Health recommended exposure limit is $0.01 \text{ ppm} (0.1 \text{ mg m}^{-3})$ for 10 h TWA.

See also: Chlorination By-products; Polybrominated Biphenyls (PBBs); Polychlorinated Biphenyls (PCBs).

Further Reading

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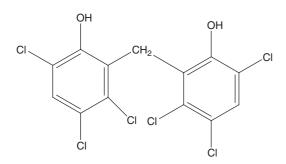
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- Podowski AA, Sclove SL, Pilipowicz A, and Khan MA (1991) Biotransformation and disposition of hexachlorocyclopentadiene in fish. *Archives of Environmental Contamination and Toxicology* 20: 488–496.
- Podowski AA and Khan MA (1984) Fate of hexachlorocyclopentadiene in water and goldfish. *Archives of Environmental Contamination and Toxicology* 13: 471–481.

Hexachlorophene

Cathy Villaroman and Robin C Guy

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 70-30-4
- SYNONYMS: 2,2'-Methylenebis(3,4,6-trichlorophenol); 2,2'-Dihydroxy-3,3',5,5',6,6'-hexachlorodiphenylmethane; bis(3,5,6-Trichloro-2-hydroxyphenyl)methane; Hexachlorophane; Hexachlorophen; bis(2,3,5-Trichloro-6-hydroxyphenyl)methane; Acigena; Exofene; Gamophene; HCP; Hexafen; Nabac
- CHEMICAL STRUCTURE:



Uses

Hexachlorophene is used as an agricultural chemical, detergent, therapeutic agent, and wood preservative. It is used as a topical antiinfective, fungicide, germicide, bactericide, and disinfectant. It is also used as an antibacterial agent in cosmetics, soaps, shampoos, and deodorants.

Exposure Routes and Pathways

Hexachlorophene can be absorbed into the body by inhalation, through the skin, and by ingestion. Exposure to hexachlorophene is usually dermal as a bactericide. It is sometimes used as a topical treatment for acne vulgaris to suppress associated staphylococci.

Toxicokinetics

Hexachlorophene is well absorbed orally and dermally and through mucosal surfaces. In rats, up to 55% of dermally applied hexachlorophene is absorbed in 24 h. Dermal absorption is enhanced by dimethylsulfoxide and dermatitis or skin abrasions. Placental transfer has been demonstrated in rats. Hexachlorophene is converted to hexachlorophene- β -D-glucuronide in the rat and rabbit. Some hexachlorophene has been found in the blood and adipose tissue. Hexachlorophene was administered intraperitoneally to rats and rabbits; excretion was slow and most (48-83%) was excreted unchanged in the feces. Hepatic function is an important determinant in the removal of hexachlorophene. In a rat study, within 3h after administration, 50% was excreted in the bile. Rats given intraperitoneal doses excreted $\sim 5\%$ of the dose in the urine and none as CO_2 ; more than 70% of the material was excreted in feces.

Mechanism of Toxicity

Following skin absorption, hexachlorophene enters the nervous system and results in intramyelinic edema, splitting the intraperiod line of myelin in both the central nervous system (CNS) and the peripheral nervous system. Experimental studies with erythrocyte membranes show that hexachlorophene binds tightly to cell membranes, resulting in osmotic swelling of erythrocyte membranes by altering their permeability to sodium and potassium. Hexachlorophene uncouples oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Pigs fed hexachlorophene for 36 days only exhibited mild neurological signs, with a no-observed-effect limit (NOEL) = $0.1 \text{ mg kg}^{-1} \text{ day}^{-1}$ and a lowestobserved-effect level (LOEL) = $0.5 \text{ mg kg}^{-1} \text{ day}^{-1}$. A subchronic dog feeding study was conducted by Nationwide Chemical Corporation (1974), where Beagle dogs (four per sex per dose) were fed hexachlorophene (0.75, 1.5, or $3.0 \text{ mg kg}^{-1} \text{ day}^{-1}$) in the diet for 13 weeks. The principal effects noted were swollen salivary glands, dry mouth, and status spongiosis in the brain, optic nerve, spinal cord, and sciatic nerve at all dose levels tested. An NOEL for this study was not established. However, the LOEL of $0.75 \text{ mg kg}^{-1} \text{ day}^{-1}$ was used to establish an oral reference dose. Applying a total uncertainty factor of 3000 (100 for inter- and intraspecies differences, 10 for the lack of an established NOEL, and 3 to account for subchronic to chronic exposure in the dog = UF of 3000) to the LOEL yielded an oral reference dose of $2.5 \times 10^{-4} \text{ mg kg}^{-1} \text{ day}^{-1}$.

Human

Acute ingestion of large amounts (\geq 30–60 m for an adult) or repeated ingestion of small amounts of hexachlorophene may cause significant toxicity or death. Exposure may also cause effects on the CNS, resulting in convulsions or respiratory failure. Dermal, gastrointestinal, and neurologic effects are the most common toxic manifestations. Cardiorespiratory arrest may occur most notably following acute ingestion of large amounts. Lethargy frequently occurs as an early manifestation of toxicity.

Chronic Toxicity (or Exposure)

Animal

Rats fed 500 ppm $(25 \text{ mg kg}^{-1} \text{ day}^{-1})$ showed weakness in their hindquarters, which progressed to

paralysis. Microscopic examination of the brain and spinal cord revealed a particular edema of white matter resembling spongy degeneration of white matter in infants. When the animals were removed from the hexachlorophene diet, they recovered gradually over a period of weeks; similar signs were noted in the monkey.

Oral administration of hexachlorophene to rats causes degeneration of spermatogenic cells. In sheep, 2500 mg kg^{-1} followed 2 days later by a dose of 50 mg kg^{-1} also caused extensive damage to spermatogonia; after 21 days there was neither sperm in epididymis nor spermatogenesis.

Oral LD₅₀s in rats were 187 and 67 mg kg⁻¹; 56 mg kg⁻¹ in the female rat; 120 mg kg⁻¹ in the rat weanling; 9 mg kg^{-1} in the rat suckling (10 days old); 63–87 mg kg⁻¹ in the female Wistar rat; and 58–87 mg kg⁻¹ in the male Wistar rat.

Oral administration of hexachlorophene in rats produced no carcinogenic effects.

Human

Repeated or prolonged dermal contact with hexachlorophene may cause skin hypersensitivity or dermatitis, while repeated or prolonged inhalation exposure may cause asthma. Repeated ingestion may result in tissue lesions or blindness. The estimated lethal dose in humans is 1–10 g. Dermal application, especially in neonates or on damaged skin, of highly concentrated ($\geq 3\%$) preparations on several occasions or repeated applications of less concentrated preparations may result in significant toxicity or death. An erythematous desquamative rash may occur following repeated dermal application, especially in neonates or in high concentrations ($\geq 3\%$).

In Vitro Toxicity Data

Hexachlorophene was not shown to be mutagenic in *Salmonella typhimurium* in tests reviewed. Cytogenetic tests with cultured human lymphocytes were also negative.

Clinical Management

Plasma hexachlorophene levels have not been demonstrated to correlate well with clinical effects. Hexachlorophene may cause seizures. The risk of seizures during emesis may preclude the use of ipecac syrup. Charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered. The usual charcoal dose is 30-100 g in adults and 15-30 g in children (1 or 2 g kg⁻¹ in infants). If seizures cannot be controlled with diazepam or they recur, phenytoin or phenobarbital should be administered. The patient should be checked for cerebral edema. Other acute symptoms after ingestion exposure include: fever, tremors, absence of light reflex, abdominal cramps, diarrhea, drowsiness, nausea, vomiting, and weakness. Vomiting should be induced in conscious persons. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. Vigorous washing with soap and water should be followed by washing with 70% isopropanol, olive oil or castor oil, followed by a second vigorous soap and water cleansing, which may increase removal of hexachlorophene. If contact is made via the skin, contaminated clothes should be removed, then the skin is rinse and wash skin with soap and water.

Environmental Fate

Hexachlorophene adsorbs very strongly to soil and is not expected to leach to groundwater. It may undergo slow photodegradation on the surface of soils and water based on its absorption of light (290 nm). No information is available on its biodegradation in soil or surface water. Hexachlorophene released in water adsorbs very strongly to sediments and may bioconcentrate in aquatic organisms. It has an estimated bioconcentration factor (BCF) of 317000. HCP is not expected to hydrolyze or to significantly evaporate from water. When released into the air, HCP is expected to be mainly in the particle-sorbed state due to its low vapor pressure and high estimated $K_{\rm oc}$. It is expected to be removed from the atmosphere primarily by dry deposition, but it is also degraded by reaction with photochemically

Hexane

Stephen R Clough and Leyna Mulholland

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- CHEMICAL NAME: *n*-Hexane
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 110-54-3
- SYNONYMS: Dipropyl; Esani; Heksan; AI3-24253; Hexanen; Hexyl hydride; NCI-C60571; HSDB 91; Skellysolve B
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aliphatic hydrocarbon
- Chemical Formula: C₆H₁₄
- CHEMICAL STRUCTURE: CH₃CH₂CH₂CH₂CH₂CH₃

produced hydroxyl radicals, with an estimated vapor phase half-life of 2.5 days.

Ecotoxicology

Hexachlorophene is very toxic to aquatic organisms and may cause long-term effects in the aquatic environment.

Other Hazards

Hexachlorophene is combustible and can give off irritating or toxic fumes (or gases), including hydrogen chloride, if heated or burned in a fire. Hexachlorophene evaporates negligibly at 20°C. However, a harmful concentration of airborne particles can be reached quickly on spraying or when dispersed as dust.

See also: Neurotoxicity.

Relevant Websites

- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Hexachlorophene.
- http://www.speclab.com Spectrum Laboratories. Chemical Fact Sheet.
- http://vm.cfsan.fda.gov US Food and Drug Administration. Code of Federal Regulations: Title 21, Volume 4. 21CFR250.250. Revised April 2002.
- http://www.state.nj.us New Jersey Department of Health and Senior Services. Hazardous Substance Fact Sheet. Hexachlorophene.

Uses

Commercial hexane is a mixture of various types of hexane isomers with minor amounts of heptane, pentane, cyclohexane, and cyclopentane isomers. It may contain concentrations of *n*-hexane ranging from 20% to 80%. *n*-Hexane is used for determining the refractive index of minerals, for calibrations, as a paint diluent, and in thermometers. It is also used as a solvent in the extraction of soybean oil, cottonseed oil, flaxseed oil, safflower seed oil, and other oil seeds. It is sometimes used as a denaturant for alcohol, and as a cleaning agent in the textile, furniture, and leather industries although it is slowly being replaced with other less toxic solvents. *n*-Hexane is also a common laboratory reagent and a component of many products associated with the petroleum and gasoline industries (one study found 1.5% of vapors encountered during gasoline handling were attributed to *n*-hexane).

Exposure Routes and Pathways

The primary exposure pathway for *n*-hexane in humans is via inhalation, and this generally is seen in an occupational setting. Minor exposures may occur when persons fill their automobiles with gasoline. Ingestion or dermal contact would be a less common exposure route, with the former expected to occur during accidental poisoning or suicide attempt and the latter occurring from a spill or the use of a hexane applicator without the proper skin protection (e.g., solvent rag without a chemically resistant glove). Exposure from contact with vapors or emissions from these refined petroleum products is the most widespread form of low-level exposure for the general population. Recent research by Ahearn et al. suggests that certain fungi may be able to produce *n*-hexane. These fungi may be common in older buildings, and in some parts of the country may provide exposures from previously unsuspected indoor sources.

Toxicokinetics

Acute exposure usually occurs by inhalation. *n*-Hexane may be absorbed orally or percutaneously. *n*-Hexane has a vapor density of 2.97 and it is heavier than air. *n*-Hexane is believed to be metabolized through the cytochrome P450 system and phenobarbital pretreatment of liver microsomes induced 2- and 3-hydroxylation of *n*-hexane sixfold. 3,4-Benzpyrene suppresses 2-hydroxylation and stimulates 3-hydroxylation of *n*-hexane. For humans, 2-, 3-hydroxyhexane is responsible for various toxicities.

Mechanism of Toxicity

n-Hexane is biotransformed to 2-hexanol and further to 2,5-hexanediol by cytochrome P450 mixed function oxidases by omega oxidation. 2,5-Hexanediol may be further oxidized to 2,5-hexanedione, the major metabolite of *n*-hexane in humans. Identification of 2,5-hexanedione as the major neurotoxic metabolite of *n*-hexane proceeded rapidly after its discovery as a urinary metabolite. 2,5-Hexanedione has been found to produce a polyneuropathy indistinguishable from *n*-hexane. 2,5-Hexanedione is many times more potent than *n*-hexane, the parent compound, in causing neurotoxicity in experimental animals. It appears that the neurotoxicity of 2,5-hexanedione resides in its y-diketone structure since 2,3-, 2,4-hexanedione and 2,6-heptanedione are not neurotoxic, while 2,5-heptanedione and 3,6-octanedione and other y-diketones are neurotoxic.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hexane has been reported to be three times as acutely toxic to mice as is pentane. A concentration of 30 000 ppm produced central nervous system (CNS) depression within 30 to 60 min. Concentrations ranging from 35 000 to 40 000 ppm produced convulsions and death. When mice were exposed to an atmosphere containing 2.5-3% of *n*-hexane for 4 days, liver enlargement was observed after 24 h. In another study, mice were exposed to commercial hexane (65-70% *n*-hexane) for 24 h a day, 6 days a week for 1 year. Exposure levels ranged from 100 to 2000 ppm. Atrophy and degeneration of hindleg muscle fibers were present in animals exposed to 1000 and 2000 ppm of *n*-hexane.

Human

Based on a national occupational survey conducted in the mid-1970s, an estimated 643 120 workers are occupationally exposed to *n*-hexane. This commonly used solvent was not regarded as an industrial hazard until the discovery of its neurotoxic potential since its acute toxicity is quite low. Because of its toxicity, the number of people presently exposed to hexane in occupational settings is expected to be much lower than the number in the 1970s. Acutely, vapor concentrations of many hundreds of parts per million are tolerated for several minutes without causing discomfort among workers.

Acute exposure to hexane causes CNS depression. Chronic exposure to an average air concentration of 450–650 ppm for as little as 2 months may result in peripheral neuropathy, characterized by muscular weakness, loss of sensation, and impaired gait. Hexane has been reported to be the most highly toxic member of the alkanes. When *n*-hexane is ingested, it causes nausea, vertigo, bronchial irritation, general intestinal irritation, and CNS effects. It poses an acute aspiration hazard. It has been reported that ~ 50 g of *n*-hexane may be fatal to humans. An exposure of 880 ppm for 15 min can cause eye and upper respiratory tract irritation in humans. Blurred vision has been mentioned in association with nhexane polyneuropathy. It was concluded that nhexane vapor levels of < 100 ppm for 8 h per day were not likely to produce a clinical neuropathy, but mild subclinical changes in muscle strength and nerve conduction velocity may occur.

In humans, 2000 ppm of *n*-hexane for 10 min resulted in no effects. However, 5000 ppm caused dizziness, giddiness, slight nausea, headache, and

eye and throat irritation. Three women had motor polyneuropathy following industrial exposure to an adhesive agent containing 80.4% *n*-hexane. In the nerves, there were polymorphous changes in the myelin sheaths and axons of large diameter fibers. Muscles showed denervation changes, with lymphocytic infiltrates and phagocytosis. Three cases of *n*-hexane neuropathy in the shoe industry were reported. In the most severe cases, symptoms consisted of dysarthria, disproportionate ataxia of gait, blurred vision, and sometimes after recovery of peripheral neuropathy, appearance of leg spasticity.

Chronic Toxicity (or Exposure)

Animal

Pregnant Fischer 344 rats were exposed to 1000 ppm *n*-hexane for 6 h per day on days 8–12 of gestation. Postnatal growth of pups born to dams exposed to 1000 ppm on days 8–16 of gestation was significantly depressed compared to controls. New Zealand rabbits exposed in inhalation chambers to 3000 ppm *n*-hexane 8 h per day for 8 days showed changes in the lungs, emphysema, necrotic phenomena in the bronchiolar epithelium, and athelectasis. Epicutaneous administration of *n*-hexane to guinea pigs caused progressing nuclear pyknosis and junctional separation between the basement membrane and the basal cells of the skin.

Male rats were exposed by inhalation to several concentrations of hexane, administered continuously or intermittently. In rats exposed to 1000 ppm hexane 24 h per day, 5 days per week for 11 weeks, the fifth component of the brain stem auditory-evoked response showed an increase in latency and decrease in amplitude, reflecting a brain stem dysfunction. Latency returned to normal within 5 weeks after termination of exposures, but amplitude did not. Latency of the compound action potential of the ventral caudal nerve of the tail of these rats was also increased and this effect was still present 22 weeks after termination of the exposure.

Adult rats were exposed to different concentrations of *n*-hexane and lung tissue was then examined. The direct toxic effect to pneumocytes could be demonstrated as definite regressive alterations, such as fatty generation and change of lamellar bodies of type II pneumocytes as well as increased detachment of cells. After chronic inhalation of solvents, conspicuous aggregation of lamellar discharge material of type II pneumocytes can be seen and, probably as a result of an irritated fat metabolism, there were large lysosome-like bodies with densely packed lipid material in type I pneumocytes.

New Zealand rabbits exposed in inhalation chambers to 3000 ppm *n*-hexane 8 h per day for 8 days showed changes in lungs, emphysema, necrotic phenomena in the bronchiolar epithelium, and atelectasis. The injection of hexane into rabbits caused edema and hemorrhaging of the lungs and tissue, with polymorphonuclear leukocytic reactions. In rabbits, dermal application of $2-5 \text{ mg kg}^{-1}$ for 4 h has resulted in ataxia and restlessness. No deaths occurred at 2 mg kg^{-1} ; however, some occurred at 5 mg kg^{-1} .

Human

Out of 1662 workers exposed to organic solvents, which consisted mainly of *n*-hexane and a small amount of toluene, 53 were found to have sensory polyneuropathy, 32 had sensorimotor polyneuropathy, and 8 had sensorimotor polyneuropathy with amyotrophy. Cranial nerve involvements, such as visual disorders and facial numbress, were observed. About 50% showed denervation and reinnervation of the nerves. Among 93 cases of *n*-hexane polyneuropathy during a large outbreak in 1968, 44 were studied. Over a few years, most of the cases completely recovered (except for a few with mild sensory impairment) after establishing 100 ppm as the maximal allowable concentration of *n*-hexane and providing well-equipped ventilation systems in individual houses. During rescreening in 1981, 21 cases with mild *n*-hexane polyneuropathy were observed, revealing mostly the same features as in the previous outbreak in 1968. These data suggest that, despite < 50 ppm of *n*-hexane concentration in a room, sandal workers have suffered from neurotoxicity from this organic solvent.

In a cross-sectional study, nerve conduction velocities were determined in 59 workers employed in press proofing factories in Taipei. Workers were divided into exposure categories on the basis of air concentrations of *n*-hexane (≥ 100 , 50–99, and < 50 ppm) and *n*-hexane concentrations in the cleaning solvent used (≥ 50 , 49–10, and < 10%). Fifteen members (25%) of the study group were found to have polyneuropathy. In one factory where all six employees developed polyneuropathy, the air concentration of *n*-hexane was determined to be 190 ppm. In other factories, workers exposed to *n*hexane at levels of < 100 ppm showed significant decreases in motor nerve conduction velocities.

n-Hexane is currently under review for its carcinogenicity; however, it is not classified as a carcinogen at the present time. A US Environmental Protection Agency (EPA) reference concentration of 0.2 mg m^{-3} was calculated based on an epidemiological inhalation study with an uncertainty factor of 300. Critical effects were reported to be neurotoxicity and electrophysiological alterations.

In Vitro Toxicity Data

Hexane was found to be negative when tested for mutagenicity using the *Salmonella* microsome preincubation assay, following the standard protocol approved by the National Toxicology Program. Hexane was tested in as many as five *Salmonella typhimurium* strains (TA1535, TA1537, TA97, TA98, and TA100) in the presence and absence of rat and hamster liver S9 at doses of 0.001, 0.0033, 0.010, 0.033, 0.100, and 0.333 mg per plate. Some cultures exhibited slight clearing of the background bacterial lawn at the two highest doses tested.

Clinical Management

Oral Exposure

In general, gastric emptying is not indicated except in selected cases in which a history of a large ingestion is obtained. An activated charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, can be administered for oral exposure. The US Food and Drugs Administration suggests 240 ml of dilutent per 30 g of charcoal. The usual charcoal dose is 30–100 g in adults and 15-30 g in children. In symptomatic patients (e.g., coughing, choking, and tachypnea), blood gases should be monitored to ensure adequate ventilation and a baseline chest X-ray should be obtained. Ventilation and oxygenation should be maintained for pulmonary edema with close arterial blood gas monitoring. Early use of positive end expiratory pressure and mechanical ventilation may be needed to maintain oxygen pressure.

Inhalation Exposure

The victim should be moved to fresh air to decontaminate. The person should also be monitored for respiratory distress. If cough or difficulty in breathing develops, respiratory tract irritation, bronchitis, or pneumonitis should be evaluated. Supplemental oxygen (100% humidified) should be administered with assisted ventilation as required.

Eye Exposure

Exposed eyes should be irrigated with copious amount of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in healthcare facility.

Dermal Exposure

The exposed area should be washed thoroughly with soap and water to decontaminate. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

In the environment, *n*-hexane does not absorb UV light (≥ 290 nm); and therefore it will not undergo direct photolysis. Some species of *Pseudomonas* bacteria, which are naturally present in soil and sediment, are able to metabolize hexane, so low-level contamination seen in groundwater is thought to naturally attenuate with time, reducing the risk to humans.

Ecotoxicology

According to the US EPA ECOTOX aquatic toxicity database, the 24 h acute LC_{50} values for saltwater organisms range from $3530 \,\mu g \, l^{-1}$ (brine shrimp) to $154\,300 \,\mu g \, l^{-1}$ (rotifer). For freshwater, the lowest respective 24 and 96 h acute LC_{50} values were for *Daphnia magna* ($50\,000 \,\mu g \, l^{-1}$) and the fathead minnow ($2100 \,\mu g \, l^{-1}$). Hexane, in all likelihood, affects aquatic organisms similarly to other volatile alkanes, which is by a narcotic mechanism (i.e., a solvent-like disruption of neuronal membranes). These concentrations are only seen in the laboratory and are many, many times higher than concentrations that would ever be anticipated in natural waters.

Exposure Standards and Guidelines

In recognition of the chronic neurotoxic property of *n*-hexane, the American Conference of Governmental Industrial Hygienists has recommended a threshold limit value of 50 ppm (180 mg m⁻³), expressed as an 8 h time-weighted average (TWA) (the National Institute for Occupational Safety and Health recommended exposure limit is also 50 ppm). The current Occupational Safety and Health Administration permissible exposure limit is 500 ppm (1800 mg m⁻³ as an 8 h TWA).

See also: Neurotoxicity; Pollution, Water.

Further Reading

Ahearn DG, Crow SA, Simmons RB, *et al.* (1996) Fungal colonization of fiberglass insulation in the air distribution system of a multi-story office building. VOC production and possible relationship to a sick building syndrome. *Journal of Industrial Microbiology* 16: 280–285.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexane.

High Production Volume (HPV) Chemicals

Pertti J Hakkinen

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High production volume chemicals ('HPV chemicals') are existing substances that are produced or imported into countries in high volumes. The definition varies by country and region. For example, in the United States, HPV chemicals are defined as those chemicals produced in or imported in amounts over one million pounds per chemical per year. The European Commission defines HPV chemicals as substances with a production or import volume in excess of 1000 metric tons (2.2 million pounds) per year. The toxicology and exposure assessment data sets available for HPV chemicals have been of increasing interest to regulatory agencies, nongovernment organizations, industry, interest groups, and others globally. A study of all US HPV chemicals found that 8.5% of the chemicals fulfilled a minimum set of data requirements, while another study found that 22% of the US HPV chemicals had 'minimal' toxicity data available. A study of publicly available data by the European Commission found that 14% of European Union HPV chemicals had data at the level of a 'base set', 65% had less than a base set, and 21% had no data. Thus, the focus of attention has been on chemicals that appear to have (1) been untested or unassessed, or (2) have less than a full basic set of data (see below for explanation of SIDS).

Since the late 1980s, governmental authorities in industrialized nations began to focus on these substances because of the presumed widespread exposure based on their large production volume. This focus began with the Organization for Economic Cooperation and Development (OECD) Screening Information Data Set (SIDS) Program in 1991, which has since been joined with similar and parallel activities such as the US HPV Challenge Program and the US Voluntary Children's Chemical Evaluation Program – both in 1998, and the plans to implement a sweeping regulatory change in the European Union (the registration, evaluation, and authorisation of chemicals (REACH) legislation).

OECD SIDS Program

The Organisation for Economic Cooperation and Development's (OECD's) 1987 Council Decision-Recommendation on the Systematic Investigation of Existing Chemicals stated, "Member Countries should establish or strengthen national programmes to systematically investigate existing chemicals." A further OECD Council Decision in 1991 focused on HPV chemicals. These decisions prompted the development of a minimum hazard data set to describe an HPV chemical – the Screening Information Data Set, or SIDS. This includes physicochemical properties (melting point, boiling point, vapor pressure, water solubility, and octanol-water partition coefficient); environmental fate (stability in water, photodegradation, biodegradation, and an estimate distribution/transport in the environment); of environmental effects (acute toxicity to aquatic vertebrates, invertebrates, and plants); and human health effects (acute toxicity, repeated-dose toxicity, toxicity to the gene and the chromosome, and reproductive and developmental toxicity).

In the OECD SIDS Program, member OECD countries are invited to 'sponsor' an HPV chemical, conduct the necessary SIDS-level testing, and present the information at an OECD meeting. The collection/ testing of information is called developing a dossier of information. Presenting the information at a meeting (a SIDS Initial Assessment Meeting or SIAM) is usually done by developing an assessment report (SIDS Initial Assessment Report or SIAR). The documentation presented at a SIAM also includes an executive summary or SIDS Initial Assessment Profile (or SIAP), which includes one of two recommendations for the meeting participants to consider: either the substance is a priority for further work or it is a low priority for further work. Since 2000, the OECD SIDS Program has been enhanced by the involvement of the Global Initiative on High Production Volume Chemicals of the International Council of Chemical Associations (ICCA) and its affiliated industry associations such as the American Chemistry Council and the European Chemical Industry Council (Cefic).

US HPV Challenge Program

In the United States, 1998 was the year that the HPV Challenge Program was established as part of a voluntary 'Chemical Right-to-Know' (RTK) Initiative between industry and the US Environmental Protection Agency (US EPA). Chemical producers and importers were requested by the US EPA via the HPV Challenge Program to voluntarily provide basic health and environmental toxicity information (the SIDS) on their HPV chemicals. Under this program, a company or consortium of companies (sometimes via a trade association) is sponsoring a chemical or category of chemicals for testing within the period specified by the US EPA.

The United States has \sim 3200 HPV chemicals and \sim 2400 have been volunteered for sponsorship in the HPV Challenge Program. The balance have been: (1) determined to be not of concern (i.e., glucose); (2) already sponsored in the OECD SIDS Program; or (3) not sponsored and thus likely targeted for possible rulemaking by the US EPA to require any missing SIDS data. The information generated through the HPV Challenge Program, including the initial testing plans and the results, is being made publicly available, and companies have compiled the existing publicly available or privately held data, and have designed and submitted test plans to address any data gaps. Further, the companies sponsoring a chemical are providing results as they become available and are preparing data summaries. A rationale for not testing a chemical for a specific endpoint can be provided in lieu of testing, for example, the use of structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs) to compare chemicals and categories of chemicals via their structures, activities, and data sets will be allowed if it meets the available US EPA guidance. Thus, testing one or a few chemicals in a category rather than each chemical in that category will be allowed if judged to be scientifically appropriate. Information on non-SIDS endpoints is also encouraged to be included when available.

In addition to fulfilling the SIDS requirements, the US program will lead to a focus of future efforts on 'priority' chemicals, that is, those that show a likelihood of posing potential harm. Further, there should be greater confidence of the manufacturers, public, and others in the nonpriority chemicals based on their low demonstrated hazard potential. In addition, the US EPA can use the data generated from the program in a risk-based process to make decisions on the need for further information such as additional types of toxicology or environmental testing, or risk management actions. Similar actions are expected outside the United States.

VCCEP

Related to the HPV chemical efforts are other programs such as the Voluntary Children's Chemical Evaluation Program (VCCEP) pilot program in the United States. VCCEP is another part of the US EPA's Chemical RTK Initiative. The goal of the VCCEP program is to enable the US public to better understand the potential health risks to children associated with certain chemical exposures. The US EPA has asked companies that manufacture or import 23 chemicals, which have been found in human tissues and the environment in various monitoring programs, to volunteer for a pilot program and sponsor Tier 1 chemical evaluations. Thirty-five companies and 10 consortia have volunteered to sponsor 20 of the 23 substances. Sponsorship requires the companies to collect or develop health effects and exposure information on their chemical(s) and then to integrate that information in a risk assessment and a 'data needs' assessment. Panels of scientific experts using a peer consultation process are discussing the assessments developed by the sponsors.

HPV Chemical-Related Activities in Europe to Monitor

Finally, major HPV chemical-related activities in Europe to utilize, or to monitor and learn from, include the development of the European Information System on 'Risks from chemicals released from consumer products/articles' (EIS-ChemRisks). EIS-ChemRisks has been designed to be a European-wide expert and stakeholders 'network of networks' to systematically exchange and assess information on risks from chemicals released from consumer products/articles. The overall objective is to develop tools and reference data to enable harmonized exposure assessment procedures in the European Union. These tools and reference data will support the development of a structured stakeholder dialog in the framework of the General Product Safety Directive (GPSD, 2001/ 95/EC) and progressively in the framework of the European Commission's REACH program as it is established and implemented.

See also: European Union and Its European Commission; Organisation for Economic Cooperation and Development.

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Relevant Websites

http://ecb.jrc.it – Allanou R, Hansen BG, and van der Bilt Y (European Commission) Public Availability of Data on EU High Production Volume Chemicals.

- http://ihcp.jrc.it European Commission's Institute for Health and Consumer Protection website (e.g., to access information on European Union activities on new and existing chemicals, implementation and harmonization of alternatives to animal testing and other testing methods, quantitative structure–activity relationship efforts, and the EIS-ChemRisks and REACH efforts).
- http://www.oecd.org Organisation for Economic Cooperation and Development (OECD) (e.g., information the Screening Information Data Set (SIDS) program); see

also 1987 Council Decision-Recommendation on the Systematic Investigation of Existing Chemicals, and a further OECD Council Decision in 1991 focused on HPV chemicals. Both documents available at the OECD website.

http://www.epa.gov – US Environmental Protection Agency: Chemical Right-to-Know Initiative; HPV Challenge Program; HPV Chemical Human Health Testing: Animal Welfare Issues and Approaches; Voluntary Children's Chemical Evaluation Program (VCCEP).

History of Toxicology See Toxicology, History of.

Holly

Ann P Slattery

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• SYNONYMS: *Ilex* species; *Ilex aquifolium* – Christmas holly, English holly, European holly, Oregon holly, and prick holly; *Ilex cornuta* – Chinese holly; *Ilex opaca* – American holly; *Ilex vomitoria* – black drink, deer holly, emetic holly, and yaupon

Uses

Evergreen shrubs and trees with stiff leathery leaves that may grow from 10 to 50 ft depending on the species. The berries are usually bright red, but some cultivars may have yellow berries. The leaves of the *aquifolium, cornuta,* and *opaca* have spiny, prickly leaves, while the leaves of the *vomitoria* are serrated, but spineless. These plants are primarily used in gardening. However, several folk remedies contain plant material or extracts from different *Ilex* species. In addition, *Ilex asprella* contains a variety of cyctotoxic compounds that have been tested on several melanoma cell lines.

Exposure Routes and Pathways

Exposure occurs via ingestion of plant material (i.e., leaves and berries).

Mechanism of Toxicity

It is believed the saponins may be responsible for gastrointestinal effects, although the exact mechanism of action is unknown. The toxins involved include ilicin, tannic acid, ilexanthin, ilicic acid, and (in *I. aquifolium*) cyanogenic glycosides. These toxins may be found in the leaves and fruit (berries) of the plant.

Acute and Short-Term Toxicity (or Exposure)

Human

Although symptoms will vary with different types of holly, the predominant toxic effect appears to be gastrointestinal irritation. Ingestion of small amounts of plant material may result in mild to moderate gastritis resulting in nausea, vomiting, abdominal pain, and diarrhea. Mild central nervous system (CNS) depression may be evident. Ingestion of the thorny leaf can cause mechanical irritation. Larger amounts have the potential to cause more severe gastrointestinal irritation along with varying degrees of CNS depression.

Clinical Management

Most unintentional holly exposures result in selflimited gastrointestinal symptoms with no specific treatment needed. The main goal of therapy for holly ingestions is fluid replacement and supportive care. There is no specific antidote available. Activated charcoal may be used for substantial recent ingestions. For patients who are symptomatic with significant gastrointestinal effects, intravenous fluid replacement may be used if oral liquids cannot be tolerated.

See also: Plants, Poisonous.

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Hormesis

Elyisha A Hanniman and Christopher J Sinal

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Introduction

Hormesis is commonly defined as a beneficial or stimulatory effect caused by exposure to low doses of an agent known to be toxic at higher doses. Conceptually, this is represented by a U-shaped doseresponse curve for toxicity where hormetic effects become smaller (after a maximum) with increasing dose up to a threshold, after which toxicity increases with dose (Figure 1a). Not surprisingly, proponents of hormesis are frequently at odds with other scientists, particularly those who favor a no threshold, linear dose-response curve (Figure 1b) or a threshold dose-response curve (Figure 1c). The beneficial effects attributed to low-dose exposure to various toxic chemical and physical agents include increased life span, improved rates of growth and development, decreased tumor incidence, and increased resistance to infection and tolerance to radiation.

Radiation Hormesis

One of the most intensively studied areas of hormesis research is the effects of exposure to low-level ionizing radiation (LLIR). The concept that LLIR can Rodriques TD, Johnson PN, and Jeffrey LP (1984) Holly berry ingestion: Case report. *Veterinary Human Toxicology* 26: 157–158.

produce beneficial effects in biological systems challenges the conventional radiation paradigm which asserts that (1) radiation exposure is harmful at all dose levels (i.e., there is no threshold); and (2) there are no effects at low doses that cannot be predicted from effects observed at high dose levels (i.e., linear or near-linear dose-response curve). The application of this paradigm extends from single cells to entire populations where it is assumed that the risk of deleterious effects to a population increases directly with the aggregate radiation exposure. In contrast, proponents of hormesis have argued that LLIR exposure produces health benefits that translate into a decreased risk (relative to zero level exposure) of the deleterious effects associated with higher doses of radiation exposure.

There is no doubt that biological systems can respond to exposure to toxic physical and chemical agents in a manner that reduces the severity of the insult. Frequently, these responses are a result of altered gene expression, such as the increased synthesis of heat shock or stress proteins following exposure to thermal or oxidant stress. Such protective effects have also been demonstrated experimentally following exposure to low-level radiation when discrete, sensitive cellular and/or biochemical end points are measured. For example, the level of oxygen free radical scavengers, which protect against the toxic effects of radiation, was shown to increase in bone marrow cells of mice after whole body exposure to as little as 0.1 gray (Gy) of cesium-137 gamma irradiation.

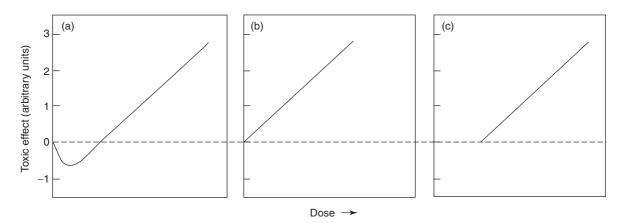


Figure 1 Idealized dose-response curves for the (a) hormesis, (b) linear, and (c) threshold hypotheses. A 'positive' toxic effect is regarded as detrimental, whereas a 'negative' toxic effect is beneficial (hormetic).

(The Gy is a unit of dose absorbed by a material and is equivalent to 1 J kg⁻¹.) Temporary suspension of DNA synthesis was also observed after 0.01 Gy exposure, an effect that might result in the formation of fewer free radical-mediated DNA lesions during the exposure and facilitate the repair of lesions caused by free radical attack. If a higher concentration of radical scavengers or antioxidants was produced or released than required for neutralization of the excess free radicals produced by irradiation treatment, this would constitute hormesis. The 'excess' radical scavengers available might produce a beneficial effect by protecting against the damage caused by background radiation, by subsequent free radical challenges induced by radiation exposure, or, alternatively, by normal cellular metabolism.

In another example, the growth of lymphocytes in the presence of $0.01-0.10 \,\mu\text{Ci}\,\text{ml}^{-1}$ of [³H]thymidine or very low (0.005-0.01 Gy) doses of X-rays, while not producing deleterious effects, has been reported to confer resistance to chromosomal breakage upon subsequent exposure to 1.5 Gy of X-rays. (The curie (Ci) is a unit of radioactive source activity and is equivalent to 3.70×10^{10} disintegrations per second.) The low-dose radiation-associated decrease in DNA damage could be eliminated by the addition of 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase when added after the high-dose exposure. This indirect evidence suggests that the LLIR exposure induces a DNA repair mechanism involving this enzyme, and thus, hormesis. Critics of these studies have suggested that the small doses of LLIR employed would result in only very small, transient increases above the normal steady-state concentrations of intracellular free radicals and thus would be unlikely to activate repair mechanisms.

Investigations with the protozoan Paramecium tetraurelia and the cyanobacterium Synechococcus lividus have provided evidence for hormesis using cell growth parameters as a biological endpoints. The growth rate of either organism within chambers shielded from normal background radiation was depressed up to 50% when compared with growth in unshielded chambers. Critical to the hormesis concept, the growth rate in shielded chambers could be restored to unshielded values when the organisms were subjected to LLIR exposure at doses comparable or slightly greater than normal background levels $(7-20 \text{ mGy year}^{-1})$. This LLIR stimulatory effect disappeared at dose rates $\geq 50 \,\mathrm{mGy}\,\mathrm{year}^{-1}$. These data have been interpreted to suggest that LLIR stimulates proliferation of these single-cell organisms, although an exact mechanism(s) has not been demonstrated.

Epidemiological studies involving large populations remain the best means available for the study of potential hormetic effects in humans. A study of atomic bomb survivors from Nagasaki suggested that significantly lower (65% of control) mortality rates from noncancerous diseases occur in males exposed to 50-149 cGy of bomb-related radiation as compared to unexposed, age-matched controls. However, these data conflict with those of other studies that have failed to produce significant evidence for beneficial effects among atomic bomb survivors exposed to low levels of radiation. Some proponents of radiation hormesis have suggested that the lack of hormetic effects among atomic bomb survivors is because the data set represents the effects of acute exposure to radiation and is, therefore, not a valid comparison to the chronic, LLIR exposure ideally associated with hormesis.

The relationship of the annual cancer incidence rate to the intensity of natural background radiation has also been studied. The annual ageadjusted mortality rate from all cancers in five major Indian cities was found to decrease at a rate of 0.3 mrem⁻¹ year⁻¹ increase in the external background radiation dose from a hypothetical incidence level of 79 per 100000 corresponding to a hypothetical 'zero environmental radiation' level. (The rem is a unit of the biological effects of radiation and is equivalent to the dose absorbed \times a quality factor specific for individual types of radiation.) A similar study from the Guangdong province of China examined two similar population groups of 60000 people living in adjacent areas but whose exposure to background radiation was 330 and 114 mrem year⁻¹, respectively. An analysis of total cancer mortality from 1970 to 1986 failed to show a significant beneficial (hormetic) effect in the high background group. When nonleukemic cancers of inhabitants aged 40-70 years from 1970 to 1986 or all cancers within the entire population from 1975 to 1978 were considered separately, however, a significant 14.6% lower cancer mortality rate in the high background group was found. Interpretations of these studies are controversial and have been criticized primarily because of the problems associated with the limited time spans evaluated, lack of an accurate assessment of radiation exposures, and confounding factors such as smoking, diet, and lifestyle.

A number of epidemiological studies have reported that inhalation of naturally occurring radon in the home is associated with an increased risk of lung cancer. In contrast, a large US-wide ecological study performed in 1995 reported an inverse relationship between the average county radon concentration and the average lung cancer rates in the county. This report of an apparent hormetic effect of radon exposure has sparked considerable debate among investigators in this area. Critics contend that consideration of the mean risk for a county rather than the more accepted practice of using individual risk has biased these results. It has also been suggested that confounding social and geographical factors were not adequately considered in these results. In spite of these criticisms, this study remains a subject of considerable discussion and intrigue within the scientific community.

Chemical Hormesis

Numerous reports of chemical-mediated hormesis in response to exposure of plants or animals to a wide variety of compounds appear in the literature. Chemical hormesis is characterized by a biphasic or Ushaped dose-response curve (Figure 1a), where very low doses of toxicants have a beneficial or stimulatory effect, relative to control exposures, and moderate to high doses are obviously toxic. The concept of chemical hormesis also challenges the linear, no threshold dose-response model (Figure 1b) and the threshold dose-response model (Figure 1c), especially with respect to carcinogens. Metals, in particular, have received much attention with respect to possible hormetic effects. Administration of 22 mg kg^{-1} of tetramethyl lead, a known central nervous system toxicant, for 60 days to pregnant rats and subsequently to their offspring was reported to produce an increase in weight gain in the absence of deleterious effects on brain weight or morphology. The authors have presumed this represents a stimulatory effect of low levels of lead on body growth, although critics have maintained that it could also result from stimulation of appetite.

Preexposure of the tidal fish *Fundulus heteroclitus* to 0.05 mg l^{-1} concentrations of cadmium prior to partial amputation of the caudal fin resulted in a 5–15% faster rate of regeneration in water containing 0.1 mg l^{-1} cadmium compared with unexposed fish allowed to regenerate in 'clean water' assumed to be cadmium free. This effect, however, was observed in only two of three experiments conducted. While the mechanism for this effect was not clear, the increased rate of regeneration was hypothesized to be due to an overcompensation of homeostatic regulation resulting in accelerated growth.

Low concentrations of carcinogenic polycyclic aromatic hydrocarbons (PAHs) have also been evaluated for a potential hormetic effect. Exposure of grunion embryos (*Leuresthes tenuis*; a freshwater teleost) to 7 ppb of benzo[*a*]pyrene resulted in significantly increased respiration rates compared with unexposed controls, whereas at higher concentrations of 24.2, 361, or 868.8 ppb, respiration rates were not significantly different from control. Although no benefit of the increased respiration rate is obvious, it may be that this or similar metabolic responses to lowlevel PAH exposure represent an overcompensatory response typical of hormesis. It might be argued, however, that an adaptive biological response to low toxicant exposure in the absence of a clearly demonstrated benefit, preferably at the molecular mechanism level, does not constitute hormesis.

Hormesis studies in humans have been performed using populations exposed occupationally to toxic chemicals. Chronic exposure to low levels (5-40 ppm) of the hepatotoxicant, trichloroethylene (TCE), has been associated with changes in cholesterol metabolism rather than causing hepatic cell damage. In particular, elevated levels of serum highdensity lipoprotein-associated cholesterol (HDL-C) were noted, while levels of the serum enzymes aspartate aminotransferase, alanine aminotransferase, and y-glutamyl transpeptidase were unaffected. Because HDL-C is thought to be protective against coronary heart disease and to improve longevity, elevated serum levels may have a beneficial effect. However, the significance of the trend for HDL-C to increase with the dose of low-level exposure was only marginal (p = 0.08). Furthermore, evidence for decreased incidence of heart disease and/or increased longevity was not part of this epidemiological study; perhaps a follow-up investigation is possible. What we are left with is the demonstration of a biological change that is apparently associated with low-level occupational exposure to TCE and that may be beneficial. However, further epidemiological studies employing definitive biological end points, with careful controls for lifestyle and other variables, are required to definitively demonstrate that chemical hormesis is a common beneficial mechanism resulting from lowlevel exposure to toxic chemicals in humans.

Conclusions

Adaptations based on changes in gene expression and/or post-translational protein modification are commonly elicited in response to exposure to toxic physical or chemical agents. It is widely believed that these responses act to reduce, but not eliminate the severity of injury caused by all doses (i.e., no threshold) of the agent. In contrast, the theory of hormesis predicts that while higher concentrations of these agents are indeed deleterious, low level exposure elicits a net beneficial response from the organism. While many of the studies that purport to show hormesis suffer from flaws in experimental design and an unimpressive response (the difference between biological significance and statistical significance is relevant here), the volume of published research material alone suggests that further investigation is warranted. Important aspects that must be evaluated include the dose–response relationship and the specific mechanism or mechanisms of the 'hormetic effect'. Additional insight might also be gained through reevaluation of data sets from previous studies in which appropriate end points have been evaluated and/or from archival databases of epidemiological data. Indeed, a number of recent studies have reexamined the data from previous chemical and radiation toxicity experiments and have identified numerous examples of possible chemical hormesis for a variety of end points studied.

In conclusion, chronic exposure of organisms to physical or chemical stressors can result in changes in gene regulation that confer a protective (detoxication) effect against the stressor in the exposed organism. The issue remains, however, whether chronic exposure to low concentrations of toxic chemicals or radiation can confer long-term beneficial effects to the organism and whether this is a general protective response. However, given the conservative nature of risk assessment, even irrefutable evidence for a hormetic response to a particular toxic agent is unlikely to lead to abandonment of the conservative linear, no threshold dose-response model.

See also: Dose–Response Relationship; Radiation Toxicology, Ionizing and Nonionizing.

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Hormone Disruptors See Environmental Hormone Disruptors.

Hormones See Anabolic Steroids; Androgens; Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals; Estrogens V: Xenoestrogens.

Host-Mediated Assay

David A Eastmond

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The host-mediated assay (HMA), developed in the late 1960s by Gabridge and Legator, is an approach for providing the *in vivo* metabolism of a whole animal (the host) for assessing effects on indicator cells that have been placed in the host during chemical exposure and then subsequently removed for *in vitro* measurements of mutagenicity. Due to the limited metabolic capacity of most bacterial and mammalian

target cells used for *in vitro* genetic toxicology, a number of chemicals found to be mutagenic and carcinogenic in whole animals were without genotoxic effects *in vitro* as the assayed cells lacked the metabolic systems which could convert the nonreactive test chemicals into the reactive electrophiles that were capable of interacting with nucleic acids and proteins. The HMA was consequently developed to overcome the metabolic limitations of commonly used test cell lines.

The HMA is of historical significance to the field of genetic toxicology primarily for two reasons: (1) its development preceded that of the now widely used exogenous liver homogenates (e.g., the 9000 g supernatant fraction, termed S9) that are added to *in vitro* test systems to approximate *in vivo* metabolic pathways; and (2) in the early 1970s, the HMA was one of three original screening tests recommended for evaluating the mutagenic effects of chemicals, together with the dominant lethal test and the *in vivo* cytogenetic assay.

In the host-mediated mutagenicity assay as initially defined, a microbial indicator organism in which mutation frequencies could be measured, was injected into the peritoneal cavity of the host, and the host was then treated with a potential chemical mutagen. Subsequently, the microorganisms were withdrawn from the host, and the induction of mutants was assessed following growth of the microorganism *in vitro*. Application of the HMA was later expanded to include additional sites of inoculation and recovery of the indicator cells (such as the blood, intestinal tract, liver, spleen, lungs, and testes) and the use of nonbacterial indicator cells, including *Neurospora*, yeast, and various types of mammalian cells.

Since its introduction, the HMA has been used to evaluate several hundred chemicals, with the measured genetic effects including forward and reverse mutations, recessive lethal mutations, mitotic gene conversion, mitotic recombination, differential DNA repair, sister chromatid exchanges, chromosome deletions and aberrations, and alterations in cellular and colony morphology. However, the HMA was not universally successful, in part because in the early 1970s it was initially coupled with mutagenesis systems that were insufficiently defined and validated. Other problems included a failure to allow for animal-to-animal variability, contamination of bacterial indicator cells with intestinal flora, and difficulties in differentiating between the mammalian indicator cells and cells from the host.

As a result, the HMA was no longer recommended for mutagenicity testing for regulatory submissions, when the technically more straightforward and less expensive use of S9 metabolic activation became available. It was also found that very few chemicals were positive in the HMA that were not also positive for mutagenicity when tested with S9, primarily due to the predominance of activating enzymes in this subcellular fraction. It should be remembered, however, that *in vitro* S9 metabolic activation systems cannot address *in vivo* processes such as absorption, storage and excretion, hormonal influences, and tissue-specific metabolism, including preferential enzymatic detoxification.

After a decade of use, host-mediated mutagenicity testing was essentially abandoned for regulatory testing. More recently, a number of the problems initially encountered with the HMA have been resolved or are capable of resolution. *In vitro* mutagenesis systems have now been extensively defined and evaluated; animal-to-animal variability can be minimized by using a sufficient number of animals per dose; and both contamination of indicator cells and difficulties in differentiating between cells from the host and mammalian indicator cells can be overcome by using devices such as diffusion chambers which can be surgically implanted into the host.

Considering these developments, the HMA could be employed more frequently. However, in practice, the HMA continues to be used infrequently in genetic toxicology and is applied primarily for specialized research needs. In addition, the need for the HMA has been supplanted in recent years by the use of metabolically competent cell lines and new *in vivo* mutation detection systems such as the transgenic Big Blue or Mutamouse systems, which in some ways may be viewed as more technologically advanced forms of the HMA.

See also: Ames Test; Carcinogenesis; Chromosome Aberrations; Dominant Lethal Tests; In Vitro Test; In Vivo Test; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Sister Chromatid Exchanges; Toxicity Testing, Alternatives.

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Hydrangea

Brenda Swanson-Biearman

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- SYNONYMS: Hydrangea arborescens; Hydrangea macrophylla; Hydrangea paniculata; Seven bark; Wild hydrangea
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Cyanogenic glycosides

Uses

Although there are no accepted therapeutic uses for hydrangea, the dried root has been reportedly used as a diuretic and cathartic.

Exposure Routes and Pathways

Exposure occurs via dermal contact and ingestion.

Toxicokinetics

Small doses of cyanide are converted to thiocyanate by an enzymatic reaction catalyzed by rhodanase. The rhodanase system can detoxify large amounts of cyanide but may not respond quickly enough to avert serious symptomatology.

Mechanism of Toxicity

The leaves and buds contain hydrangin, which has the potential to produce cyanide. When the plant material is ingested, it reacts slowly in the acid pH of the stomach. Once transportation into the alkaline medium of the duodenum occurs, the process is hastened. Hydrocyanic acid is produced forming a stable complex with the ferric iron and cytochrome oxidase, thereby inhibiting the activity of the enzyme and aerobic metabolism. Cells containing cytochrome oxidase become hypoxic because they are unable to utilize available oxygen. However, symptoms of

Hydraulic Fluids

Richard D Phillips

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Uses

The most common hydraulic fluids are generally of three types: mineral oil, polyalphaolefin (PAO, synthetic oil), or organophosphate ester-based with a cyanide poisoning from exposure to *Hydrangea* species have not been reported in modern medical literature.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity associated with hydrangins is not known in animals, but ruminal microorganisms have the ability to degrade cyanogenic glycosides causing the release of hydrogen cyanide. However, no recent cases of toxicity have been described.

Human

Cyanide toxicity due to accidental exposure is unexpected. Patients ingesting hydrangea may develop vomiting and epigastric soon after exposure. Allergic contact dermatitis due to the sensitizer, hydrangenol, has been reported.

Clinical Management

In asymptomatic patients, activated charcoal and observation may be all that is necessary. In the unlikely event that patients exposed to hydrangea develop symptoms consistent with cyanide poisoning, ignore gastric decontamination procedures until life-support measures have been instituted. Cyanide antidote kit administration may be necessary.

See also: Cyanide.

Further Reading

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small percentage of additives. The majority of hydraulic fluids are mineral oil-based. Their primary function has typically been to transfer pressure from one point to another in machinery or mechanical operations. They also serve as lubricants depending on the specific application. They are used for automobile automatic transmissions, brakes and power steering, as well as heavy machinery.

Exposure Routes and Pathways

Exposure to hydraulic fluids occurs mainly in workers using hydraulic equipment and in maintenance workers on cars, tractors, airplanes, or similar equipment. The components of hydraulic fluids are in other lubricant products as well so exposure is not limited to hydraulic fluids. However, exposure is primarily to the major components (i.e., mineral oil, PAO, or organophosphate ester). Exposures would result from contact with the skin or inhalation of oil mist. In rare instances, oral exposure could occur by accident or eating contaminated food.

Toxicokinetics

The toxicokinetics of hydraulic fluids will be dependent on the type of base oil and the key additives, which in many cases are proprietary. Hydraulic fluids or some of their components are likely to be poorly absorbed from the gastrointestinal (GI) tract or lungs. Accumulation of oil in lungs could occur at high and fairly prolonged exposure. Absorption through the skin would likely be limited and dependent on duration of contact. Hydrocarbons found in mineral oils are not expected to undergo extensive metabolism. The same would be true for PAO-based hydraulic fluids. Some chemical components of organophosphate ester hydraulic fluids can enter the body from the lungs and GI tract.

Mineral oils and PAOs found in hydraulic fluids are not anticipated to undergo appreciable metabolism. There is some evidence that organophosphate esters are oxidized by cytochrome P450 and then form conjugates and are excreted.

Mechanism of Toxicity

Mineral oil and PAO-based hydraulic fluids are generally not toxic. They are expected to be absorbed only to a limited extent by lungs, skin, and the GI tract.

Certain organophosphate esters cause neurotoxicity due to cholinesterase inhibition. Current manufacturing processes for organophosphate esters used in hydraulic fluids are designed to minimize the production of toxic isomers such as tri-o-cresyl phosphate.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute toxicity reports on hydraulic fluids are very limited. Fairly high exposure levels would not be expected to be lethal ($LC_{50} > 1000 \text{ mg m}^{-3}$). Exposure to high concentrations of mist could cause damage to the lungs and may irritate the respiratory

tract. Aspiration into lungs following oral exposure could cause severe lung damage and even death.

Organophosphate ester-based hydraulic fluids could cause neurologic effects but only if the toxic isomers are present in the fluid. Specific organophosphate esters have been synthesized to be toxic to insects. These organophosphate esters inhibit neural acetylcholinesterase and at toxic levels can cause increased salivation, watering of the eyes, perspiration, dilated pupils, nausea, vomiting, diarrhea, slowing of heart rate, and frequent urination. Symptoms can also include muscle cramps, weakness, and paralysis. Organophosphate esters can also cause a syndrome referred to as organophosphate-induced delayed neuropathy. This is a syndrome observed in humans and some animal models after exposure to certain organophosphate esters such as tri-o-cresyl phosphate.

Chronic Toxicity (or Exposure)

Animal

Hydraulic fluids have not been shown to be carcinogenic in animal cancer bioassays or mutagenic in other test systems.

Human

Hydraulic fluids are not likely to cause chronic toxicity or cancer as currently manufactured and used. Only one epidemiology study has been conducted for a mineral oil-based fluid, and there were no associations between exposure and cancer. The mineral oils used in hydraulic fluids are highly refined and not mutagenic or carcinogenic in animals. The same would be expected for PAOs.

Clinical Management

Gastric emptying by either lavage or vomiting is contraindicated since there is a danger of pulmonary aspiration and subsequent pneumonitis. If a person is overexposed to oil mist, the victim should be moved to fresh air as quickly as possible and monitored to make sure that chemical pneumonitis is not present. If acute effects of central nervous system depression are present, the appropriate treatment may be indicated.

Washing with soapy water is suggested following dermal contact, and ocular washing with water following eye contact.

Environmental Fate

Hydraulic fluids represent a wide range of products which are formulated to conform to performance specifications and not to specific chemical or fate analysis. However, some conclusions can be made based on what's known regarding the major component (i.e., the base oil). The carbon number present in mineral oil hydraulic fluids ranges from C_{15} to C_{50} . These oils have low water solubility and will tend to partition to sediments if released. These oils will degrade over time and show little tendency to bioaccumulate.

Hydrazine

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 302-01-2
- SYNONYMS: Diamine; Hydrazine anhydrous
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Miscellaneous nitrogen compounds
- CHEMICAL FORMULA: H₄N₂

Uses

Hydrazine is a chemical intermediate for explosives. It is used in soldering fluxes, as a laboratory reagent, and as a substance to reduce metals of silver, gold, and copper. Hydrazine is also used in the manufacture of certain drugs, dyes, rocket fuel, photographic supplies, insecticides, plastics, corrosion inhibitors, and textiles.

Exposure Routes and Pathways

Exposure to hydrazine may occur by inhalation, dermal contact, ingestion, and injection.

Toxicokinetics

Hydrazine is a colorless oily liquid that fumes and is flammable. Absorption occurs by all routes of administration. Several possible metabolic pathways exist for hydrazine and include hydrolysis, acetylation, and the splitting of hydrazine into two equal amines. Acetylation appears to have a major role in humans and slow acetylators may be more susceptible to toxic effects. Hydrazine and its metabolites are excreted by the kidneys.

Mechanism of Toxicity

Hydrazine has strong caustic action on the skin and mucus membranes. Hydrazine produces a functional pyridoxine deficiency that can lead to seizure activity. Hydrazine causes hepatic necrosis. The gastrointestinal See also: Fuel Oils; Organophosphates.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hydraulic Fluids.

effects are due to local irritation and corrosive effects if ingested.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation exposure to guinea pigs and dogs resulted in rapid weakness, significant liver damage, and injury to the kidneys and lungs. Muscular tremors have been noted in dogs following inhalation exposure. Exposure to pregnant toads to hydrazine resulted in teratogenic effects. Pregnant rats exposed to hydrazine had dose-related embryolethality and maternal toxicity. There was increased tumor incidence in mice (pulmonary tumors), rats (liver tumors), and hamsters (liver tumors). Hydrazine also reduces the latency period associated with the development of tumors in exposed rats. Hydrazine has been shown to be mutagenic in bacteria, viruses, and mammalian assays.

Human

Hydrazine's odor is ammonia-like or 'fishy' and is detectable by smell at 1–10 ppm. Because hydrazine is a marked corrosive, it can cause chemical burns of the skin. Hydrazine vapors may cause irritation of the mucus membranes of the eyes, nose, throat, and respiratory tract. Inhalation of vapors can produce cough, dyspnea, and pulmonary edema. Eye exposure to vapors or liquid can result in conjunctivitis, corneal damage, and blindness. Other clinical effects include nausea, vomiting, tremors, dizziness, hyperreflexia, seizures, hypotension, liver necrosis, methemoglobinemia, and hemolysis. The National Institute for Occupational Safety and Health recommends that the level of hydrazine in workplace air not exceed 0.03 parts of compound per million parts of air (0.03 ppm) for a 2 h period. The Occupational Safety and Health Administration (OSHA) limits the amount of hydrazine in workplace air to 1 ppm for an 8h workday. The Environmental Protection Agency (EPA) requires that spills or accidental releases into the environment of 1 pound or more of hydrazine be reported to the EPA.

Chronic Toxicity (or Exposure)

Human

Chronic dermal exposure to hydrazine can result in eczema and skin sensitization. The International Agency for Research on Cancer has determined that hydrazine is a possible human carcinogen. The EPA has determined that hydrazine is a probable human carcinogen. The American Conference of Governmental Industrial Hygienists currently lists hydrazine as suspected carcinogens, but has recently recommended that the listing of hydrazine be changed to that of animal carcinogen, not likely to cause cancer to people under normal exposure conditions.

In Vitro Toxicity Data

Hydrazine is mutagenic in several *Salmonella* models as well as in an *H. influenza* model. However, the *H. influenza* model, hydrazine was postulated to have both mutagen and antimutagen effects.

Clinical Management

If dermal or eye contact with the liquid occurs, the affected areas should be flushed thoroughly with

Hydrobromic Acid 537

water for at least 15-30 min and then observed for resulting irritation. In case of inhalation, the victim should be moved to fresh air and the patient should be monitored for respiratory irritation and pulmonary edema. If ingestion occurs, basic and advanced life-support measures should be utilized as necessary. Due to its potential caustic effects, gastrointestinal decontamination procedures and charcoal should be avoided. The use of methylene blue should be considered in the treatment of hydrazine-induced methemoglobinemia. Seizures should be treated with high-dose pyridoxine (vitamin B₆) at doses of 1-5 g intravenous and treated with benzodiazepines.

See also: Pyridoxine.

Further Reading

- Clark DA, Bairrington JD, and Bitter HL (1968) Pharmacology and toxicology of propellant hydrazines. *Aeromedical Reviews* 11: 1–126.
- Kirklin JK, Watson M, and Bondoc CC (1976) Treatment of hydrazine-induced coma with pyridoxine. *New England Journal of Medicine* 294: 938–939.

Hydrobromic Acid

Mary Lee Hultin

Published by Elsevier Inc.

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 10035-10-6
- SYNONYMS: Hydrogen bromide; Hydrogen bromide, anhydrous; Anhydrous hydrobromic acid
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Inorganic acid; Corrosive
- CHEMICAL FORMULA: HBr
- Chemical Structure: H–Br

Uses

Hydrobromic acid is used in the manufacture of inorganic bromides for use in photography, pharmaceuticals, industrial drying, textile finishing, engraving, lithography, and in fire retardants. It is also used as a reagent in analytical chemistry.

Exposure Routes and Pathways

Exposure may occur by the oral, inhalation, dermal, and ocular routes.

Toxicokinetics

No information is available regarding the toxicokinetics of hydrogen bromide in humans or animals.

Mechanism of Toxicity

The primary mechanism by which hydrogen bromide exerts its toxic effects is via irritation upon contact with tissues. Hydrogen bromide is a potent irritant of the tissues of the mouth, nose, eyes, and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

In a comparative toxicity study, hydrogen bromide caused more severe burns to the skin than hydrogen chloride or hydrogen iodide. In another study comparing the acute toxic effects of hydrogen fluoride, hydrogen bromide, and hydrogen chloride, rats were exposed to 1300 ppm hydrogen bromide for 30 min. Rats were separated into two groups. The first group consisted of ordinary mouth-breathing rats and the second group consisted of rats fitted with an apparatus to simulate nose breathing. More than twice as many rats in the pseudo-mouth-breathing group died as in the nose-only group and none died in the control group. The location of the lesion was found in the nasal passages of the nose-breathing group and in the trachea of the pseudo-mouth-breathing group. The inhalation (LC_{50}) in rats is 2858 ppm for 1 h. The inhalation LC_{50} in mice is 814 ppm for 1 h.

Human

Subjective responses of six human volunteers exposed to levels ranging from 2 to 6 ppm for several minutes were reported as follows: exposure to 5 or 6 ppm resulted in nasal irritation in all volunteers and throat irritation in one volunteer. Eye irritation was not reported at any of the tested concentrations. One individual noted nasal or throat irritation at the 3 ppm level. Odor was detectable at all concentrations tested. Higher levels of inhalation exposure can produce pulmonary edema. If a solution is splashed on the skin or eyes, it will cause a burn. Ingestion can cause burns of the stomach.

Two clinical cases of reactive airways dysfunction syndrome were reported after inhalation exposure in a hot tub where hydrobromic acid and bromine were generated from the disinfectant used.

Clinical Management

Personnel not wearing personal protective equipment should be restricted from areas of spills or leaks until cleanup has been completed. In the case of exposure to the eyes or skin from hydrogen bromide solutions, contaminated areas should be flushed with copious amounts of water for at least 15 min. If an individual inhales large amounts of hydrogen bromide, this person should be moved to fresh air at once. Artificial respiration should be performed if breathing has stopped. In the event of ingestion of hydrogen bromide solution, large quantities of water or milk should be given immediately, provided the individual is conscious. Vomiting should not be induced.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 3 ppm (10 mg m^{-3}) as an 8 h, time-weighted average. The American Conference of Governmental Industrial Hygienists and National Institute for Occupational Safety and Health recommend 3 ppm as a ceiling concentration. The Revised (1996) IDLH level is 30 ppm. The temporary emergency exposure limits (TEELs) are: TEEL-0, TEEL-1, and TEEL-2 (μgm^{-3}) 10000; TEEL-3 (μgm^{-3}) 100 000.

The US Department of Energy classifications for TEELs are:

- TEEL-0 is the threshold concentration below which most people will experience no appreciable risk of health effects.
- TEEL-1 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing other than mild transient adverse health effects or perceiving a clearly defined, objectionable odor.
- TEEL-2 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action.
- TEEL-3 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing life-threatening health effects.

See also: Acids; Corrosives.

Further Reading

Braker W and Mossman A (1980) Matheson Gas Data Book, 6th edn., p. 373. New York: McGraw-Hill.

Hydrochloric Acid

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7647-01-0
- SYNONYMS: Aqueous hydrogen chloride; Chlorohydric acid; HCl; Hydrochloride; Hydrogen chloride; Muriatic acid; Spirits of salt
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Inorganic acid; Corrosive

Uses

Hydrochloric acid (HCl) is commonly used for the neutralization of alkaline agents, as a bleaching agent, in the synthesis of dyes and chemicals, and in metal refining.

Exposure Routes and Pathways

Exposure may occur via dermal, ocular, enteral, parenteral, or inhalation routes.

Toxicokinetics

HCl is colorless to light-yellow as both a gas and liquid with an irritating, pungent odor. HCl dissociates in water to hydronium and chloride ions. HCl is highly water soluble.

Mechanism of Toxicity

HCl causes local pH changes and denatures proteins. This leads to edema formation and tissue necrosis. HCl produces a coagulation necrosis characterized by the formation of an eschar. Ingested HCl may give rise to damage of the esophagus and stomach. Gastric damage may occur secondary to pooling of HCl in the antrum as a result of pylorospasm. Patients who survive ingestions of HCl may develop stricture formation, gastric atony, and gastric outlet obstruction. When inhaled, HCl typically deposits in the upper respiratory tract and causes damage. Concentrated HCl can penetrate to the level of the brochioles and alveoli and cause subsequent damage to these regions.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, HCl is a severe irritant of the eyes and respiratory system. The 30 min LC_{50} values in rats and mice are 4701 and 2644 ppm, respectively. Animals exposed to high concentrations of HCl gas developed necrosis of the tracheal and bronchial epithelium, pulmonary edema, atelectasis, emphysema, and damage to the pulmonary blood vessels and liver. Chronic exposure to 10 ppm for 6 h day⁻¹ for life did not cause neoplastic lesions or serious irritant effects in the nasal epithelium of rats. In experimental animals, exposure to a concentration of 1350 ppm hydrogen chloride gas caused clouding of the cornea after 1.5 h and exposure to 3000 ppm for 6 h caused slight erosion of the corneal epithelium.

Human

Acute eye exposure to HCl gas or solutions of HCl may cause eye irritation and permanent damage with loss of sight. Dermal exposure may cause burns, with degree depending upon the concentration and duration of the exposure. Inhalation of HCl immediately causes severe irritation with cough and choking sensation. Inflammation and ulceration of the upper respiratory tract may occur. Pulmonary edema can develop if HCl gas is inhaled deeply. Excessive exposures (e.g., 1000–2000 ppm) for a few minutes can cause life-threatening pulmonary edema. Severe breathing difficulties may be delayed in onset. The current Occupational Safety and Health Administration permissible exposure limit (PEL) for hydrogen chloride is 5 ppm (7 mg m⁻³) as a ceiling limit. Ingestion may cause corrosion of the mucous membranes, esophagus, and stomach with dysphagia, nausea, vomiting, abdominal pain, and hematemesis. Circulatory collapse and death may occur.

Chronic Toxicity (or Exposure)

Animal

Vapor concentrations of hydrochloric acid 100 ppm daily for 50 days in guinea pigs, pigeons, and rabbits resulted in symptoms of only minor irritation.

Human

Chronic exposure to HCl may cause erosion of the teeth, bronchitis, and gastritis. Repeated exposure of the skin to dilute solutions of hydrogen chloride may cause a rash.

In Vitro Toxicity Data

Low concentrations of HCl were required to dramatically reduce the spore concentration of *Bacillus subtilus*. Concentrations as low as $6 \text{ mg} \text{l}^{-1}$ can inhibit some plant stem growth.

Clinical Management

The first priority in management of patients exposed to HCl is assuring patency of the airway. Direct visualization of the pharynx and vocal cords with fiberoptic devices may be necessary after inhalational and oral exposures to assure lack of injury. If signs of airway edema are present, intubation should be considered as swelling may progress over ensuing minutes and lead to airway obstruction. Fiber-optic intubation or orotrachael intubation with a laryngoscope can be attempted. Emergent surgical airway intervention may be necessary. Vomiting should not be induced. Gastric lavage, syrup of ipecac, activated charcoal, and cathartics should be avoided. There is questionable efficacy in giving water or milk to an awake and minimally symptomatic person who has ingested HCl. The utility of dilution decreases with time and should not be administered to persons with vomiting, airway compromise, significant abdominal pain, or altered mental status.

Following ophthalmic exposure to HCl, the eyes should be irrigated immediately with water for at least 15 min and continued until pH neutralization. If skin exposure occurs, contaminated clothing should be removed and the exposed areas flushed with water. The exposed person should be moved to fresh air.

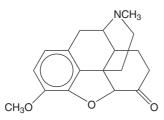
See also: Acids.

Hydrocodone

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 125-29-1 (Hydrocodone); CAS 143-71-5 (Anhydrous hydrocodone tartrate); CAS 34195-34-1 (Hydrocodone tartrate)
- SYNONYMS: Anexsia; Calmodid; Curadol; Damason-P; Dihydrocodeinone acid tartrate; Dihydrocodeinonum bitartaricum; Duodin; Hycodan; Hydrocodonhydrogentartrat; Hydrocodoni; Hydrocodonum; Hydrocodonium; Hydroconi; Hydroconum; Kolikodal; Lortab; Orthoxycol; Panacet; Procodal; Vicodan
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Opiate agonist



Uses

Hydrocodone is used as an analgesic and as an antitussive. It has also been diverted as a drug of abuse.

Exposure Routes and Pathways

Ingestion is the most common route of exposure to hydrocodone. It is available as tablets and syrup. Hydrocodone has been solubilized and used parenterally as a drug of abuse.

Further Reading

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- Bond GG, Flores GH, and Stafford BA (1991) Lung cancer and hydrogen chloride exposure: Results from a nested case-control study of chemical workers. *Journal of Occupational Medicine* 33: 958–961.
- Dilawari JB, Singh S, and Rao PN (1984) Corrosive acid ingestion in man, a clinical and endoscopic study. *Gut* 25: 183–187.

Toxicokinetics

Hydrocodone is well absorbed from the gastrointestinal tract. At therapeutic doses of regular release products, peak serum levels occur within 1 h after ingestion and peak analgesia occurs within 2 h after ingestion. Extended release products can provide up to 12 h of antitussive effects. Hydrocodone is metabolized in the liver by O- and N-demethylation and 6-keto-reduction. The primary metabolites of hydrocodone are norhydrocodone, hydromorphone, 6-hydrocodol, and 6-hydromorphal. All metabolites have active pharmacologic activity. The volume of distribution is $3.3-4.71 \text{ kg}^{-1}$. The kidneys are the main site of excretion. Unchanged drug (~12%) and metabolites are excreted in the urine. The elimination half-life is ~4 h.

Mechanism of Toxicity

Hydrocodone is a semisynthetic, centrally acting narcotic analgesic and antitussive agent. It is postulated that the drug's antitussive effects come from its direct depression of the medulla. Analgesic effects are related to the stimulation of opiate receptors in the central nervous system (CNS). Interaction with the opioid receptors mimics the actions of endogenous enkephalins and endorphins. These actions result in analgesia, sedation, euphoria, and decreased gastrointestinal motility.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hydrocodone is used in small animal veterinary practice for the management of pain and for its antitussive properties. Hydrocodone should be used with caution in cats due to likelihood of the development of stimulant effects (e.g., excitement, muscle spasms, seizures).

Dogs act similarly to humans – symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at 0.02 mg kg^{-1} if needed.

Human

CNS depression is the most frequently reported clinical effect. The typical overdose patient may present with extreme somnolence that may progress to frank coma. Miosis is usually present unless the individual is acidotic or has suffered hypoxic brain injury. Respiratory depression can occur and may progress to respiratory arrest. Pulmonary edema may be seen. Bradycardia, hypotension, and hyperthermia can develop. Hydrocodone is often combined in products with acetaminophen; therefore, patients should be evaluated for hepatotoxicity secondary to acetaminophen overdose. Available opiate immunoassays cross-react unreliably with hydrocodone. Peak therapeutic serum levels are $0.024 \text{ mg} \text{l}^{-1}$; toxic levels have been reported to reach $0.1-1.3 \,\mu g \, m l^{-1}$, but are of little prognostic or therapeutic value.

Chronic Toxicity (or Exposure)

Animal

Studies have been conducted in animals to attempt to clarify the role of varying rates of drug metabolism in humans and animals and the likelihood for development of addiction/dependence to narcotics. Hydromorphone has been studied in several animal models because it is metabolized in humans by specific cytochrome P450 isoenzymes. So far, studies have looked at administration of agents that either block or promote P450 isoenzymes responsible for hydrocodone metabolism and the impact of enhanced or degraded metabolism of hydrocodone on those animal models. Results in rats and rhesus monkeys have demonstrated little effect from modifying hydrocodone metabolism.

Human

Hydrocodone has the potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Abrupt cessation can cause withdrawal, yielding restlessness, insomnia, hypertension, tachycardia, tachypnea, vomiting, and diarrhea. Chronic use of hydrocodone in humans has also been associated with hearing loss.

Clinical Management

In patients presenting with hydrocodone toxicity, the airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO₂ narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal (1 gm kg^{-1}) may be administered with substantial recent ingestions. Syrup of ipecac is contraindicated after overdose with the hydrocodone due to the potential for rapid clinical deterioration. Gastric lavage should be avoided.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of hydrocodone. A dose of 0.4–2.0 mg is given intravenously slowly, titrated to resumption of adequate respirations, and can be repeated as needed. The therapeutic effect of naloxone may be of shorter duration than that of hydrocodone activity; therefore, it is imperative that hydrocodone intoxicated patients who demonstrated improvement after naloxone be closely monitored for resedation. Vital sign measurements and neurological checks should be monitored frequently.

See also: Acetaminophen; Drugs of Abuse; Hydromorphone.

Further Reading

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- Evans LE, Swainson CP, and Roscoe P (1973) Treatment of drug overdosage with naloxone, a specific narcotic antagonist. *Lancet* 1: 452–455.

Hydrofluoric Acid

Samantha E Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7664-39-3
- SYNONYMS: Hydrogen fluoride; Hydrofluoride; HFA
- CHEMICAL FORMULA: HF

Uses

Hydrofluoric acid (HFA) is the inorganic acid of elemental fluorine. HFA is used in fluorocarbons, fluoropolymers, aluminum production, stainless steel pickling, uranium processing, glass etching, oil well acidizing, gasoline production, removal of sand and scale from foundry castings, and as a laboratory reagent. Anyone using HFA should understand the safety measures required to protect human health, for example, read the relevant Material Safety Data Sheet (MSDS), call the supplier for additional information if necessary, and confirm that the personal protective equipment (PPE) that has been shown to effectively protect against HFA exposure. In addition, the PPE should be checked carefully before each use, for example, a pinhole-sized hole could cause problems since HFA can penetrate deeply into skin and muscle tissue and simply flushing the area with water is not enough.

Exposure Routes and Pathways

Accidental dermal exposure is the most common route for human exposure; inhalation and ingestion are also possible. Occupational sources include the manufacture of chemicals, photographic film, solvents, and plastics.

Toxicokinetics

HFA rapidly corrodes and penetrates the skin and mucous membranes. Fluoride ions are then readily absorbed but are rapidly almost completely bound to available calcium and magnesium ions. The resulting salts are excreted.

Mechanism of Toxicity

HFA is toxic by ingestion, inhalation, and (most commonly) by dermal exposure. It is highly corrosive to the skin and mucous membranes with very short

(5 s or less) exposure concentrations of 0.003% and above, acting by protonation of tissues. It causes a liquefying necrosis at the site of contact. Absorption of fluoride ions leads to systemic fluoride poisoning, in turn leading to hypokalemia and hypomagnesemia potentially resulting in neuromuscular paralysis and cardiac arrhythmias.

Acute and Chronic Toxicity

Animal

Signs of acute systemic fluoride intoxication include increased salivation, lacrimation, vomiting, diarrhea, muscular fibrillation, and respiratory, cardiac, and general depression. The inhalation LC_{50} is 1774 ppm in monkeys, 1276 ppm in rats, 4327 ppm in guinea pigs, and 342 ppm in mice. The intraperitoneal LD_{Lo} in rats is 25 mg kg^{-1} . Repeated inhalation exposure to concentrations in the range of 20-25 ppm produces injury to the lungs, liver, and kidneys. Dermal concentrations as low as 0.001% result in injury. Chronic exposure of guinea pigs and rabbits has caused injury of the cornea and mucous membranes. Repeated inhalation of 17 ppm HFA resulted in damage to the lungs, liver, and kidneys of animals, but inhalation of 8.6 ppm failed to elicit significant pathologic change in these tissues.

Human

HFA toxicity occurs after ingestion, inhalation, or ocular or dermal contact. HFA is one of the strongest and most corrosive acids known, and is highly irritating, corrosive, and poisonous. Therefore, special safety precautions are necessary when using this chemical. Inhalation of anhydrous HFA or HFA mists or vapors can cause severe respiratory tract irritation that could be fatal. The inhalation TC_{Lo} is $100 \,\mathrm{mg \, m^{-3}}$ and the LC_{Lo} is 50 ppm. Ingestion of HFA produces pain and corrosion of the oral mucous membranes, esophagus, and stomach, and fatalities have occurred. The oral TC_{Lo} is 143 mg kg^{-1} . Systemic exposure can precipitate cardiovascular collapse quickly, with systemic hypokalemia and prolonged QTc interval. Chronic exposure via inhalation or ingestion can lead to fluorosis, with symptoms such as weight loss, malaise, anemia, leukopenia, discoloration of teeth, and osteosclerosis.

Clinical Management

Most burns are minor if they involve only small parts of the body surface area. When larger parts of the skin are burnt, morbidity and mortality significantly

increase, for example, if more than 20% of the body surface area is burnt with high concentration HFA, mortality approaches 100%. In major HFA burns, death almost always results from severe electrolyte imbalance. Exitus letalis (i.e., a fatal outcome) has been reported after a burn with 70% HFA involving as little as 2.5% of the body surface area. Unlike most minor HFA burns, which can be successfully managed by topical and regional therapy as well as close monitoring, major HFA burns require immediate critical care treatment. If the exposure is to the skin, all clothing should be removed from the affected region. The region should be copiously irrigated with water and then treated with a calcium gluconate paste. For exposure by any route, a 10% solution of calcium gluconate should be slowly infused (intravenously) to a total of 0.5 ml kg^{-1} .

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists ceiling limit for exposure is 3 ppm. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h time-weighted average, is 3 ppm. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day, is 3 ppm, the NIOSH ceiling limit for a 15 min exposure is 6 ppm, and the NIOSH 'immediately dangerous to life or health' value is 30 ppm.

See also: Acids; Corrosives; Fluoride; Fluorine.

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Hydrogen Cyanide See Cyanide.

Hydrogen Peroxide

David Eldridge and Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7722-84-1
- SYNONYMS: Albone; Carbamide peroxide; Hydrogen dioxide; Hydroperite; Hydroperoxide; Inhibine; Perhydrol; Peroxan; Urea hydrogen peroxide; Urea peroxide
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Antiinfective, topical antiseptic, cleansing agent
- CHEMICAL FORMULA: H₂O₂

Uses

Dilute concentrations of hydrogen peroxide (up to 6%) are used as an antiseptic. In this capacity, it can be used as a mouthwash and topical wound cleanser. It is useful in loosening cerumen and clearing its impaction from the auditory canal. Dentists use

hydrogen peroxide for teeth whitening. When a toxic ingestion is suspected, dilute hydrogen peroxide is commonly given orally by veterinarians to household pets in order to induce vomiting and achieve gastric decontamination.

Exposure Routes and Pathways

Ingestion is the most common route of reported toxic exposure. However, hydrogen peroxide exposure and toxicity can be multifaceted with absorption via inhalation, dermal, or ocular contact. Less common routes include direct instillation into body cavities and intravenous administration.

Toxicokinetics

With any direct exposure, absorption and most subsequent clinical effects are generally rapid.

Mechanism of Toxicity

One mechanism of toxicity by hydrogen peroxide involves gas release. Hydrogen peroxide, as an oxidizing agent, liberates oxygen upon contact with tissue. This release is considerable as each volume of 3% solution produces 10 volumes of oxygen. This volume is problematic if this gas cannot easily escape. Another major concern, particularly in the case of industrial strength concentrations (>10%), is local, caustic tissue injury. Localized injury can occur with direct exposure to the skin, the eyes, and the gastrointestinal and respiratory tracts.

Acute and Short-Term Toxicity (or Exposure)

Human

Household strength (3%): Ingestion of small amounts usually causes only mild irritation to the mucosa and results in nausea and vomiting. Rarely, ingestions of household hydrogen peroxide has been linked to gastrointestinal erosions and ulcerations in children. Gastrointestinal distention and, in extreme cases, rupture of a hollow viscous can occur in the face of confined gas release. Eye exposure results in immediate but generally transient pain. Dermal exposure causes burning, tingling, and a temporary white discoloration of the skin. Colitis has been documented after use of dilute hydrogen peroxide enemas.

Industrial strength (>10%): More serious complications generally occur at strengths at >10%. Even small amounts of solutions containing >30%hydrogen peroxide have resulted in death when ingested. Ingestions cause corrosive burns to the mouth, throat, and gastrointestinal tract. Inflammation in this setting can also involve airway compromise from direct injury. Again the gas released may lead to viscous distension and rupture. In this setting, gas emboli of blood vessels are well-documented occurrences. These may travel to the brain with subsequent neurological sequelae. Cerebral edema and seizures have been noted with ingestion. Burns to the eyes and skin can be severe. Inhalation of concentrated hydrogen peroxide can potentially cause pulmonary irritation. As hydrogen peroxide is water soluble, its effects generally result in inflammation and irritation of the upper airway when inhaled.

Chronic Toxicity (or Exposure)

Animal

Dogs exposed to 7 ppm of 90% hydrogen peroxide 5 days a week for 6 months developed irritant symptoms on the skin, sneezing, lacrimation, irritated lungs at necropsy, and bleaching of the hair.

Human

A link between interstitial lung disease and chronic exposure to high aerosol hydrogen peroxide levels has been suggested in one case report. Some have suggested that chronic use of dilute hydrogen peroxide mouthwash may cause hypertrophied papillae of the tongue.

In Vitro Toxicity Data

Hydrogen peroxide is mutagenic in *Salmonella* and *Escherichia coli* models.

Clinical Management

Ingestion

No intervention is generally required for asymptomatic patients who have ingested small amounts of household hydrogen peroxide. If the patient has significant symptoms (bloody vomitus, abdominal distension, or discomfort), he/she should seek medical attention. Ingestion of milk or water in an attempt to dilute the hydrogen peroxide has not been proven to be beneficial. Similar exposure to concentrated solutions (>10%) requires more aggressive treatment. These patients are at a greater risk of becoming symptomatic and developing hematemesis, drooling, and stridor. Prompt medical evaluation is a necessity. Syrup of ipecac and activated charcoal are contraindicated. The patient's airway and vital signs should be closely monitored. A careful physical exam is performed initially to assess the extent of injury. This may need to be followed with endoscopic exam to more thoroughly characterize the extent and location of gastrointestinal injury. Hyperbaric oxygen therapy should be considered in cases of gas embolus, especially in patients with proof of cerebral involvement or central nervous system symptoms.

Inhalation

Removal from the source of injury is the primary treatment, and the patient should be promptly moved to fresh air and monitored for respiratory distress. More aggressive support such as supplemental oxygen and assisted ventilation may be used as indicated.

Eye Exposure

The primary intervention consists of irrigation with copious amounts of tepid water for at least 15 min. If symptoms are severe or persist, examination by a physician is indicated.

Dermal Exposure

First, contaminated clothing should be quickly removed. Then, the affected areas should be washed thoroughly with soap and water. If irritation or pain persists, or a chemical burn is present, examination by a physician is recommended.

See also: Mouthwash.

Hydrogen Sulfide

Betty J Locey

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7783-06-4
- SYNONYMS: Sulfur hydride; Dihydrogen sulfide; Hydrosulfuric acid; Stink damp; Sewer gas
- CHEMICAL FORMULA: H₂S
- CHEMICAL STRUCTURE: H:S:H

Uses

Hydrogen sulfide is formed naturally as well as produced by a number of commercial methods. It is commonly found in petroleum deposits, volcanic gases, natural sulfur springs, natural gas deposits, and anywhere an organic matter is decaying (e.g., manure and sewage). It may be released into the environment during operations at manufactured gas plants, paper mills, petrochemical plants, at tanneries, and production of heavy water for nuclear reactors. The primary source of hydrogen sulfide is reported to be as a by-product of purification of natural and refinery gases. It may be produced when sulfide or sulfuric acid is used in mixtures or when tanning leather, during glue making, metal recovery, and rubber vulcanizing.

Hydrogen sulfide is used in metallurgy, as a reagent in analytical chemistry, in the manufacture of sodium sulfide, in the production of heavy water, and, at one time, as an agricultural disinfectant. It is a major source of elemental sulfur and sulfuric acid.

Background Information

Hydrogen sulfide gas is highly toxic and can cause death within minutes if exposure is high enough. It is an important cause of morbidity and mortality in the workplace. Workers may be exposed to high levels in certain work environments, including poorly

Further Reading

- Cina SJ, Downs JCU, and Conradi SE (1994) Hydrogen peroxide – A source of lethal oxygen embolism – Case report and review of the literature. *American Journal of Forensic Medical Pathology* 15: 44–50.
- Henry MC, Wheeler J, and Mofenson HC (1996) Hydrogen peroxide 3-percent exposures. *Clinical Toxicology* 34: 323–327.

ventilated areas of wastewater treatment plants, in stagnant wells, in certain areas of petroleum refineries, and near fermenting manure. Hydrogen sulfide had been reported at concentrations greater than 700 mg m^{-3} (~500 ppm) at a wastewater treatment plant; greater than 310 mg m^{-3} (~221 ppm) in a stagnant well; and ranging from 70 to 280 mg m^{-3} (~50–200 ppm) at an oil refinery in open maintenance ports.

Hydrogen sulfide has a distinctive and disagreeable odor (often described as smelling like rotten eggs). There is a wide range for reported odor thresholds in air. The American Conference of Governmental Industrial Hygienists (ACGIH) reported a geometric mean for odor thresholds as 0.0045 ppm $(\sim 0.0063 \,\mathrm{mg}\,\mathrm{m}^{-3})$ and an accepted values range from 0.001 to 0.13 ppm (\sim 0.0014–0.18 mg m⁻³) for those studies reported as reviewed. Odor cannot be used to warn of overexposure. The sense of smell becomes rapidly fatigued and cannot be relied on to warn of the continuous presence of hydrogen sulfide. Loss of sense of smell has been observed to occur after exposure to atmospheres containing between 50 and 150 ppm (\sim 70 and \sim 211 mg m⁻³) hydrogen sulfide. Hydrogen sulfide levels ranging from 100 to 200 ppm $(\sim 140-281 \text{ mg m}^{-3})$ have been reported to cause loss of the sense of smell and lead to olfactory paralysis.

Exposure Routes and Pathways

Hydrogen sulfide is a colorless gas and most human exposures occur via inhalation. However, exposure may also occur via ingestion or skin contact. Exposure to high concentrations is most likely to occur in the workplace. The general public may be exposed from industrial operations, from natural gas wells during drilling operations, and from natural sources. Exposure may be to low-levels, long-term, or high levels during an accidental release.

Exposure to hydrogen sulfide may also occur when precursors enter the body and are changed to produce hydrogen sulfide. Certain sulfhydryl-containing amino acids (e.g., cysteine) may be metabolized by bacteria in the gut or in the mouth to produce hydrogen sulfide. It may also be produced, and then released after ingestion of soluble inorganic sulfide salts. Soluble salts, administered by injection, have been used in laboratory animals to study the effects of exposure to hydrogen sulfide. Some smooth muscle tissues (e.g., ileum, thoracic aorta, portal vein) and the brain have enzymes that produce hydrogen sulfide.

Toxicokinetics

Hydrogen sulfide gas is quickly absorbed through the lungs. Absorption may also occur through gastrointestinal tract. Absorption through the skin is limited, but does occur. Once absorbed into the body, hydrogen sulfide may be metabolized (changed by the body) or may remain unchanged until eliminated. The major metabolic pathway is oxidation in the liver. However, the kidneys also contain a sulfide oxidizing system. Oxidation may occur by nonenzymatic or enzymatic mechanisms. The primary product of oxidation is sulfate. Hydrogen sulfide may oxidize to the sulfate of thiosulfate in certain tissues. Thiosulfate can bind methemoglobin and form sulfmethemoglobin, which autooxidizes. Hydrogen sulfide may also be metabolized by methyolation and/or reactions with metal-containing proteins (metalloproteins).

Hydrogen sulfide and its metabolites may be eliminated from the body in the urine, through the lungs, or in the feces. The amount excreted by a particular route is influenced by the route of exposure. Hydrogen sulfide that is not metabolized (unchanged) is eliminated either in the volatile form through the lungs or in the feces. After oral exposure, most hydrogen sulfide is eliminated from the body in the urine as sulfate.

Mechanism of Toxicity

Contact with hydrogen sulfide can be irritating to tissues. Systemic effects are generally believed to be caused by changes that occur at the cellular level. Hydrogen sulfide inhibits cellular (mitochondrial) respiration. It binds proteins that are important to mitochondrial electron transport (cytochrome *aa* and cytochrome oxidase (last step in mitochondrial oxidative metabolism) and inhibits the conversion of molecular oxygen to water and the generation of adenosine triphosphate (ATP). ATP provides the energy required for many cellular functions. Hydrogen sulfide stops cellular respiration and leads to hypoxia (decrease of oxygen) at sufficiently high doses. The mechanism of toxicity is believed to be similar to that of cyanide.

Inhibition of respiration on the cellular level inhibits cellular function. Systems in the body with the highest oxygen demand are most vulnerable to its effects. These include the central nervous system, particularly the area that controls breathing, and the heart. High-level acute exposure may cause death due to a depressant effect on the respiratory center in the brain (area that controls breathing). Exposure to high levels leads to respiratory paralysis, pulmonary edema, and death. Chemoreceptors in the carotid body (small body of vascular tissue that is sensitive to changes in the concentration of oxygen in the blood) are believed to be responsive to sulfide in the blood and react to cause changes in breathing (rapid and/or deep breathing), respiratory depression, and apnea (transient cessation of breathing).

The following may contribute or cause the neurotoxicity associated with exposure to hydrogen sulfide.

- Inhibition of cellular respiration in tissues such as lung can lead to hypoxia (decrease of oxygen) and local tissue and cell damage.
- Hypotension (low blood pressure) and ischemia (restricted blood flow) associated with exposure reduces the delivery of oxygen to tissues and cells and may lead to sever damage and/or death of nerve cells (neuronal necrosis).
- High concentrations of hydrogen sulfide gas may alter pulmonary surfactants leading to pulmonary edema. Pulmonary edema can be the primary cause of death.
- Sulfide can irritate certain pulmonary nerves (e.g., afferent endings of the pulmonary vagi) and high doses can paralyze the ventilatory center.
- Sulfides may be selectively taken up by the rat brainstem. Since the brainstem plays a significant role in regulation of respiratory rhythm (catecholaminergic innervation of the brain begins within the brain stem and catecholamines and serotonin affect respiratory rhythm), changes in the levels of neurotransmitters may cause or contribute to loss of central control of breathing.

Acute and Short-Term Toxicity (or Exposure)

Hydrogen sulfide is a highly toxic gas. Inhalation can result in collapse and death in minutes if concentrations are high enough. The likelihood of adverse effects due to exposure to hydrogen sulfide depends on (1) level of exposure (e.g., concentration in the air), (2) how long the individual is exposed, and (3) the individual's sensitivity to the effects of the chemical. For example, an individual with lung disease (e.g., emphysema or asthma) may exhibit certain symptoms before a healthy individual. Generally, the shorter the duration of exposure, the higher the concentration associated with adverse effects. Effects in animals and humans are generally similar.

Adverse effects may occur at the site of contact (e.g., lungs, eyes, and skin) or systemically. Hydrogen sulfide is a local irritant at low concentrations and may be irritating to all contact surfaces (eyes, skin, and respiratory tract). Contact with the vapor may cause irritation and/or conjunctivitis to the eyes ('gas eye') and lesions in the nasal tract. Prolonged or high-level exposure may cause more serious local effects (e.g., pulmonary edema). Exposure to hydrogen sulfide can cause neurotoxicity.

Systemic effects are serious and can be life threatening. Signs and symptoms of exposure also may include headaches, fatigue, dizziness, confusion, cardiac effects (e.g., tachycardia and bradycardia), cough, respiratory depression, nausea, cyanosis, and shortness of breath. Symptoms may progress to include pulmonary edema, apnea, seizures, coma, and death.

Animal

There is a fairly large body of animal data characterizing the toxicity of hydrogen sulfide. Studies have been conducted in monkeys, dogs, rabbits, mice, rats, and guinea pigs in which animals have been exposed via both inhalation and dermal contact. Inhalation studies have documented responses at different atmospheric concentrations over varying periods of time. Both local and systemic effects have been observed.

Single dose, short-term, and medium-term exposure to hydrogen sulfide via inhalation have caused a number of effects in test animals. These include nasal and respiratory tract irritation and damage, cardiovascular damage, neurotoxicity, changes in the immune system, and developmental toxicity. The respiratory tract has been identified as the most sensitive target organ and nasal lesions have been identified as the adverse effect occurring at the lowest exposure by several regulatory agencies.

Examples of results of animal studies include the following:

- Many studies have been completed to evaluate the concentration that causes death in study animals. Several examples are presented below.
 - \circ Rat (Sprague–Dawley) 2 h lethal concentration in half the study animals (LC₅₀ study) was reported as 820 mg m⁻³ (~586 ppm).

- $^{\circ}$ Rat (Fischer-344) 4 h LC₅₀ was reported as 700 mg m $^{-3}$ (\sim 500 ppm).
- Rat (Long-Evans) 6 h LC₅₀ was reported as 470 mg m^{-3} (~336 ppm).
- Rats and mice exposed via inhalation to hydrogen sulfide at concentrations varying from 0.01 to 101 ppm (~0.014–142 mg m⁻³) for various time periods exhibited irritation of the respiratory tract, anorexia, reduced weight gain, weight loss, changes in the lung, nerve cell abnormalities, changes in certain blood cells (increase in reticulocytes and changes in the mean corpuscular volume), and death.
- Rabbits exposed via inhalation to hydrogen sulfide at 72 ppm (~102 mg m⁻³) exhibited disturbed liver, brain, kidney metabolism, serum protein, enzyme and mineral changes, decreased myocardial enzymes, cardiac irregularities, and unconsciousness. Dermal exposure resulted in changes in blood chemistry.
- Monkeys exposed via inhalation to hydrogen sulfide at 504 ppm (~710 mg m⁻³) for various time periods exhibited eye irritation, changes in gray matter (brain tissue), necrosis in certain areas of the brain, moderate changes in the liver, hyperemia, ataxia, anorexia, sudden loss of consciousness, and cardiac arrest.
- Dogs exposed via inhalation to a range of concentrations of hydrogen sulfide from 100 to 1800 ppm (~141-2535 mg m⁻³) exhibited effects ranging from local irritation to respiratory paralysis and immediate death.
- Rats and mice exposed via inhalation in several subchronic studies report olfactory nasal mucosa as the principal target site affected.

The potential for exposure to hydrogen sulfide to cause developmental effects has been studied. Several studies did not report effects, however, some suggest hydrogen sulfide may be a developmental neurotoxin. *In utero* and postpartum exposure was associated with significant changes in the levels of certain neurotransmitters (e.g., norepinephrine and serotonin) as well as certain amino acids (e.g., aspartate, glutamate) in several regions of the brain.

Human

Effects observed in animals are generally relevant to humans. Exposure to hydrogen sulfide may cause irritation, neurotoxicity, respiratory distress, pulmonary edema, headache, nausea, shortness of breath, dizziness, ataxia, chest pain, collapse, coma, and death.

Certain effects have been observed after acute and/ or short-term exposure at ranges of concentrations. Ranges reported in the literature do vary and the duration of exposure is important to the likelihood of seeing effects. The following provide examples.

- Exposure to very high concentrations (1000–2000 ppm or greater) can cause collapse in seconds (called 'knockdown concentration'). Exposure at 'knockdown' concentrations can cause paralysis of the respiratory center; breathing may stop, leading to collapse, and death within minutes. Another source reported a 'knockdown' concentration range of 500–1000 ppm.
- Exposure to 700 ppm can be rapidly fatal.
- Pulmonary edema may occur after short-term exposure to 250–600 ppm concentration.
- The sense of smell may be lost shortly after exposure to atmospheres ranging from 50 to 150 ppm. Other sources indicated the sense of smell is lost after exposure to 100–200 ppm.
- Prolonged exposure to 50 ppm has also been associated with pulmonary edema.
- Exposure for 1 h to 50–100 ppm may cause eye and respiratory tract irritation.
- Exposure to 14–25 ppm may cause burning eyes, headache, loss of appetite, weight loss, and dizziness.
- Conjunctivitis (eye irritation) has been reported after exposure to atmospheres containing 10–14 ppm hydrogen sulfide.

Data have been collected on how hydrogen sulfide is processed in healthy volunteers (primarily undergraduate and graduate students). Subjects were exposed to 7 or 14 mg m^{-3} (~5–10 ppm) (mouth breathing) for two 30 min sessions while engaged in aerobic exercise. Blood lactate concentrations increased, and there was a decrease/inhibition of the aerobic capacity of the exercising skeletal muscle. Men were more sensitive to this effect than women.

Hydrogen sulfide was released intermittently from an industrial source in the City of Terre Haute, Indiana, over a period of 2 months. Ambient air concentrations were reported to range from 0.002 to 8 ppm (\sim 0.0028–11 mg m⁻³). Twenty-seven residents complained of nausea, headache, shortness of breath, sleep disturbance, and throat and eye irritation during this time.

Chronic Toxicity (or Exposure)

Animal

Long-term animal studies were not identified.

Human

There is some evidence that chronic exposure and/or short-term higher level exposure (e.g., accidental incidents in the workplace) can result long-term effects (e.g., neurophychological and neurobehavorial changes). Long-term exposure to hydrogen sulfide has been reported to cause fatigue, headache, sleep disturbances, irritability, emotional disturbances, diminished memory, dizziness, nausea, vomiting, loss of appetite, weight loss, irregular heartbeat, lung congestion, and nerve damage. There is one report where exposure was believed to have lead to dementia.

Several epidemiological studies have been completed to evaluate the effect of long-term exposure to hydrogen sulfide in humans. In general, these studies did not have good information on the levels and duration of exposure. There is some evidence that exposure in the workplace was associated with increased incidence of spontaneous abortion. There are limited data and information that can be used to determine whether children are more susceptible that adults to the effects of hydrogen sulfide. Most data are anecdotal.

Hydrogen sulfide is not regulated a potentially carcinogenic to humans. None of the generally accepted sources classifies hydrogen sulfide as likely to cause cancer in humans. In 2003, the US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) assessment described evidence as inadequate for an assessment of the carcinogenic potential. There are several epidemiological studies that address the carcinogenic potential of hydrogen sulfide. A cohort study of residents (Alberta, Canada) living downwind from natural gas refineries did not show an increase in cancer (from 1970 to 1984). A retrospective epidemiological study of residents of Rotorua, New Zealand, a city that uses geothermal energy for industrial and domestic heating, was conducted to study cancer in this population (from 1981 to 1990). The authors concluded it was not possible to evaluate the carcinogenic potential of hydrogen sulfide on the basis of human studies.

In Vitro Toxicity Data

Data indicate hydrogen sulfide is not mutagenic. Hydrogen sulfide was negative when tested in the Ames test with *Salmonella typhimurium* with and without s9 liver fractions. Hydrogen sulfide gas potentiated the mutagenicity of hydrogen peroxide as measured in *S. typhimurium*.

Clinical Management

The victim should be removed to fresh air and away from the source of exposure. Caution should be exercised by rescuers as high concentrations of hydrogen sulfide can cause collapse in seconds. Oxygen should be provided if there is respiratory distress. Providing life support quickly may be critical to patient survival in certain cases. Administration of naloxone and dextrose may be indicated. For irritation, contaminated clothing should be removed and contaminated skin washed. Medical attention should be sought immediately for all symptomatic exposures. Patients who have had significant exposures should be closely monitored in a hospital.

Symptoms resemble those observed in cyanide poisoning and cyanide antidote kits may be useful for emergency treatment. Amyl nitrate by inhalation and intravenous sodium nitrite may be appropriate for certain patients. In laboratory animals, inducing methemoglobinemia with nitrates provides protection (even antidotal properties) against sulfide poisoning because the hydrosulfide anion (HS⁻) can bind methemoglobin and form sulfmethemoglobin. This treatment has been used in some instances in humans.

Measurement of blood sulfide or urinary thiosulfide levels within 2 h can be used to confirm exposure to hydrogen sulfide if needed.

Environmental Fate

Hydrogen sulfide is colorless gas under normal environmental conditions. When released to the environment it will move with, and disperse in, the ambient air. It is estimated to remain in the atmosphere for an average of 18 h. This is generally longer in the winter. Once released to the ambient air, it may become oxidized and form sulfuric acid and/or sulfur dioxide. Oxidation may occur quickly by combination with hydroxide radicals or more slowly by combination with molecular oxygen. Sulfuric acid and sulfur dioxide may contribute to 'acid rain'. Hydrogen sulfide in the air may sorb to soil. Most is then converted to elemental sulfur. Several microorganisms can degrade hydrogen sulfide to sulfate or elemental sulfur (e.g., a heterotrophic fungi, a heterotrophic bacterium (Xanthomona), and a marine isopod (Saduria (Mesidotea) entomon).

Hydrogen sulfide is soluble in alcohol, ether, glycerol, gasoline, kerosene, crude oil, and carbon disulfide. It may also be dissolved in water (solubility at 20°C is reported as 1g per 242 ml of water). In surface water it is generally oxidized by combining with oxygen or hydrogen peroxide. Oxidation is pH dependent. When dissolved in sewage water, sulfur is produced at pH ranging from 6 to 7, however if the pH ranges from 7 to 9 polysulfides, thiosulfates, and ultimately sulfates are formed. In some warm, damp environments (e.g., gravity sewers) it may be oxidized by autotrophic bacteria to sulfuric acid. The potential for evaporation is influenced by temperature and pH. Low pH and high temperature tend to favor evaporation.

As the pH increases, more sulfhydral radical (SH^-) is present. In general, the SH^- is not as biologically available (does not cross biological membranes as easily as hydrogen sulfide) and is therefore considered to pose less of a hazard.

Ecotoxicology

There are locations where hydrogen sulfide concentrations are naturally high (e.g., some geothermal vents, bogs of swamps with local area of decaying matter). In these areas, animal and plant life appear to be able to adapt.

For example, natural hydrocarbon seeps (oil and gas flow out of the ocean floor) have been reported to support dense biological communities. Hydrocarbon seeps produce methane and hydrogen sulfide. Certain bacteria can live on compounds like methane and hydrogen sulfide. Certain species (e.g., tube worms and mussels) can establish a symbiotic relationship with these bacteria and not only survive, but thrive in deep sea seeps. These populations may provide the basis for diverse community in the seep environment. The following are examples of life in areas that have naturally high levels of hydrogen sulfide.

- A seep identified in the Gulf of Mexico (at depths greater than 500 m) was reported to be populated with a worm species, living in a dense network of burrows.
- A sulfurous lake in New Zealand lake that is charged by an active underground geothermal vent reportedly averages hydrogen sulfide concentrations ranging from 5 to 3900 ppb. Studies do not report visible effects on local plant and bird populations.

At higher pH sulfhydral radicals (SH^-) in hydrogen sulfide impacted surface water may be toxic to fish.

Other Hazards

Hydrogen sulfide is a flammable gas. Toxic sulfur oxide fumes are produced when it is heated to decomposition. It can cause a flash fire and is a flash back hazard. In emergency situations full protection (such as positive pressure, pressure-demand, full-face piece, self-contained breathing apparatus (SCBA)) is recommended.

Hydrogen sulfide is heavier than air and tends to sink. Persons closer to the ground (e.g., fallen injured, children) may be exposed to higher concentrations during a release. Hydrogen sulfide is incompatible with a number of materials, including strong oxidizers, certain metals, and strong nitric acid.

Exposure Standards and Guidelines

Criteria are usually developed for particular populations and are generally based on prevention of an effect that occurs at lower doses ('critical effect'), which, if the levels do not exceed criteria, is expected to prevent the occurrence of more serious effects known to occur at higher doses. Criteria are available that are protective of acute, short-term, and chronic exposure for workers and the general public for hydrogen sulfide. Different agencies have developed exposure criteria and standards for hydrogen sulfide. Selected criteria and guidelines are provided below.

Criteria for workplace exposure:

- The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) ceiling of 20 ppm with a 50 ppm concentration for a maximum of 10 min was set to protect workers against risk of 'eye irritation and conjunctivitis'.
- The ACGIH threshold limit value (TLV) is 14 mg m^{-3} , with a short-term exposure limit (STEL) of 21 mg m^{-3} .
- The National Institute of Occupational Safety and Health (NIOSH) recommended exposure limit (REL) ceiling of 10 ppm (~14 mg m⁻³) for 10 min.

Criteria protective of the general public

 The US EPA has developed a chronic reference concentration (RfC) of 2×10⁻³ mg m⁻³ (~0.0014 ppm) based on a critical effect of nasal lesions in the olfactory mucosa in rats (updated assessment in 2003). The RfC represents a daily exposure that is expected to be protective of the general public over a lifetime of exposure.

- The Agency for Toxic Substances and Disease Registry (ATSDR) has developed acute and intermediate inhalation minimal risk levels (MRLs) of 0.07 and 0.03 ppm ($\sim 0.099-0.042 \text{ mg m}^{-3}$), respectively.
- The acute MRL was established based on effects reported in humans with the critical effect of respiratory effects-bronchial obstruction (30% change in airway resistance). It is intended to protect for exposure occurring up to 14 days.
- The intermediate MRL was established based on respiratory effects reported in mice. Details on how these values were derived are provided in ATSDR's toxicological profile for hydrogen sulfide.
- The World Health Organization (WHO) air quality guideline is 0.15 mg m^{-3} (~0.11 ppm) (24 h average concentration). The guideline is based on protection of eye irritation. In addition, WHO recommends the average ambient air concentration, averaged over 30 min, of $7 \mu \text{g m}^{-3}$ to avoid odor annoyance (published in 2000).

Acute exposure guideline levels (AEGLs) are airborne emergency exposure guidelines established for the general population (including sensitive populations) for periods ranging from 10 min to 8 h.

• AEGL-1: If exceeded, may cause discomfort, irritation, or effects. Effects are not expected to be disabling. Effects are expected to be short term and reversible.

Agency	Standards and guidelines (ppm)	Averaging time	
OSHA	Ceiling	20	NA
OSHA	Maximum peak	50	10 min maximum peak
NIOSH	Ceiling	10	10 min
NIOSH	IDLH	100	NA
ACGIH	TLV TWA	10	8 h over a lifetime of work
ACGIH	STEL	15	15 min
NRC	EEGL	50	10 min
NRC	EEGL	10	24 h
ATSDR	MRL	0.03	14-365 days (intermediate exposure)
ATSDR	MRL	0.07	0-14 days (acute exposure)
US EPA	RfC	0.002	Daily dose over a lifetime exposure

NA, not applicable or not available; ppm, parts per million; OSHA, Occupational Safety and Health Act; NIOSH, National Institute for Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; IDLH: immediately dangerous to life or health; EPRGs, Emergency Planning Response Guidelines (2003); NRC, National Research Council; EEGL, Emergency Exposure Guidance Level; MRLs, minimal risk levels; ATSDR, Agency for Toxic Substances and Disease Registry; US EPA, United States Environmental Protection Agency; RfC, reference concentration.

Table 2	US EPA Acute Exposure Guideline Levels (AEGLs)
(Interim) a	s presented on the Internet

Duration of exposure	AEGL 1 (ppm)	AEGL 2 (ppm)	AEGL 3 (ppm)
10 min	0.75	41	76
30 min	0.6	32	59
60 min	0.51	27	50
4 h	0.36	20	37
8 h	0.33	17	31

- AEGL-2: If exceeded, may cause irreversible or serious, long-term adverse health effects. May impair the victim's ability to escape.
- AEGL-3: If exceeded, may cause life-threatening effects or death.

Representative inhalation criteria for hydrogen sulfide are summarized in Tables 1 and 2. Table 2 provides a summary of the US EPA's AEGLs for hydrogen sulfide.

Hydroiodic Acid

Mary Lee Hultin

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 10034-85-2
- SYNONYMS: Anhydrous hydroiodic acid; Hydrogen iodide; Hydrogen monoiodide
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Inorganic acid; Corrosive
- CHEMICAL FORMULA: HI

Uses

Hydroiodic acid was formerly used as an expectorant in chronic bronchitis and bronchial asthma. It is used in the manufacture of disinfectants. It is also used for analytical purposes (e.g., as a chemical intermediate for inorganic iodides and organic synthesis).

Exposure Routes and Pathways

Exposure may occur via ingestion, dermal or ocular contact, or inhalation.

Mechanism of Toxicity

Hydroiodic acid is a strong irritant. When used as an expectorant, hydroiodic acid is believed to act by

See also: Cyanide; Neurotoxicity; Occupational Exposure Limits; Respiratory Tract; Sensory Organs.

Further Reading

Agency for Toxic Substances and Disease Registry (AT-SDR) (1999) Managing Hazardous Materials Incidents. Volume III – Medical Management Guidelines for Acute Chemical Exposures: Hydrogen Sulfide. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Relevant Websites

- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hydrogen Sulfide.
- http://www.epa.gov US Environmental Protection Agency. Toxicological review of hydrogen sulfide in support of summary information on Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC. EPA/635/R-03/005. June 2003.

irritating the gastric mucosa, which then stimulates respiratory tract secretion.

Acute and Short-Term Toxicity (or Exposure)

Animal

A study compared the toxicity to rat skin of hydrogen iodide, hydrogen bromide, and hydrogen chloride. Hydrogen bromide caused the most severe burns in the rats. No data are available on animal studies examining effects from inhalation or ingestion.

Human

Inhalation of hydrogen iodide can cause irritation of the upper respiratory tract. A concentration of 35 ppm has been shown to cause irritation of the throat after short exposure. More severe exposures may result in pulmonary edema and laryngeal spasms. As with other acids, oral ingestion may produce oral and esophageal burns with more severe burns occurring in the stomach. Initial signs and symptoms may not reliably predict the extent of injury to the gastrointestinal tract. Tachycardia, hypotension, and circulatory collapse may occur as a result of the ingestion of concentrated corrosive iodine solutions. Severe burns may occur with dermal exposure. Systemic toxicity could result in acute hepatic injury.

Chronic Toxicity (or Exposure)

Human

Repeated exposures to fumes may cause erosion of the teeth. Chronic exposure may result in the development of bronchitis.

Clinical Management

In the case of inhalation exposure, the victim should be moved to fresh air. Trained personnel may administer oxygen and monitor the patient for signs of respiratory distress. Contaminated clothing and shoes should be removed and isolated. In the case of skin or eye contact, the skin or eyes should be flushed with running water for at least 15 min. Contact lenses should not be worn when working with this chemical. In the case of accidental ingestion, vomiting should not be induced. Bicarbonate should not be given to neutralize the acid. From 4 to 8 oz (i.e., 118 to 237 ml) of water or milk should be given to adults (2–4 oz (i.e., 59–118 ml) to children) for dilution. The victim should be kept quiet and normal body temperature maintained.

Exposure Standards and Guidelines

The temporary emergency exposure limits (TEEL) are: TEEL-0=35 ppb; TEEL-1=10 ppb; TEEL-2=500 ppb; TEEL-3=5000 ppb. The TEEL-i, i = 0-3, classification is discussed elsewhere in this encyclopedia.

See also: Acids; Corrosives.

Further Reading

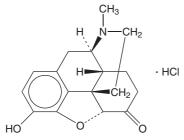
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Hydromorphone

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 466-99-9 (Hydromorphone); CAS 71-68-1 (Hydromorphone hydrochloride)
- SYNONYMS: Dihydromorphinone; Dilaudid; Dimorphone
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Opiate agonist
- CHEMICAL STRUCTURE:



Uses

Hydromorphone is prescribed as an analgesic. It has also been diverted as a drug of abuse.

Exposure Routes and Pathways

Hydromorphone is available in tablet, liquid, suppository, or parenteral formulations. Hydromorphone tablets have been solubilized and used parenterally as a drug of abuse.

Toxicokinetics

At therapeutic doses, hydromorphone has an oral and rectal bioavailability of 51% and 36%, respectively. Peak serum levels occur within 2 h after ingestion and within 1 h after intramuscular administration. Hydromorphone is metabolized primarily in the liver, where it undergoes conjugation with glucuronic acid to form hydromorphone-3-glucuronide. Hydromorphone's volume of distribution is 2.91 kg^{-1} . Unchanged drug (~6%) and metabolites are excreted in the urine. The elimination half-life of the parent compound is ~2.5 h.

Mechanism of Toxicity

Hydromorphone is a semisynthetic, centrally acting opioid analgesic. It is nearly 10 times as potent as morphine on a milligram-to-milligram basis. Analgesic effects are related to the stimulation of opiate receptors in the central nervous system (CNS). Interaction with the opioid receptors mimics the actions of endogenous enkephalins and endorphins. These actions result in the analgesia, sedation, euphoria, and decreased gastrointestinal motility.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs act similarly to humans. Symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at 0.02 mg kg^{-1} if needed.

Human

Due to hydromorphone's potency, numerous adverse effects have been reported. CNS depression is the most frequently reported clinical effect. The typical overdose patient may present with extreme sommolence and may progress to coma. Miosis is usually present unless the individual is acidotic or has suffered hypoxic brain injury. Respiratory depression can occur and may progress to respiratory arrest. Pulmonary edema may be seen. Bradycardia, hypotension, and hyperthermia can develop. Available opiate immunoassays cross-react unreliably with hydromorphone.

Chronic Toxicity (or Exposure)

Human

Hydromorphone has the potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Abrupt cessation can cause withdrawal, yielding restlessness, insomnia, hypertension, tachycardia, tachypnea, vomiting, and diarrhea.

In Vitro Toxicity Data

Hydromorphone has showed binding affinity to the delta opioid receptor that was equivalent to the binding affinities of etorphine, butorphanol, and lofentanil.

Clinical Management

In patients presenting with hydromorphone toxicity, the airway should be patent and adequate

ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO₂ narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal (1 g kg^{-1}) may be administered to patients who have ingested hydromorphone and present early. Syrup of ipecac is contraindicated after overdose with hydromorphone due to the potential for rapid clinical deterioration. Gastric lavage should be avoided.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of hydromorphone. A dose of 0.4–2.0 mg is given intravenously slowly, titrated to resumption of adequate respirations, and can be repeated as needed. The therapeutic effect of naloxone may be of shorter duration than that of hydromorphone activity; therefore, it is imperative that hydromorphone intoxicated patients who demonstrated improvement after naloxone be closely monitored for resedation. Vital sign measurements and neurological checks should be monitored frequently.

See also: Drugs of Abuse; Morphine.

Further Reading

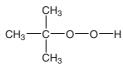
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Hydroperoxide, tert-Butyl

Samantha E Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 75-91-2
- SYNONYMS: 2-Hydroperoxy-2-methylpropane; 1,1-Dimethylethyl hydroperoxide; TBHP, TBH
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Peroxides
- CHEMICAL FORMULA: C₄H₁₀O₂
- CHEMICAL STRUCTURE:



Uses

tert-Butyl hydroperoxide (TBHP) is an intermediate in the production of propylene oxide and *t*-butyl alcohol from isobutane and propylene. It is primarily used as an initiator and finishing catalyst in the solution and emulsion polymerization methods for polystyrene and polyacrylates. Other uses are for the polymerization of vinyl chloride and vinyl acetate and as an oxidation and sulfonation catalyst in bleaching and deodorizing operations. It is a strong oxidant and reacts violently with combustible and reducing materials, metallic and sulfur compounds.

Exposure Routes and Pathways

Dermal contact and inhalation are primary routes of exposure. Occupational exposure to TBHP may occur through these routes at workplaces.

Toxicokinetics

TBHP can be absorbed into the body by inhalation, through the skin, and by ingestion.

Mechanism of Toxicity

TBHP accelerates oxidation of glutathione and decreases the metabolism of sodium hexabarbitol in rat livers and is a strong oxidation agent.

Acute and Short-Term Toxicity (or Exposure)

Animal

TBHP is a strong irritant to eye and skin. The rat oral LD_{50} is 560 mg kg⁻¹, and the rat intraperitoneal

 LD_{50} is 87 mg kg⁻¹. It is moderately toxic when ingested. The ability of TBHP to cause chromosome aberration was evaluated in bone marrow cells of Sprague–Dawley rats receiving up to 100 ppm inhalation exposure for 6 h day⁻¹ for up to 5 days. None of the treatments produced chromosomal aberrations or damage in the bone marrow cells.

Human

TBHP causes eye and skin irritation, and its inhalation causes lung damage at high concentrations.

Chronic Toxicity (or Exposure)

Animal

A 45 day oral study found treatment-related changes in the form of tubular nephrosis in male rats receiving up to 30 mg kg^{-1} body weight. In a combined repeated dose and reproduction/teratogenic study, male and female rats were given up to 30 mg kg^{-1} body weight orally. No effects on male and female reproduction were observed. In an oral dosage study, up to 50 mg kg^{-1} body weight was administered to mated female rats on days 6–15 of gestation. Neither embryotoxic nor teratogenic effects were found up to the highest dose.

In Vitro Toxicity Data

TBHP did not produce a genotoxic response in the cell transformation assay, but did produce a mutagenic response in the Ames (*Salmonella*) and mouse lymphoma mutagenesis assays. TBHP was tested for the induction of sex-linked recessive lethal mutations in *Drosophila melanogaster*, and was positive at a dose of 2000 ppm when administered to males by feeding.

Clinical Management

Respiratory therapy should be administered to exposed individuals. Contaminated clothing should be removed. Exposed skin should be washed with soap and water. Exposed eyes should be flushed with water for at least 15 min.

Environmental Fate

TBHP may be released to the environment through various waste streams. Chemical degradation is expected to be the dominant fate process in water because of reaction with organic matter and therefore, it is doubtful that unreacted TBHP would be biologically available. A bioconcentration factor (BCF) of 3 has been calculated for TBHP, and this BCF suggests the potential for bioconcentration in aquatic organisms is low. If released to air, TBHP will exist solely as a vapor in the ambient atmosphere. Vapor-phase TBHP will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 5 days.

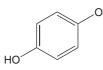
See also: Glutathione; Oxidative Stress.

Hydroquinone

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 123-31-9
- SYNONYMS: 1,4-Benzendiol; *p*-Benzendiol; Dihydroquinone; Quinol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Hydrocarbon; Ketone; Reducing agent
- Chemical Formula: $C_6H_6O_2$
- CHEMICAL STRUCTURE:



Uses

Photographic reducer and developer; reagent for the determination of small quantities of phosphate, dye intermediate; stabilizer in paints and varnishes; motor fuels and oils; antioxidant for fats and oils, and a polymerization inhibitor. Therapeutically used topically for depigmentation to treat skin blemishes, for example, hypermelanosis. Hydroquinone occurs naturally, as a conjugate with β -D-glucopyranoside, in the leaves, bark, and fruit of a number of plants, and its presence may be an important factor in fire-blight resistance in the pear. It may also play an important part in the defense mechanisms of some insects.

Exposure Routes and Pathways

The common exposure routes are dermal, by inhalation, and by ingestion. Hydroquinone exposure is also possible among people developing photographic film. Hydroquinone is not found naturally in the body.

Further Reading

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Relevant Websites

- http://www.chem.unep.ch Organisaton for Economic Cooperation and Development (OECD). *t*-Butyl Hydroperoxide (OECD Screening Information Data Set).
- http://www.inchem.org International Programme for Chemical Safety (IPCS). *tert*-Butyl Hydroperoxide (ICSC 0842) (from IPCS).

Toxicokinetics

Absorbed from the gastrointestinal (GI) tract and possibly the skin, and appears to act on the body by first being oxidized to quinone. Hydroquinone is excreted in the urine as either a glucuronide or a sulfate.

Mechanism of Toxicity

Benzene, phenol, and hydroquinone are metabolized *in vivo* to benzoquinone and excreted as the mercapturate, *N*-acetyl-*S*-(2,5-dihydroxyphenyl)-L-cysteine. Hydroquinone is a reducing cosubstrate for peroxidase enzymes, and the resultant semiquinone and *p*-benzoquinone may bind to DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD₅₀ values for rats, mice, guinea pigs, cats, and dogs range from 70 to $550 \,\mathrm{g \, kg^{-1}}$ of hydroquinone, with the cat having the greatest sensitivity. Hyperexcitability, tremors, convulsions, salivation, and emesis were observed in cats within 90 min of administration of lethal doses, and death occurred after several hours.

Experimental exposure of rabbit eyes to high concentrations of the vapor resulted in conjunctivitis, corneal edema, and necrosis. Acute neurotoxic effects reported in animals include activity increase, hyperactive reflexes, hypersensitivity, convulsions and paralysis.

Human

Ingestion of one g by an adult may cause tinnitus, nausea, dizziness, a sense of suffocation, an increased

rate of respiration, vomiting, pallor, muscle twitchings, headache, dyspnea, cyanosis, delirium, and collapse. The urine is usually green or brownish green in color and continues to darken on standing. Markedly elevated methemoglobin levels may occur with hydroquinone exposure. Increased blood cell fragility, hemolytic icterus, anemia, leukocytosis, reticulocytosis, and hypoglycemia may occur with subacute hydroquinone poisoning. Hydroquinone dust is irritating to eyes, nose, and mucous membranes, and hydroquinone is classified as a strong eye and skin irritant, and can cause hypomelanosis and delayed hyperpigmentation. Further, it is a dermal sensitizer. Ingestion of 5–12 g causes hemolysis, renal and hepatic failure, and death.

Chronic Toxicity (or Exposure)

Animal

In a rabbit study, hydroquinone at $150 \text{ mg kg}^{-1} \text{ day}^{-1}$ produced minimal developmental alterations in the presence of maternal toxicity. The no-observedeffect level for developmental toxicity was $75 \text{ mg kg}^{-1} \text{ day}^{-1}$. In rat studies, maternal toxic effects from exposure to hydroquinone included changes in the ovaries, fallopian tubes, and menstrual cycle. Postimplantation mortality was also observed in rat studies. Observed paternal toxic effects from exposure to hydroquinone included changes in the testes, epididymis, sperm duct, prostate, seminal vesicle, Cowper's gland, accessory glands, and male fertility index. Further, exposure to hydroquinone produced skeletal malformations in chickens and ocular and skeletal malformations in rabbits. Hydroquinone can induce renal tubule adenomas, bladder carcinomas, hepatocellular neoplasms, and mononuclear cell leukemia in experimental animals.

Human

Humans have been reported to be able to ingest 300– 500 mg daily for several months without adverse effects. Several hundred crewmen on a US Navy vessel were reported to have developed GI symptoms (acute onset of nausea, vomiting, abdominal cramps, and diarrhea) due to hydroquinone contamination of the water system from automatic photo developing systems. Vision disturbances are among the chronic toxic effects, for example, discoloration, distortion, and opacification of the corneas of workers exposed long-term to low levels. Workers exposed chronically may develop a reddish discoloration of the hair, and brown and orange-brown nail discoloration. Further, workers exposed chronically to hydroquinone may develop a reddish discoloration of the soles and palms. There is inadequate evidence in humans for the carcinogenicity of hydroquinone, and hydroquinone is in the International Agency for Research on Cancer's group 3 list (not classifiable as to its carcinogenicity to humans).

In Vitro Toxicity Data

Hydroquinone was not mutagenic in *Salmonella typhimurium* strains with or without exogenous metabolic activation. It induced trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation. An equivocal response was obtained in tests for induction of sex-linked recessive lethal mutations in *Drosophila* administered hydroquinone by feeding. Hydroquinone induced sister chromatid exchanges in Chinese hamster ovary cells with or without exogenous metabolic activation and caused chromosomal aberrations in the presence of activation.

Clinical Management

Clinical management should be symptomatic and supportive. A benzodiazepine can be administered to control seizures. Methylene blue can be used to treat methemoglobinemia.

Environmental Fate

In the soil, hydroquinone is expected to biodegrade under aerobic conditions. It may be removed from the soil by oxidation processes or by direct photolysis on the surface. Volatilization would be minimal. In the water, it would degrade under either aerobic or anaerobic conditions. In the air, hydroquinone undergoes photochemical degradation. It is listed as undergoing rapid biodegradation in a commercial activated sludge unit under aerobic conditions. The estimated and experimental bioconcentration factors for hydroquinone of 40–65 have been obtained. These data indicate that hydroquinone is not expected to significantly bioconcentrate in fish and aquatic organisms.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h timeweighted average (TWA) is 2.0 mg m⁻³, and this is also the US Occupational Safety and Health Administration permissible exposure limit, 8 h TWA. Hydroquinone is listed as a US Environmental Protection Agency (EPA) hazardous air pollutant "generally known or suspected to cause serious health problems." The Clean Air Act, as amended in 1990, directs the EPA to set standards requiring major sources to sharply reduce routine emissions of toxic pollutants. Hydroquinone is a US Food and Drug Administration (FDA) indirect food additive for use only as a component of adhesives.

See also: Benzene; Quinone.

Hydroxylamine

Samantha E Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7803-49-8
- SYNONYM: Oxammonium
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Reducing agent
- CHEMICAL FORMULA: NH₂OH
- CHEMICAL STRUCTURE: H–NH–OH

Uses

Hydroxylamine is used as a reducing agent in photography, in synthetic and analytical chemistry, to purify aldehydes and ketones, as an antioxidant for fatty acids and soaps, and as a dehairing agent for hides. In addition, hydroxylamine is used in the production of cyclohexanone oxime or caprolactam. Its potential uses must be carefully evaluated since hydroxylamine has been reported to pose a dangerous fire hazard when exposed to heat and flame, and may ignite spontaneously in air if a large surface area is exposed. Further, it ignites on contact with copper(II)sulfate; metals (e.g., sodium); oxidants (e.g., barium peroxide, barium oxide, lead dioxide, potassium permanganate, chlorine); phosphorus chlorides (e.g., phosphorus trichloride, phosphorus pentachloride).

Exposure Routes and Pathways

Routes of exposure include dermal contact, inhalation, and (potentially) ingestion. The most extensive exposure is occupational, for example, through inhalation of dust particles and dermal contact during production or in loading and unloading of crystallizers and centrifuges, and in the packaging of finished product.

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Mechanism of Toxicity

Hydroxylamine acts as a reducing agent when absorbed systemically, producing methemoglobin and the formulation of Heinz bodies in the blood. It can induce hemolytic anemia. It inhibits platelet aggregation and is a 'nitric oxide' vasodilator. Oxylmines such as hydroxylamine and methoxylamine disturb DNA replication and act as potent mutagens, causing nucleotide transition from one purine to another or one pyrimidine to another.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hydroxylamine is a positive dermal sensitizer in guinea pigs and mice. The intraperitoneal LD_{50} is 60 mg kg^{-1} in mice and 59 mg kg^{-1} in rats. Hydroxylamine is a strong dermal and ocular irritant.

Human

Hydroxylamine produces methemoglobin when systemically absorbed, potentially resulting in cyanosis, convulsions, hypotension, and coma. It is a marked irritant (also reported to be corrosive) to eyes, skin, and mucous membranes.

Chronic Toxicity (or Exposure)

Animal

It is reported to be a teratogen in rabbits but not in rats.

Human

Long-term exposures at lower levels can induce hemolytic anemia. It is a dermal and pulmonary sensitizer.

In Vitro Toxicity Data

It is an *in vitro* mutagen in most test systems, at levels as small as $10-20 \text{ mmol l}^{-1}$ in some systems. Industrially used hydroxylamines were studied in human blood cells *in vitro*. The parent compound hydroxylamine and the O-ethyl derivative gave very similar results. Both compounds induced a high degree of methemoglobin formation and glutathione depletion. Cytotoxicity was visible as Heinz body formation and hemolysis.

Clinical Management

Treatment consists of administration of methylene blue (1% solution), 0.1 ml kg^{-1} intravenously over a 10 min period.

Environmental Fate

Hydroxylamine's production and use may result in its release to the environment through various waste streams. Hydroxylamine will exist solely as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 18 h. Abiotic degradation of hydroxylamine by photochemically produced peroxy radicals is an important environmental fate process in surface waters, with the half-life of this reaction measured at ~ 2 h. An estimated bioconcentration factor of 3 suggests the potential for bioconcentration in aquatic organisms is low.

See also: Blood.

Further Reading

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- http://www.inchem.org Hydroxylamine (International Chemical Safety Cards 0661) (October 1995).

Hymenoptera

Gary W Everson

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• SYNONYMS: Bees; Hornets; Wasps; Ants

Exposure Routes and Pathways

Hymenoptera envenomation results from the subcutaneous injection of venom through a stinging apparatus.

Mechanism of Toxicity

The constituents of Hymenoptera venom differ among bees, wasps, and ants. As an example, honeybee venom includes phospholipase A, hyaluronidase, and various other allergens. Local skin reactions occur as a result of endogenous inflammatory response to the injected foreign proteins and enzymes. Multiple stings can result in a generalized systemic reaction due to the large volume of venom injected and absorbed. Following multiple stings, large amounts of injected venom may cause the release of vasoactive substances, which can lead to hypotension and shock. Allergic (anaphylactic) reactions may also occur as a result of antibody production from a previous sensitization (previous sting). The resulting IgE mediated reaction triggers the release of such vasoactive substances as histamine and leukotrienes.

Acute and Short-Term Toxicity (or Exposure)

Human

Clinical manifestations following a bite or sting can be classified into three groups. The first and most common reaction is a mild local tissue reaction characterized by minor swelling, redness, itching, and pain at the site of the sting. The second involves a systemic reaction resulting from multiple stings (typically requiring > 50–100 stings). Symptoms may include nausea, vomiting, headache, and loss of consciousness. Renal failure and seizures are rare, but have been documented. Lastly, allergic reactions may develop as a result of an individual's prior sensitization to the venom. This may range from a simple urticarial reaction to anaphylaxis. The latter is characterized by laryngeal edema, bronchospasm, difficulty in breathing, wheezing, hypotension, cardiovascular collapse, and death. Anaphylactic reactions may occur within 15–30 min following a sting. Although unrelated to toxicity, any bite or sting may also result in local infection.

Chronic Toxicity (or Exposure)

Animal

Bees have been used therapeutically in some animal populations for treatment of a variety of disorders. A recent study examined the effects of bee stings on sows with hypogalactia syndrome postpartum. Complications expected from this type of therapy are primarily local effects as well as more systemic hemorrhagic effects.

Human

One small epidemiologic study linked arthritis of the hands with exposure to bee stings in patients occupationally exposed to bee venom (bee keepers).

Clinical Management

Basic and advanced clinical life support may be required for those individuals exhibiting anaphylactic reactions following a bite or sting. As opposed to wasps, honeybees possess barbed stingers that remain imbedded in the skin along with the venom sac. Following a bee sting, the stinger should be removed using a stiff card (e.g., credit card) scraped across the skin at an angle. The stinger should not be grasped since doing so will contract the attached venom sac and force more venom into the skin. Nonallergic local reactions following small numbers of bites or stings can generally be managed outside of the hospital setting. Home remedies such as the application of meat tenderizer to the sting site are not effective. Best results are obtained by washing the site well with soap and water, applying a disinfectant such as iodine or alcohol at the site, and using a cold compress to decrease swelling and pain. Antihistamines, like diphenhydramine, may help relieve itching and swelling. Stings to the oral mucosa have occurred during the process of accidentally swallowing a bee or wasp. Swelling of the oropharynx may occur and impair breathing. These cases represent a medical emergency and should be managed in a health care facility. Patients with multiple stings (>50-100), those with a history of anaphylaxis, and those exhibiting an allergic reaction to a sting should be managed in an emergency department setting. Patients with multiple stings require general supportive care and observation. Urticarial reactions may be managed with antihistamines with or without subcutaneous epinephrine depending on the severity of the reaction. Anaphylaxis must be treated promptly and aggressively. Intravenous fluids should be started immediately. Respiratory status should be evaluated and an airway established with supplemental oxygen, if necessary. Subcutaneous epinephrine 1:1000 should be administered immediately. Intravenous diphenhydramine may be used but it is of secondary importance. A vasopressor is occasionally required in addition to intravenous fluids to manage hypotension.

See also: Animals, Poisonous and Venomous; Diphenhydramine.

Further Reading

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Hypersensitivity, Delayed Type

Leigh Ann Burns-Naas

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In general terms, the purpose of the immune system is to provide protection for the individual against disease, whether infectious, parasitic, or tumorigenic. In doing so, the body must recognize what is 'self' from what is foreign (e.g., nonself). In most situations, the immune system acts appropriately. However, there are times when the immune system acts in an exaggerated manner leading to tissue damage. This is referred to as hypersensitivity. In the classic Coombs and Gell classification system, there are four types of hypersensitivity reactions. The first three (types 1–3) are mediated by antibody (e.g., IgG, IgE). The fourth type (type 4) is mediated by antigenspecific T cells and is also known broadly as delayedtype hypersensitivity (DTH). It is 'delayed' because the reaction appears hours to days after antigen crosses into the skin. Though often thought of as adverse effects (probably due to the association with contact hypersensitivity), DTH responses are probably important in natural host defense to intracellular infectious organisms, such as mycobacteria, listeria, candida, and certain viruses. DTH reactions are also associated with certain diseases, such as Wegener's granulomatosis and sarcoidosis. The prototypical DTH response is the tuberculin reaction, though the more commonly recognized is cutaneous (contact) hypersensitivity mediated by small molecular weight chemicals. Some DTH responses are also considered to be allergic responses; specifically, chemical allergy that may lead to the development of allergic contact dermatitis.

Contact Hypersensitivity

Of the various DTH responses, contact hypersensitivity possibly affects the greatest number of individuals. It is a common occupational health problem in a variety of industrial settings and it can occur following chemical exposures in the home and in the environment. One of the most recognized contact hypersensitivity reactions affecting many people is that which develops in response to cutaneous exposure to poison ivy (pentadecacatechol). Contact hypersensitivity reactions are initiated by topical exposure to the skin (often by a small molecular weight chemical), and the subsequent response is primarily epidermal.

Contact (or chemical) hypersensitivity reactions develop in two phases: induction and elicitation (Figure 1). Induction is the development of the initial sensitization. In this phase, the chemical penetrates the epidermis. Chemicals are haptens because they are, by themselves, unable to elicit an immune response. As such, they must associate with proteins in the skin to stimulate a specific immune response. The hapten–protein complex is then processed by antigen presenting cells in the skin, transported and presented to T cells in the draining lymph nodes. This interaction leads to a proliferative response and the development of memory T cells that distribute systemically within the body.

Elicitation is the clinical response to subsequent challenge with the antigen or hapten. Penetration into the skin leads to encounters with antigenspecific memory T cells. These cells release cytokines such as IFN- γ and IL-17 that recruit other cells to the site and stimulate the production of chemoattractants and proinflammatory cytokines (e.g., IL-8, IL-1, TNF- α , IL-6) from multiple cell types, including T cells and keratinocytes. The resulting effect is an infiltration of inflammatory cells into the tissue and a localized increase in vascular permeability leading to the development of erythema and edema, and/or the appearance of cutaneous vesicles or papules. Although this is typically thought of as a local effect, it may be systemic as well.

In summary, chemical sensitization is dependent upon intact immunological function and the integrity of T lymphocyte responses. It is also dependent upon the ability of the hapten–protein complex to stimulate (in a susceptible individual) an immune response of sufficient vigor and of the right quality such that when that individual is exposed to the inducing chemical for the second time (by an appropriate route) they will mount a more accelerated and more aggressive inflammatory response.

Tuberculin-Type Hypersensitivity

In contrast to contact hypersensitivity, tuberculintype hypersensitivity reactions are primarily dermal and result from intradermal injections into the skin. In people that have had tuberculosis or have been exposed to the bacterium through infection or BCG immunization, a cell-mediated immune response to the bacterium develops. When small amounts of tuberculin (a complex mixture of antigenic material derived from Mycobacterium tuberculosis) are subsequently injected into the skin, a localized T celldependent inflammatory response develops in the dermis. Within 24–72 h of injection, individuals with prior exposure to the bacterium display a raised, red, indurated area on the skin at the injection site. The lack of a response suggests no prior exposure to the bacterium.

Methods to Assess Delayed-Type Hypersensitivity

There are several acceptable ways to evaluate DTH responses in nonclinical species. Of these, the most common are the guinea pig assays used to assess contact sensitization. Both the Magnusson and Kligman model (guinea pig maximization test) and the Buehler model measure the elicitation phase of the hypersensitivity response, though the tests vary in their methods of chemical application and utilization of adjuvants. Most recently, the local lymph node assay has been accepted as a stand alone test for chemical hypersensitivity. This assay is conducted in mice and measures the induction phase of sensitization. In humans, the most common methods to assess delayed hypersensitivity are the patch test (contact sensitivity; for diagnostic purposes) and the human repeat insult patch test (contact sensitivity; for predictive purposes). Additionally, intradermal

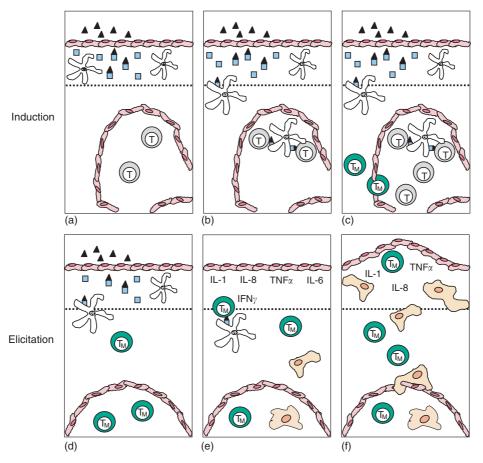


Figure 1 Development of Chemical-Induced (Contact) Hypersensitivity. (a) Following dermal exposure, the chemical (hapten) penetrates the epidermis and complexes with proteins in the skin. (b) The hapten–protein complex is recognized and processed by antigen presenting cells (APCs; Langerhan's cells) in the skin. The APCs then begin to migrate to the draining lymph nodes. (c) Upon entering the draining lymph nodes, the APCs interact with T cells, causing their subsequent activation and proliferation. These antigen-specific T cells develop into memory T cells which then distribute systemically in the body. (d) Upon subsequent exposure to the chemical, APCs in the skin again recognize, process, and present the hapten–protein complex on their surface. (e) Some of these APCs interact with resident, antigen-specific, memory T cells. Activated memory T cells release cytokines causing the release of other proinflammatory mediators and chemoattractants from other cell types in the skin, and beginning the migration of additional memory T cells and proinflammatory cells from the circulation. (f) Memory T cells and inflammatory cells continue to migrate to the epidermis, resulting in erythema and edema at the site of chemical exposure. (Reproduced from Burns-Naas LA, Meade BJ, and Munson A (2001) Toxic responses of the immune system. In: Klaassen C (ed.) *Casarett & Doull's Toxicology. The Basic Science of Poisons*, 6th edn., pp. 419– 470. New York: Academic Press.)

injection of an antigen is used to determine if individuals have developed active immunity (e.g., memory T cells) to infectious agents, such as *M. tuberculosis* (e.g., tuberculin skin test).

See also: Immune System; Skin; Toxicity Testing, Sensitization.

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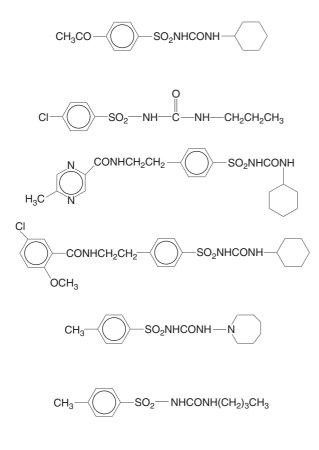
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Hypoglycemics, Oral

Henry A Spiller

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- REPRESENTATIVE CHEMICALS: Sulfonylureas and glinides
- Synonyms:
 - First-generation sulfonylureas: Acetohexamide, Dylmelor[®] (CAS 968-81-0); Chlorpropamide, Diabenese[®] (CAS 94-20-2); Orinase[®] (CAS 64-77-7); Tolazamide, Tolynase[®] (CAS 1156-19-0); Tolbutamide
 - Second-generation sulfonylureas: Glipizide, Glucotrol[®] (CAS 29094-61-9); Glyburide (glibenclamide); Diabeta[®] (CAS 102.38-2.1-8); Glimepiride, Amaryl[®]
 - Glinides: Repaglinide, Novonorm[®] or Prandin[®]; Nateglinide, Starlix[®]
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: All sulfonylureas are arylsulfonylureas, with substitutions on the benzene and the urea groups producing the different drugs. Repaglinide is a nonsulfonylurea oral hypoglycemic. Nateglinide is an amino acid derivative oral hypoglycemic agent
- CHEMICAL STRUCTURES:



Uses

Sulfonylureas and glinides are used in the treatment of (Type II) noninsulin-dependent diabetes mellitus.

Exposure Routes and Pathways

All sulfonylureas are available as tablets only, with the exception of tolbutamide, which is available as tablets and a sterile solution for injection. The glinides are available as tablets only.

Toxicokinetics

The only significant difference in the sulfonylureas and the glinides is in the toxicodynamic parameters of onset of action and duration of action. Sulfonylureas are readily absorbed from the gastrointestinal tract with the exception of tolazemide, which is absorbed somewhat more slowly. The glinides are rapidly absorbed with peak serum levels in 30-60 min. Peak plasma levels of the sulfonylureas occur within a range of 1 h. However, in overdose the duration of effect is a more important factor than onset of action. There is extensive biotransformation of sulfonylureas and the glinides by the liver. Sulfonylureas with active metabolites are acetohexamide and chlorpropamide. There are no active metabolites for repaglinide or nateglinide. The sulfonylureas and glinides are highly protein bound, from 92% to 99%, except for acetohexamide, which is 65-90%. Glyburide may accumulate in deep body compartments, allowing for later redistribution after withdrawal of the drug. Sulfonylureas and nateglinide are cleared primarily in the urine as metabolites. Repaglinide is cleared primarily via the feces. Glyburide is also cleared to a significant amount as the parent drug in the feces via biliary secretion. The half-lives of sulfonylureas are generally less important clinically than durations of action, which range from 8 to 72 h. Both nateglinide and repaglinide have a short duration of action of ~ 4 h.

Mechanism of Toxicity

The sulfonylureas and the glinides bind to the sulfonylurea receptor on the cell membrane of the beta cells of the pancreatic islets, which causes the ATPsensitive potassium channel to close. The closed potassium channel increases membrane potential causing the voltage-sensitive calcium channel to open. The sudden influx of calcium begins a cascade of events including kinase activation, which results in release of preformed insulin into circulation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Sulfonylureas are not routinely used in animals. Hypoglycemia, lethargy, and seizures can occur.

Human

The cascade of symptoms from a sulfonylurea or glinide overdose will reflect the patient's hypoglycemic state secondary to hyperinsulinemia. Initially, the patient may present with restlessness, diaphoresis, altered mental status, combative behavior, tremors, or confusion. An infant or small child may be difficult to feed. Nausea, vomiting, or abdominal pain may also occur. This will be followed by increasing central nervous system depression, seizures, and coma if the patient's blood glucose continues to fall. Most other effects reflect persistent hypoglycemia. In small children and poorly nourished patients, the onset of hypoglycemia may be sudden. In severe cases of persistent and prolonged hypoglycemia, hypotension, tachycardia, and eventually cardiac arrest may occur. Metabolic acidosis may also occur. In sulfonylurea overdose, for patients without concomitant intravenous glucose supplementation, an 8-h observation period should be sufficient to detect those patients who will become hypoglycemic. Those patients who do show evidence of hypoglycemia should be monitored for 24 h due to the potential for prolonged duration of effect. In a glinide overdose, due to their more rapid onset and shorter duration of action, a 3-4 h period of observation would be sufficient. A 24 h observation period is not expected to be necessary for the glinides. A disulfiram-like reaction may occur with concomitant ethanol and sulfonylurea use.

Chronic Toxicity (or Exposure)

Animal

Rats and mice exposed to chlorpropamide at doses greater than 6000 ppm for 2 years found no evidence of increased rates of tumor development.

Human

The primary toxic effects of oral hypoglycemic agents are related to their effects of decreasing blood sugar. Patients have also reported gastrointestinal

effects (nausea, vomiting, diarrhea), rare hepatic toxicity, and hypersensitivity reactions.

In Vitro Toxicity Data

Chlorpropamide was mutagenic in Chinese hamster cells but negative in Ames *Salmonella* assays.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as appropriate to the patient's level of consciousness and the history of the ingestion. Activated charcoal effectively binds sulfonylureas. The cornerstone of therapy is glucose replacement or in severe cases inhibition of insulin secretion. Continuous intravenous 10% glucose in water via a peripheral line is usually sufficient to maintain euglycemia. However, patients may require additional boluses of D25W or D50W to maintain adequate blood glucose. In cases of symptomatic sulfonylurea overdose, due to their pronged effects, frequent blood glucose monitoring is recommended. Patients are at greatest risk during or after periods of fasting, such as sleep. Delayed or prolonged effects are not expected in glinide overdose. Glucose therapy should be titrated to the patient's serial blood glucose measurements. Additional oral glucose via frequent snacks and meals will be helpful but usually not sufficient by themselves. In cases of recurrent or refractory hypoglycemia octreotide may be helpful by altering calcium influx in the beta cell and, therefore, reducing insulin secretion. Octreotide, a long-acting somatostatin analog, may be administered subcutaneously or intravenously. Subcutaneous administration may be 50-100 µg every 6-12 h as needed in adults or $1 \mu g k g^{-1}$ every 6–12 h as needed in children. Continuous intravenous administration can be given at a rate of $15-30 \text{ ng kg}^{-1} \text{min}^{-1}$.

See also: Diabetes, Effect of Toxicity.

Further Reading

- Palatnick W, Meatherall RC, and Tenenbein M (1991) Clinical spectrum of sulfonylurea overdose and experience with diazoxide therapy. Archives of Internal Medicine 151: 1859–1862.
- Spiller HA, Villalobos D, and Krenzelok EP (1997) Prospective multicenter study of sulfonylurea ingestion in children. *Journal of Pediatrics* 131: 141–146.

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latrogenic Disease

Beck Bertine Goldberg

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Iatrogenic disease is any disease, sickness, disfigurement, or death that occurs during the practice of acceptable medical care. The word iatrogenic comes from 'iatros', which is Greek for medical or medicinal, and 'genic', which is Greek for 'caused by'. In the United States, death by iatrogenic causes is the third leading cause of death after deaths from heart disease and cancer. These include adverse reactions to prescriptions, medical mistakes, unnecessary surgery, errors in hospitals, and nosocomial infections. It has been estimated in the United States that 106 000 deaths per year occur from nonerror, adverse effects of medications and 7000 deaths per year from medication errors in hospitals.

Three Toxicological Examples

1. Merbromin is a weak antiseptic that contains mercury and is used in the treatment of minor dermal injuries, and in the escharotic treatment of large omphaloceles in neonates. An omphalocele is an abnormality found in neonates where the infant's intestine or other abdominal organs protrude from the infant's navel. Merbromin can be absorbed topically through the omphalocele sac and cause mercury toxicity and/or death. Alternatives lacking the toxicity of merbromin are silver sulfadiazine and gentian violet.

- 2. Patients taking methotrexate, an antimetabolite used for controlling the symptoms of conditions such as rheumatoid arthritis and psoriasis, can cause suppression in bone marrow. To reduce the incidence of bone marrow suppression, this drug is only given once weekly ($\sim 30 \text{ mg a week}$) along with folic acid supplements.
- 3. Dental amalgam restorations leak small amounts of elemental mercury vapor into the oral cavity of the mouth. The released mercury can be taken up by the saliva and then distributed to various organs and compartments throughout the body. Daily mercury uptake rates from amalgam are estimated to range from 2 to $25 \,\mu g \, Hg/24 \, h$ with the 'worst case' individual estimated to have an uptake of $70 \,\mu g \, Hg/24 \, h$. Mercury is a known neurotoxicant and the off-gassing of mercury over time may cause dementia like conditions in some people.

See also: Interactive Toxicity; Mercury; Monoamine Oxidase Inhibitors.

Further Reading

- Sarvana S and Lalukotta K (2003) Myelotoxicity due to methotrexate An iatrogenic cause. *European Journal of Haematology* 71: 315–316.
- Starfield B (2000) Is US health really the best in the world? Journal of the American Medical Association 284: 483– 485.

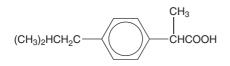
Ibotenic Acid See Mushrooms, Ibotenic Acid.

Ibuprofen

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 15687-27-1
- SYNONYMS: 2-(4-Isobutylphenyl) propionic acid; Advil; Bayer Select; Dayquil Sinus; Dimetapp Sinus; Dristan Sinus; Excedrin IB; Haltran; Medipren; Motrin; Midol 200; Nuprin; Pamprin IB; Profen; Rufen
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: A substituted phenylalkanoic acid; Nonsteroidal antiinflammatory and analgesic agent
- CHEMICAL STRUCTURE:



Uses

Ibuprofen is used for analgesic, antipyretic, and antiinflammatory purposes.

Exposure Routes and Pathways

Ibuprofen is available in tablet and liquid dosage forms. Ingestion is the most common route of both accidental and intentional exposures to ibuprofen.

Toxicokinetics

Ibuprofen is rapidly absorbed after ingestion with peak plasma concentrations obtained within 1-2 h. Ibuprofen is highly protein bound (99%) and occupies only a fraction of the total drug binding sites during therapeutic use. The volume of distribution is $0.1-0.21 \text{ kg}^{-1}$. Ibuprofen passes slowly into synovial spaces and may remain there in higher concentration as the concentrations in plasma decline. Ibuprofen passes readily across the placenta. Ibuprofen is extensively metabolized, yielding four urinary metabolites formed by hydroxylation. The excretion of ibuprofen is rapid and complete. Ibuprofen's elimination half-life is 1-2h. Approximately 90% of an ingested dose is excreted in the urine as metabolites or their conjugates, and 10% is eliminated as free drug.

Mechanism of Toxicity

The mechanisms of ibuprofen-induced toxicity have not been clearly defined. Acute renal failure is postulated to result from decreased production of intrarenal prostaglandins via inhibition of the cyclooxygenase pathway. In turn, this will decrease the renal blood flow and glomerular filtration rate. Ibuprofen also interferes with prostaglandin synthesis in the gastrointestinal system that can contribute to its irritating effect on the mucosa of the gastrointestinal tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ibuprofen is not recommended for use in animals. Dogs appear to be exquisitely sensitive to the propionic acid group of nonsteroidal anti-inflammatory drugs (NSAIDs) and easily develop gastric ulcers and renal failure. Seizures have been reported in both dogs and cats after ingestion of ibuprofen.

Human

The majority of patients who acutely overdose on ibuprofen remain asymptomatic. In one retrospective study of ibuprofen overdoses, only 19% of patients developed symptoms. Abdominal pain, nausea, vomiting, lethargy, and drowsiness are the most frequently reported symptoms. In rare instances of massive acute overdose, apnea, seizures, hypotension, metabolic acidosis, renal failure, and coma have occurred.

Chronic Toxicity (or Exposure)

Human

The chronic ingestion of excessive amounts of ibuprofen may produce similar toxicity as acute but in a more insidious fashion. Gastritis and renal dysfunction may be seen.

In Vitro Toxicity Data

Studies of ibuprofen and other NSAIDs have produced toxic effects at concentrations 10 times therapeutic in cultured hepatocytes. No adverse effect on cell survival was noted at therapeutic concentrations of ibuprofen in this model, although increases in lactate dehydrogenase leakage were prominent.

Clinical Management

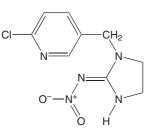
Treatment of acute overdoses of ibuprofen should consist of symptomatic supportive care. Ingestions $> 250 \text{ mg kg}^{-1}$ may require hospital evaluation and treatment. Children ingesting $> 400 \text{ mg kg}^{-1}$ have the greatest risk for serious toxicity. Activated charcoal may be used to adsorb ibuprofen or concomitant ingestants if given within 1 h of the exposure. Adequate hydration should be assured. Serum ibuprofen levels are not readily available and do not influence patient management.

Imidacloprid

Larry P Sheets

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 138261-41-3
- SYNONYMS: 1-[(6-Chloro-3-pyridinyl)methyl]-*N*nitro-2-imidazolidinimine; Confidor[®]; Gaucho[®]; Admire[®]
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Neonicotinoid insecticide
- CHEMICAL FORMULA: C₉H₁₀ClN₅O₂
- CHEMICAL STRUCTURE:



Uses

Imidacloprid is a neonicotinoid insecticide that is registered for many uses, including grub and termite control, crop protection, and to control fleas and ticks on companion animals. Its insecticidal activity is attributed to nicotinic activity on post-synaptic receptors.

Exposure Routes and Pathways

Occupational exposure may occur through dermal contact where imidacloprid is produced or used. Exposure to the general population may occur through dermal contact or consumption of food residues. Imidacloprid is not a primary irritant and does not See also: Propionic Acid.

Further Reading

- Hall AH, Smolinske SC, and Conrad FL (1986) Ibuprofen overdose: 126 cases. *Annals of Emergency Medicine* 15: 1308–1313.
- McElwee NE, Veltri JC, and Bradford DC (1990) A prospective, population-based study of acute ibuprofen overdose: Complications are rare and routine serum levels not warranted. *Annals of Emergency Medicine* 19: 657–662.

cause damage at the point of contact (i.e., skin, eyes, lungs and gastrointestinal tract). Penetration of the skin is poor but may be facilitated by formulating agents in commercial products.

Toxicokinetics

There are two principal routes of metabolism in mammals. The first involves oxidative cleavage to imidazolidine and 6-chloronicotinic acid, with urinary excretion of imidazolidine moiety. The nicotinic moiety is degraded by glutathione-conjugation to a mercapturic acid derivative and then to methyl mercaptonicotinic acid, which is conjugated with glycine to form a hippuric acid conjugate for excretion. The second involves hydroxylation of the imidazolidine ring, followed by the elimination of water and formation of an unsaturated metabolite. More than 90% of the dose is eliminated within 24 h, with total excretion by 48 h; 80% of the dose is excreted via the urine, with 20% eliminated via the feces. In rats, imidacloprid is absorbed and distributed to organs within 1 h following oral administration. It is not distributed to fatty tissues, the central nervous system (CNS) or bone, so it tends not to accumulate or affect the CNS. Poor penetration of the blood-brain barrier also occurs with other neonicotinoids. Reduced access to receptors in the CNS contributes to its low potential for centrally mediated effects.

Mechanism of Toxicity

Mammalian tissues contain many subtypes of nicotinic receptors that are located in various tissues, including autonomic ganglia, skeletal muscle (neuromuscular junction), spinal cord, and various brain regions. Differences in binding properties to the various receptor subtypes contribute greatly to the much lower activity of imidacloprid and other neonicotinoids in vertebrate tissues, as compared to insects. The relative specificity for the nicotinic receptor in insects is used to select neonicotinoids for commercial development. This attribute, combined with poor penetration of the blood-brain barrier to access tissues with the most sensitive receptors, contributes to high margins of safety.

The acute toxicity (i.e., lethal potency) of imidacloprid, other neonicotinoids, and related analogs in mammals is most closely related to potency at the α_7 nicotinic receptor subtype, followed in order by potency at α_4 , β_2 , α_3 , and α_1 nicotinic receptors, respectively. However, acute toxicity in mammals involves complex actions (agonist and antagonist) at multiple receptor subtypes and these actions vary greatly with minor changes in chemical structure.

Acute and Short-Term Toxicity (or Exposure)

There are few published studies on the toxicity of imidacloprid or other neonicotinoid insecticides and the ones that are available are generally limited to an examination of acute lethal potency (e.g., LD_{50}). Casida and coworkers reported tremor in mice treated with an acute oral dose of imidacloprid and other neonicotinoids, providing evidence of nicotinic stimulation at near-lethal or lethal dose levels. The limited information on the toxicology of imidacloprid that has been published contrasts with the extensive database generated for the registration of commercial products. These studies were performed in accordance with regulatory guidelines (e.g., US Environmental Protection Agency, Organization for Economic Cooperation and Development, and Japanese MAFF), in compliance with GLP standards.

Animal

Imidacloprid is not an irritant and does not produce evidence of dermal sensitization. Acute exposure to imidacloprid produces minimal toxicity by dermal and inhalation routes of exposure and moderate toxicity by oral administration. The acute (4 h exposure) LC_{50} by inhalation is >69 mg m⁻³ air (droplets) and >5323 mg m⁻³ air (dust). The LD₅₀ in rats by oral and dermal exposure is 450 and >5000 mg kg⁻¹, respectively. Acute administration of an aqueous suspension by gavage to adult rats had no effect at 50 and 100 mg kg⁻¹ in males and females, respectively, while higher doses of up to 315 mg kg⁻¹ produced clinical signs, without mortality. Treatment-related deaths at higher doses generally occurred within 3–7 h following treatment. Signs associated with treatment include tremor, gait incoordination, decreased activity, as well as nasal and urine staining. Signs of intoxication occurred within 15–40 min following oral administration and, except for stains, were reversible within 8–24 h after treatment. This profile is consistent with rapid distribution and metabolism.

Human

Little is known about the acute toxicity of imidacloprid in humans.

Subchronic Toxicity

Imidacloprid was administered through the diet for 13 weeks to young-adult Wistar rats. Clinical signs associated with treatment were not evident at exposures as high as $300 \text{ mg kg}^{-1} \text{ day}^{-1}$. The liver was the principal target organ, with hypertrophy of hepatocytes and sporadic cell necrosis in high-dose males only. Liver pathology was mild at study termination and fully reversible within the 4 week recovery period. The no-observed-effect level (NOEL) was 14 and 83 mg kg⁻¹ day⁻¹ in males and females, respectively.

Imidacloprid was administered through the diet to young-adult beagles for 13 weeks. Tremor was seen in males and females that received 600 and 1800/ 1200 ppm (dietary level reduced after week 4 due to decreased food consumption and weight loss), however, this finding was not substantiated at comparable doses in other studies. There was no evidence of tissue damage by clinical chemistry, gross necropsy examination, tissue weight or microscopic examination at any dietary level. The NOEL was 200 ppm in both sexes.

Chronic Toxicity (or Exposure)

Animal

Rat and Mouse A chronic toxicity/carcinogenicity study was performed, with imidacloprid administered to male and female Wistar rats for 12 months and 2 years at dietary levels of 100, 300, 900, and 1800 ppm. These dietary concentrations resulted in average daily dosages of 5.7, 17, 51, and 103 mg kg^{-1} for males, and 7.6, 25, 73, and 144 mg kg^{-1} for females. The thyroid was a target organ, with mineralization of the colloid, fewer colloid aggregation sites, and parafollicular hyperplasia sites at $144 \text{ mg kg}^{-1} \text{ day}^{-1}$. This lesion did not affect thyroid function (e.g., plasma T3, T4, and TSH levels were normal). Mineralization of the colloid was also evident in males at 300 ppm and in both sexes at 900 ppm. There was no change in liver morphology at any dietary level. The NOEL was $5.7 \text{ mg kg}^{-1} \text{ day}^{-1}$ and there was no evidence of carcinogenicity.

The oncogenic potential of imidacloprid was investigated in B6C3F1 mice that were exposed through the diet for 12 or 24 months, at concentrations that resulted in average daily doses of 20, 66, 208 or 414 mg kg⁻¹ for males and 30, 104, 274 or 424 mg kg⁻¹ for females. There were no clinical signs due to treatment and no effect on survival at any level. Metabolic adaptation was apparent at the highest dose only as low-grade periacinary hepatocyte hypertrophy. There were no effects on serum chemistry, tissue weight, or tissue morphology (by gross and microscopic examination) at any dietary level. There was no evidence of carcinogenic potential and the overall NOEL was 66 mg kg⁻¹ day⁻¹.

Based on results in both species, imidacloprid is classified in category 'E', which indicates there is evidence of non-carcinogenicity for humans.

Dog Imidacloprid was administered to young-adult beagles for 52 weeks at dietary levels corresponding to dosages of 6.1, 15, and $41/72 \text{ mg kg}^{-1} \text{ day}^{-1}$ (the dietary level was increased at week 17). The tremor that was evident in the aforementioned subchronic canine study was not evident here at any dietary concentration. Effects in high-dose animals included a slight increase in plasma cholesterol (females only) and a slight increase in hepatic cytochrome P-450 activity (both sexes). The liver was the principal target organ, with increased liver weight and induction of cytochrome P-450 enzymes. The NOEL in this study was 15 mg kg⁻¹ day⁻¹.

Human

Little is known regarding the chronic effects of imidacloprid in humans.

Mutagenicity

Imidacloprid has been evaluated for mutagenicity using a full complement of *in vitro* and *in vivo* tests required for registration. All tests, including the *in vitro* point mutation tests, *in vivo* chromosomal aberration tests, a mitotic recombination test in yeast, a rec assay with *Bacillus subtilis*, and the unscheduled DNA synthesis (UDS), were negative.

Developmental Toxicity

The potential for imidacloprid to produce developmental toxicity, including teratogenicity, was examined in the rat and rabbit. In the rat, fetal malformations were not evident at any dose, the maternal NOEL was $10 \text{ mg kg}^{-1} \text{ day}^{-1}$ and the fetal NOEL was $30 \text{ mg kg}^{-1} \text{ day}^{-1}$. In the rabbit, embryotoxicity was only evident at a maternally toxic dose and fetal malformations were not evident at any dose; the maternal NOEL was $8 \text{ mg kg}^{-1} \text{ day}^{-1}$ and the fetal NOEL was $24 \text{ mg kg}^{-1} \text{ day}^{-1}$. The results in these species indicate that imidacloprid is not a primary embryotoxicant and is not teratogenic.

Reproductive Toxicity

Effects on reproduction and development were examined in a two-generation, two-litter study in Wistar rats (30/sex/dietary level in the parental generation), at dietary concentrations of 100, 250, and 700 ppm. Liver enzymes (cytochrome P-450, O-demethylase, and N-demethylase) were induced in high-dose maternal animals. Reproduction and development were not affected at any dietary level and there was no evidence of pathology, in the form of malformations, gross lesions, changes in tissue weight or histopathology, at any dose. The NOEL in this study was $6.7 \text{ mg kg}^{-1} \text{ day}^{-1}$ for adults and 12.5 mg kg⁻¹ day⁻¹ for the offspring.

Neurotoxicity

Acute neurotoxicity was evaluated in adult rats that received a single dose of imidacloprid by gavage as an aqueous suspension at doses of 20 (females only), 42, 151, or $307 \,\mathrm{mg \, kg^{-1}}$ (both sexes). The only effect at 42 mg kg^{-1} was a slight decrease in the activity of females in an automated test device. Effects at $150 \,\mathrm{mg \, kg^{-1}}$ included tremor (one female), a slight decrease in body temperature and red nasal stain. The high dose produced severe toxicity, including lethality (two males and eight females) within 4-24 h after treatment and 4h after treatment, tremor was apparent in all surviving animals and body temperature was reduced. Overt toxicity at this lethal dose included motor incoordination (e.g., incoordinated gait and impaired aerial righting), autonomic signs (e.g., perianal and urine stains), and evidence of CNS depression (e.g., minimal activity and a diminished response to stimuli). Clinical signs generally resolved in surviving animals within 8-24 h following treatment. Neuropathology was not evident. The NOEL for males and females was 42 and 20 mg kg⁻¹, respectively.

Neurotoxicity was also evaluated in young-adult rats, with imidacloprid administered via the diet for 13 weeks at dietary concentrations of 150, 1000, and 3000 ppm. These dietary levels resulted in average daily exposures of 9.3, 63, and 196 mg kg^{-1} for males, and 10.5, 69, and 213 mg kg⁻¹ for females.

There was little evidence of toxicity at any dietary level. Effects at 1000 and 3000 ppm consisted of decreased food consumption and an associated decrease in body weight gain. Effects were not evident by cage-side observation or automated test of activity at any dietary level. At week 13, there was a modest increase in the incidence of high-dose males with a slightly uncoordinated righting response. There was no evidence of neuropathology. The NOEL was 9.3 and 10.5 mg kg⁻¹ day⁻¹ for males and females, respectively.

In Vitro Toxicity Data

As noted above, *in vitro* studies of the selectivity of imadocloprid for nicotinic receptors has been useful in explaining its selective toxicity. *In vitro* mutagenic assays were all negative.

Clinical Management

The recommended treatment in cases of acute poisoning is symptomatic. It is important to monitor and support breathing if signs of respiratory paralysis appear and to monitor blood pressure and pulse rate, since bradycardia and hypotonia may occur. Since imidacloprid does not inhibit acetylcholinesterase activity, treatment with a reactivating oxime (e.g., pralidoxime) is not indicated. Furthermore, treatment with a nicotinic antagonist may be ineffective or potentially harmful since symptoms of poisoning may be mediated by stimulation or inhibition of various nicotinic receptor subtypes or by other possible mechanisms.

Environmental Fate

Numerous laboratory and field studies have been conducted with imidacloprid, providing a comprehensive understanding of its behavior in the environment. Imidacloprid has a low octanol-water partition coefficient ($K_{ow} = 3.72$), which is consistent with it not accumulating in biological tissue or the food chain. Imidacloprid has an extremely low vapor pressure and therefore does not volatilize into the atmosphere. Imidacloprid is generally not persistent in aquatic environments and is quickly degraded by sunlight (4.2 h). In simulated pond studies, imidacloprid quickly degraded, with a half-life of 1.4 days. Imidacloprid also has rather unique soil binding characteristics; the longer imidacloprid ages in the field and the lower the initial residue concentration, the tighter it binds to soil. Field dissipation studies conducted in the USA have shown that the parent compound and its metabolites have limited mobility in soil and are not likely to leach to groundwater.

Ecotoxicology

Avian Species

The LD_{50} values for bobwhite quail and Japanese quail are 152 and 31 mg kg^{-1} , respectively. Redwinged blackbirds and brown-headed cowbirds have been observed to avoid imidacloprid-treated seeds after experiencing transient gastrointestinal distress (retching) and ataxia (loss of coordination). And so, it was concluded that treated seeds represent a minimal risk to birds.

Aquatic Organisms

Imidacloprid is moderately toxic to fish, with 96 h LC_{50} values of 211 mgl^{-1} for rainbow trout, $280 \text{ mg} \text{ l}^{-1}$ for carp and $237 \text{ mg} \text{ l}^{-1}$ for golden orfe. Imidacloprid is slightly toxic to *Daphnia magna* (48 h $EC_{50} = 85 \text{ mg} \text{ l}^{-1}$) but is highly toxic to certain other aquatic invertebrates.

Beneficial Insects

Imidacloprid is highly toxic to bees if used as a foliar application, especially during flowering, but is not considered a hazard to bees when used as a seed treatment.

See also: Acetamiprid; Neonicotinoids; Nithiazine.

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Immediately Dangerous to Life or Health (IDLH) Values

Alan J Weinrich

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The immediately dangerous to life or health (IDLH) air concentration values have been recommended by the US National Institute for Occupational Safety and Health (NIOSH) as respirator selection criteria. The current NIOSH definition for an IDLH condition is a situation "that poses a threat of exposure to airborne contaminants when that exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment." NIOSH's stated purpose for establishing IDLH values is to "ensure that the worker can escape from a given contaminated environment in the event of failure of the respiratory protection equipment." The complete introduction and documentations to the 398 existing IDLH values can be read or downloaded at the NIOSH Internet website.

At the time of writing, NIOSH is reconsidering several aspects of the IDLH definition and the criteria used in developing IDLH values.

Background

The concept of using respirators to protect workers in IDLH situations was discussed at least as early as the 1940s. The following is from a US Department of Labor bulletin:

The situations for which respiratory protection is required may be designated as, (1) nonemergency and (2) emergency. Nonemergency situations are the more or less normal ones that involve exposure to atmospheres that are not immediately dangerous to health and life, but will produce marked discomfort, sickness, permanent harm, or death after a prolonged exposure or with repeated exposure. Emergency situations are those that involve actual or potential exposure to atmospheres that are immediately harmful and dangerous to health or life after comparatively short exposures.

The US Occupational Safety and Health Administration (OSHA) defines an IDLH concentration in their hazardous waste operations and emergency response regulation (29 CFR 1910.120) as follows:

An atmospheric concentration of any toxic, corrosive or asphyxiant substance that poses an immediate threat to life or would cause irreversible or delayed adverse health effects or would interfere with an individual's ability to escape from a dangerous atmosphere. The OSHA regulation on permit-required confined spaces (29 CFR 1910.146), defines an IDLH condition as follows:

Any condition that poses an immediate or delayed threat to life or that would cause irreversible adverse health effects or that would interfere with an individual's ability to escape unaided from a permit space.

History

IDLH values were first developed by NIOSH in the mid-1970s based on an earlier concept by the US Bureau of Mines.

In 1974, NIOSH and OSHA jointly initiated development of occupational health standards for substances with then-existing OSHA permissible exposure limits (PELs). This joint effort was called the Standards Completion Program (SCP). The SCP developed 387 substance-specific draft standards with supporting documentations that became the basis for the original 1978 NIOSH/OSHA Pocket Guide to Chemical Hazards and the Occupational Health Guidelines for Chemical Hazards. As part of the respirator selection process for each draft standard, an IDLH value was established. The purpose for establishing an IDLH value was to determine a concentration from which a worker could escape without injury or without irreversible health effects in the event of respiratory protection equipment failure. At that time, NIOSH did not recommend a respirator for use at concentrations above the IDLH, with the exception of an escape respirator. Respirator selection was based on the reliability of the respirator; the perceived danger was respirator failure.

In determining IDLH values, NIOSH considered a worker's ability to escape without loss of life or irreversible health effects, along with severe eye or respiratory irritation and other deleterious effects that could prevent escape. To provide a safety margin, NIOSH based IDLH values on effects that might result from exposures as long as 30 min.

In 1985, NIOSH changed its respirator recommendations for IDLH environments from 'highly reliable', based on the danger of respirator failure, to 'most protective', based on health effects, and included a recommendation for 'emergency or planned entry in unknown concentration or IDLH conditions'. These highly protective respirators included either:

• self-contained breathing apparatus (SCBA) with a full facepiece and operated in a pressure-demand or other positive-pressure mode, or

 a supplied-air respirator with a full facepiece and operated in a pressure-demand or other positivepressure mode in combination with an auxiliary SCBA operated in a pressure-demand or other positive-pressure mode.

Criteria for Revised IDLH Values

In 1994, NIOSH used the following hierarchy to develop a preliminary, revised IDLH value for each chemical:

- 1. Human acute toxicity data were used if sufficient.
- 2. When sufficient human data were unavailable, mammalian acute lethal concentration (LC) data were considered. The lowest reliable LC data were used, with LC_{50} data preferred. If acute LC data determined from a 30 min exposure were not available, the data were adjusted to an equivalent 30 min value using the following relationship, proposed in 1986 by ten Berge and colleagues:

Adjusted LC₅₀ (30 min) = LC₅₀(t) × (t/0.5) × (1/n)

where $LC_{50}(t) = LC_{50}$ determined over *t* hours and where *n* is an experimentally derived constant. The adjusted or experimentally derived 30 min LC values were divided by a factor of 10 to determine a preliminary IDLH value.

- 3. When neither human data nor animal LC data were sufficient, NIOSH considered animal lethal dose (LD) data. NIOSH used the LD data to estimate the equivalent total dose to a 70 kg worker. The 30 min LC was estimated by dividing by 10 m³, even though a worker breathing at a rate of 50 l min⁻¹ for 30 min would inhale 1.5 m³ of air. NIOSH determined a preliminary IDLH by dividing this estimated LC value by a factor of 10.
- 4. Chronic toxicity data were considered if no relevant acute toxicity data existed.
- 5. When relevant toxicity data for the specific chemicals in question were lacking, analogies to substances with similar acute toxic effects were considered.

All preliminary IDLH values derived during this update were checked against the following factors prior to establishing the final revised IDLH value:

1. Ten per cent of the lower explosive limit (LEL). (Note: OSHA considered concentrations in excess of 10% of the LEL to be a hazardous atmosphere in confined spaces.)

- 2. RD_{50} data: an estimate of severe respiratory irritation measured as the 10 min exposure concentration producing a 50% respiratory rate decrease in rodents.
- 3. Other short-term exposure guidelines, such as the American Industrial Hygiene Association's emergency response planning guidelines (ERPGs) and the National Research Council's emergency exposure guidance levels (EEGLs); short-term public emergency guidance levels (SPEGLs); and occupational exposure standards or recommendations such as OSHA PELs, NIOSH recommended exposure limits (RELs), or the American Conference of Governmental Industrial Hygienists (ACGIH[®]) threshold limit values (TLVs[®]).
- 4. Based on NIOSH respirator decision logic, the revised IDLH values could not be greater than 2000 times the NIOSH REL or OSHA PEL.
- 5. The revised IDLH values would not be greater than the original IDLHs derived during the SCP.

See also: National Institute for Occupational Safety and Health; Occupational Safety and Health Act, US; Occupational Safety and Health Administration; Occupational Toxicology; Occupational Exposure Limits.

Further Reading

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Relevant Website

http://www.cdc.gov/niosh – NIOSH Pocket Guide to Chemical Hazards (NIOSH Publication Number 97–140). Michael P Holsapple and Norbert E Kaminski

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Introduction

The role of the immune system may be stated succinctly as the preservation of integrity. This system is charged with identifying that which is 'self' and that which is 'nonself'. Examples of self are all the tissues, organs, and cells of the body. Examples of nonself are a variety of opportunistic pathogens, including bacteria and viruses, and transformed cells or tissues (i.e., tumors). The great complexity of the mammalian immune system is an indication of the importance, as well as the difficulty, of this task. If the immune system fails to recognize as nonself an infectious entity or the neoantigens expressed by a newly arisen tumor, then the host is in danger of rapidly succumbing to the unopposed invasion. This aspect of immunocompetence is the reason why the immune system is often made synonymous with 'host defense'. Alternatively, if some integral bodily tissue is not identified as self, then the immune system is capable of turning its considerable defensive capabilities against that tissue, and an autoimmune disease may be the end result. This aspect of immunocompetence emphasizes the tremendous destructive potential which is associated with the host defense mechanisms of the immune system. The cost to the host of these mistakes, made in either direction, may be quite high. The fact that mistakes can occur in either direction is discussed below as a continuum. Because the cost of mistakes in immunocompetence can be so high, and since there is tremendous diversity involved in the identification of self versus nonself, a complex array of organs, cells, soluble factors, and their interactions has evolved to regulate this system and minimize the frequency of errors in either direction.

Definition of Immunotoxicology

Studies in animals and humans have indicated that the immune system, like most organs, is a potential target organ, and that damage to this system can be associated with morbidity and even mortality. These studies coupled with tremendous advances made in immunology and molecular biology have led to a steady and exponential growth in our understanding of immunotoxicology during the past 20 years. In addition, recognition by regulatory agencies that the immune system is an important as well as sensitive target organ for chemical- and drug-induced toxicity (i.e., as described in greater detail later in this chapter) almost insures that this subdiscipline of toxicology will continue to flourish and grow in the foreseeable future. Immunotoxicology can be most simply defined as the study of adverse effects on the immune system resulting from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, and, in some instances, biological materials. Collectively, these agents are frequently referred to as xenobiotics, where 'xeno-' means foreign and '-biotic' means anything affecting biology.

Adverse Effects

A critical component of this definition is the term 'adverse'. The need to determine whether an effect is adverse is what differentiates toxicology from other branches of biomedical science. Consequently, immunotoxicology is not merely the demonstration of treatment-related changes in a component of the immune system. Not all treatment-related changes are adverse. Some changes may be beneficial, some may be indifferent, and some are of unknown or uncertain consequence. It is inappropriate to declare an effect to be adverse simply because an adverse consequence cannot be ruled out. The long-term credibility of any scientific discipline depends on involved scientists being forthcoming when there is uncertainty, or when the effects have no known adverse consequence. Some have attempted to classify as adverse any effect which is undesired or unwanted. This definition of adverse effect is social or cultural in nature and has little scientific utility because social definitions may vary as a matter of individual preferences. The term adverse has been defined in classical toxicology as the undesired side effects of a drug, which are deleterious. This definition of an adverse effect differentiates between undesired (unwanted) effects that are deleterious and those that are not, thereby avoiding the quagmire associated with social definitions.

The evolution of immunotoxicology has been based in large part on the design and validation of critical experimental approaches to most clearly answer the question, Is an observed effect adverse or is it not? For the purposes of this entry, an adverse effect in immunotoxicology will be defined as a xenobioticinduced change in the ability to perform an immune function. A suppression in immune functional ability would obviously be deleterious if the host were to encounter an opportunistic pathogen. As noted previously, an exaggerated immune response can also be deleterious. The utility of this definition is that it focuses attention on a specific functional ability. This definition does not explicitly address the issue of a xenobiotic-induced change in a structural component of the immune system, that is, such as a change in the weight of an immune organ or a histopathological change in an immune organ. This omission is a deliberate one because a structural change without a concomitant change in a functional component would fall into the category of a treatment-related effect of unknown or uncertain consequence. As such, by this definition, the demonstration of a treatment-related structural change in itself cannot be considered an adverse immunotoxicological effect.

Immunotoxicology as a Continuum

Because the primary role of the immune system is the discrimination of self versus nonself, immunotoxic effects can occur in either direction. As such, immunotoxicology can be thought of as a continuum, which is depicted as the solid dark vertical arrow in **Figure 1**. A xenobiotic-induced change in immunocompetence from the normal range, which is manifest as an underactive immune system, is shown as a 'suppressed immune response', while a xenobiotic-induced change manifested as an over-

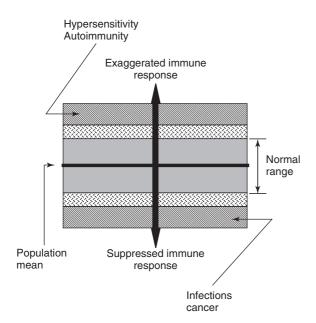


Figure 1 Immunotoxicology is a continuum. There are adverse consequences associated with both suppressed and exaggerated immune responses.

active immune system is shown as an 'exaggerated immune response'. The adverse (i.e., deleterious) consequences of suppressed immune function would reflect an inability to recognize nonself and are depicted as infections and cancer. The adverse consequences of exaggerated immune function would reflect an inability to recognize self and are depicted as hypersensitivity and autoimmunity. A hypersensitivity response is most simply defined as an acquired immune response which occurs in an exaggerated or inappropriate form, causing tissue damage. Autoimmune disease occurs when the reactions of the immune system are directed against the body's own tissues.

Treatment-related effects which are not clearly manifested as adverse are depicted in Figure 1, as the lightly shaded areas both above and below the normal range. The consequences of immunotoxicology are also described in greater detail in later sections of this entry. It is also important to emphasize that whether a treatment-related effect on the immune system is manifest as adverse or not in a given individual is dependent on where that individual is positioned in relationship to the normal range and the population mean. For example, an individual with a suppressed immune system, such as someone with the acquired immunodeficiency syndrome (AIDS) or any of a number of congenital immune deficiencies, could benefit by being exposed to a drug or chemical which causes immunostimulation. Similarly, an individual with an autoimmune disease can be treated therapeutically with an immunosuppressive drug. It is also important to note that, it is routine to treat an individual scheduled for an organ/ tissue transplant with an immunosuppressive drug. The goal of this therapy is to purposefully lower that individual's capacity to identify self versus nonself in order to decrease the chances that the transplant (i.e., an example of nonself) will be rejected. However, there are consequences associated with this treatment which are consistent with the continuum depicted in Figure 1.

Organization of the Immune System

The mammalian immune system is a complex system which is dependent on the integration of, and orchestrated cooperativity among, several organs, cells, and soluble factors. Components of the immune system are distributed throughout the body. An appreciation of the organization of the various components of the immune system is essential to understand immunotoxicology. By knowing the role played by a given component of the immune system in host defense mechanisms, one can begin to understand the

Organs of the Immune System

The organs of the body which comprise the immune system and/or contribute to immune function include the bone marrow, spleen, thymus, lymph nodes, a network of lymphoid tissue along secretory surfaces (i.e., the so-called mucosa-associated lymphoid tissue, MALT), and the skin. Lymphoid organs can be classified in two ways. The first classification is based on the role that organs play in the development of the immune system and/or its ability to elicit a response.

Primary and Secondary Lymphoid Organs

Primary lymphoid organs are those organs in which the production of the cells of the immune system takes place. For example, bone marrow is a primary organ and contains a pluripotent stem cell which serves as the precursor to red blood cells (i.e., erythrocytes) and myeloid progenitors (which ultimately differentiate into granulocytes, mast cells, monocytes, and platelets), in addition to lymphoid progenitors (which ultimately differentiate into the various types of lymphocytes). Hematopoiesis is a general term used to refer to the production of the cells of the blood, and it can be subdivided into erythropoiesis, myelopoiesis, and lymphopoiesis, respectively, based on the cell lineages described previously. Lymphoid progenitors will emerge from the bone marrow and travel to other primary lymphoid organs where the final stages of lymphocyte maturation take place. As described later, mature lymphocytes play a major role in discriminating between self and nonself because they are endowed with surface receptors characterized by tremendous specificity. Lymphoid progenitors which receive their final education in the thymus are called thymus-derived lymphocytes or T cells. The other major subtype of lymphocyte is the B cell, so named because it was originally characterized in the chicken as a lymphoid progenitor which receives its final education in a primary lymphoid organ called the Bursa of Fabricious, an outpocket of the gastrointestinal epithelium. Although there is no Bursa in mammals, fetal liver, spleen, and adult bone marrow are considered the 'bursal equivalents' and function as the primary lymphoid organs for the production of B cells. The process of lymphopoiesis takes place within specific regions of the thymus and bursal equivalents called microenvironments and is regulated by specialized cells (bone marrow stromal cells and thymic epithelial cells) and their soluble factors (including the interleukins IL-3, IL-7, IL-9, and IL-12; see **Table 1**), which comprise the microenvironments. The stages of lymphopoiesis are generally believed to be antigen independent, where antigen is defined as any substance which can stimulate a specific immunological reaction. The surface receptors of lymphocytes, mentioned previously, are directed toward 'antigen'. Although lymphopoiesis is neither antigen dependent nor antigen driven, a role for antigen cannot be excluded because factors secreted during an antigen-specific reaction in the periphery can promote various forms of hematopoiesis in the bone marrow.

Moreover, although antigen is not a driving force for lymphocyte development, the antigen receptors on the surface of lymphocytes play a critical role. Many immature lymphocytes have the potential to respond to self products and therefore pose a threat. During development, immature B cells in the bone marrow and immature T cells in the thymus will have an opportunity to interact with neighboring cells which express surface proteins indicative of self. These self proteins are encoded for by the major histocompatibility complex (MHC). If the antigen receptor on any lymphocyte binds too effectively to these MHC-derived proteins, then that cell will be eliminated. This process of negative selection is thought to be mediated by one of two possible mechanisms. Either this inappropriate recognition of self proteins triggers apoptosis, which is programmed cell death, and the lymphocytes are eliminated, or these cells become anergic, which is an induced lack of responsiveness to antigenic stimulation. This process of negative selection is not limited to self proteins obviously associated with the microenvironments of the bone marrow and thymus. Self proteins from different parts of the body are actually transported to these primary lymphoid organs to probe lymphocytes for reactivity against distant tissues.

Secondary lymphoid organs are those organs in which the antigen-dependent proliferation and differentiation of specific lymphocytes takes place. These organs are responsible for the dissemination of an antigen-specific immune response and include lymph nodes, spleen, and the various types of MALT, which are further defined below. An appreciation for the role that secondary lymphoid organs play in the immune system can be derived from the fact that swollen lymph nodes (i.e., as a consequence of the antigen-specific lymphoproliferation) are a hallmark indicator of certain types of infections.

Table	1	Cytokine	network
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Cytokine	Other names	Cell source	Cell target and actions
Interferon-α (IFN-α)		Leukocytes	B cells: proliferation and differentiation NK cells: stimulates cytolytic activity T _c cells: increases generation APCs: increases MHC I and II expression Others: increases MHC I and FcR expression; induces antiviral state
IFN-β IFN-γ		Fibroblasts T _H cells	 Similar to IFN-α B cells: stimulates IgG2a synthesis and inhibits IL-4- induced IgE/IgG1 synthesis APCs: increases MHC I and II expression Macrophages (macs): activates cytolytic activity NK cells: stimulates cytolytic activity Others: increases MHC I expression; induces antivira
Interleukin 1 (IL-1)	Endogenous pyrogen	Monocytes/macs	 state T_H cells: stimulates production of lymphokines, especially IL-2 and expression of IL-2R B cells: proliferation and differentiation Macs: stimulates production of cytokines, IL-1, IL-6, and tumor necrosis factor-α (TNF-α)
IL-2	T cell growth factor (GF)	T _H cells	 Brain: fever response T_H cells: stimulates proliferation and release of lymphokines (especially T_{H1} cells) B cells: proliferation and differentiation NK cells: activates
IL-3	Multicolony stimulating factor (MSF)	T _H cells	Bone marrow (BM): promotes growth of stem cells to granulocytes, macs, and mast cells
IL-4	BCGF; B-cell- stimulating factor (BSF)	T _H cells (B cells)	B cells: stimulates IgE and IgG1 production and increases MHC II expression
IL-5	T-cell-replacing factor (TRF); BCGF II	T _H cells	T _H cells: promotes generation; synergizes with IL-2 B cells: proliferation and differentiation; stimulates IgA production
IL-6	IFN- γ_2	T _H cells Monocytes Endothelial cells	T cells: proliferation and differentiation B cells: proliferation and differentiation Others: similar profile of activity to IL-1; synergizes with IL-1
IL-7	Lymphopoietin	Fibroblasts BM stroma	T cells: induces growth of immature cells B cells: induces growth of immature cells
IL-8	Neutrophil-activating factor (NAF)	Monocytes	Neutrophils: chemotaxis; granular exocytosis; respiratory burst
IL-9		T _H cells	 BM: stimulates growth of erythroid and megakaryocyte precursors Others: promotes mast cell growth B cells: acts synergistically with IL-4 in production of IgE and IgG1
IL-10		T _H cells (B cells)	T_{H1} cells: inhibits lymphokine synthesis T_{H2} cells: promotes generation Monocytes: inhibits cytokine synthesis T_{C} cells: stimulates IL-2-dependent growth Mast cells: stimulates growth
IL-11		Fibroblasts	BM: stimulates T-dependent antibody response; resembles IL-6
IL-12	NK cell stimulatory	BM stroma Monocytes/macs	NK cells: activates cytotoxicity
	factor (NKSF)	B cells	T_{H1} cells: stimulates proliferation and lymphokine production, especially IFN- γ T_{H2} cells: inhibits generation (negative feedback) T_{C} cells: activates; synergizes with IL-2
IL-13	P600	T cells	 B cells: promotes growth and differentiation macrophages; inhibits inflammatory cytokine production T_{H1} cells; inhibits cytokine release

Table 1 Continued

Cytokine	Other names	Cell source	Cell target and actions
IL-15	T-cell growth factor	T cells	T cells: stimulates growth NK cells; stimulates growth epithelial cells; stimulates growth
IL-16	T cells; mast cells; eosinophils		CD4 ⁺ T cells: chemoattractant monocytes; chemoattractant eosinophils; chemoattractant T cells, anti-apoptotic for IL-2-activated cells
IL-17	MCTLA-8	CD4 + memory cells	Epithelial cells: induces cytokine production endothelial cells; induces cytokine production fibroblasts; induces cytokine production
IL-18	Interferon-γ inducing factor		
Lymphotoxin	Tumor necrosis factor- β (TNF- β)	T cells	Target cells: kills
Macrophage-activating factor	MÀF	T _D cells	Macs: activates cytotoxicity and proinflammatory actions
Macrophage-inhibiting factor	MIF	T _D cells	Macs: inhibits migration
Transforming growth factor- β (TGF- β)	TGF-β	Lymphocytes	B cells: suppresses growth; inhibits IgM and IgG production; decreases MHC II expression
· · · · · · · · · · · · · · · · · · ·		Macs	T cells: suppresses growth Monocytes: inhibits TNF production; chemotaxis; induces IL-1 and IL-6 expression
Tumor necrosis factor- α (TNF- α)	Cachectin (TNF-a)	Monocytes/macs	Tumor cells: cytotoxicity
· · · ·			Others: similar profile of activity of IL-1; promotes antiviral state

Internal and External Lymphoid Organs

The second classification of lymphoid organs is based on their location, with some being classified as internal organs and others being classified as external organs. The internal lymphoid organs include the bone marrow, thymus, spleen, and some lymph nodes. The external lymphoid organs include all the components of MALT as well as the lymph nodes draining MALT. As indicated previously, MALT is defined as lymphoid tissue associated with mucosa. This tissue can be subdivided into more specific regions based on the anatomical location, and includes gut-associated lymphoid tissue (including Peyer's patches and the appendix) and bronchus-associated lymphoid tissue. The skin is another example of an external organ whose contribution to the immune system is sometimes underappreciated. Although the skin does not contain organized lymphoid tissue, there are immune components associated with the skin that are interconnected with other immune organs, leading to the concept of the socalled skin-associated lymphoid tissue. An appreciation for the important role that skin plays as a 'first line of defense' in the immune system can be derived from the fact that when this barrier is breached, as occurs following an abrasion and especially so after a severe burn, a serious consequence is an increase in the incidence and severity of infections.

The importance of this second classification of lymphoid organs is that the two locations behave somewhat independently in host defense. An immune response mediated primarily by internal lymphoid organs is generally referred to as a systemic immune reaction or systemic immunity, while an immune response mediated primarily by external lymphoid organs is generally referred to as a local immune reaction or local immunity. As will be described in greater detail, there are also differences in the specific effector functions associated with systemic and local immunity.

Cells of the Immune System

The most obvious example of a cellular component of the immune system is the lymphocyte and includes all the various subtypes of T and B cells. As indicated previously, the fundamental distinction between T cells and B cells is based on the specific primary lymphoid organ in which the final stages of lymphopoiesis takes place. As described below, these cell types can also be distinguished based on their respective functions within the immune system as well as by phenotypic characteristics. For the purposes of this entry, a phenotype will be defined as a marker expressed on the surface of a cell which is genetically determined and which is frequently associated with the specific function of that cell. Other important examples of cellular components of the immune system include monocytes, macrophages, granulocytes, mast cells, and natural killer (NK) cells. As with the lymphocytes, the specific functions of these cells as they relate to the immune system will be described in greater detail. Some of these cells are oftentimes found circulating in the blood. Examples of circulating cells include monocytes, granulocytes, and NK cells.

Other cells important to the immune system are typically tissue bound and include mast cells and macrophages. Macrophages present in tissue constitute the mononuclear phagocytic system, which was formerly known as the reticuloendothelial system. Macrophages located in specific anatomical regions have been frequently given distinct names, including Kupffer cells (liver), Langerhan cells (skin), microglia (brain), osteoclasts (bone), follicular dendritic cells (B-cell regions of lymphoid tissue), and interdigitiating dendritic cells (T-cell regions of lymphoid tissue). The issue of whether a lymphocyte is circulating or noncirculating is a bit more complex than for most of the other cellular components of the immune system. The blood circulation contains only a minor part of the body's total lymphocyte count (estimated at $\sim 1\%$), which is a relatively select population, the so-called recirculating lymphocyte pool. As such, an assessment of only the blood lymphocyte pool provides an incomplete inventory of the body's immune system as it relates to lymphocytes because it ignores the activities of the nonrecirculating cells. In general, the recirculating lymphocyte pool does not include cells that are in a state of activation, proliferation, or differentiation. As indicated previously, the dissemination of an antigen-specific reaction takes place in secondary lymphoid organs, especially lymph nodes. The lymphatic system represents a second circulatory system of conducting vessels (i.e., lymphatic capillaries and/or lymphatics), loose aggregates of lymphoid tissues (i.e., nodules), and more highly organized and structured organs (i.e., lymph nodes). Through this system passes lymph, a collection of tissue fluids rich in globulins and lymphocytes. As lymph passes through draining lymph nodes, it becomes progressively more enriched with lymphocytes. Lymphocytes within lymph can empty back into the blood circulation via the thoracic duct. After entry into the blood, these lymphocytes will eventually find their way into a lymph node, at which point they may either remain in the circulating blood or reenter the lymphatic circulation. Therefore, lymphocytes and their products are transported between lymphoid organs and throughout the body via the blood and the

lymph. However, it is again important to emphasize that the percentage of the total lymphocyte count in the blood is normally very small.

Soluble Products of the Immune System

Soluble products also play an important role in the immune system and are oftentimes the primary mediator of a given effector function of the immune system. Some soluble products of the immune system are secreted by lymphocytes. For example, immunoglobulins (Igs) are secreted by B cells. It is important to emphasize that an antibody is an Ig molecule secreted by a B cell during an immune response that specifically reacts with the antigen. Therefore, 'Ig' and 'antibody' are distinct terms that are often used somewhat interchangeably as they pertain to effector functions by the immune system. A second example of lymphocyte-derived soluble products is the variety of substances secreted by T cells called lymphokines. Although lymphokines have been described as being hormone-like, they are probably more analogous to neurotransmitters because of their very localized sites of action and because of the signal transduction pathways they trigger in responsive cells. Specific examples of lymphokines, including the interleukins, will be provided and are summarized in Table 1. It is important to emphasize that lymphokines are a subgroup of a larger family of soluble factors secreted by a variety of cells, called cytokines, which can modulate immune function, and that both lymphokines and cytokines can affect cells, organs, and tissues outside of the immune system.

Some soluble products are secreted by cells of the immune system other than lymphocytes. For example, monocytes/macrophages are also capable of secreting cytokines, which are sometimes referred to as monokines and which include some interleukins, such as IL-1, IL-6, IL-8, and IL-12, and other factors such as tumor necrosis factor (TNF). Some soluble products with important roles in the immune system originate outside of the immune system. One example is interferon (IFN), which is actually a heterogeneous family of proteins of two types. Type I or viral IFNs are induced by infection and consists of IFN- α (interferon- α) and -IFN- β , which are secreted by leukocytes, and fibroblasts or epithelial cells, respectively. Type II or immune IFN, consists of IFN- γ , which is secreted by T cells (therefore, IFN- γ can be classified as a lymphokine) in response to specific antigens. A second example of a soluble product which originates outside of the immune system is complement, which is primarily produced in the liver. Complement is actually a group of ~ 20

proteins, including several proteases, that activate and split each other in sequential order. The specific functional roles played by complement and the other soluble products of the immune system will be discussed later.

Immune Response

Innate versus Acquired Immunity

Foreign substances, including the various examples of nonself indicated in the Introduction, can provoke two basic types of immune responses, innate (also called nonspecific) immunity and acquired (also called specific) immunity. One of the easiest ways to present and understand the functions of the various cells and soluble products of the immune system is in the context of these two types of immune responses, which each make significant contributions to host defense capability. The principal difference between these two types of immunity is the role of the antigen, which was defined previously as any substance which can trigger a specific immunological reaction. In this context, it is important to note that an antigenic determinant on the surface of a microbe or tumor cell is usually about 10 amino acids in size and can be made up of polypeptides, carbohydrates, or lipids, and that a given type of microbe or tumor cell can express several different types of antigens as well as multiple copies of a given antigen. Innate immunity is considered to be antigen independent and occurs without prior exposure to antigens. Acquired immunity is considered to be antigen dependent and comprises all of the specific immunological reactions alluded to in the definition of antigen. Acquired immunity can be subdivided into humoral immunity and cellmediated immunity, which are described in greater detail later.

Because innate immunity can be triggered upon the initial encounter with a foreign substance, its components are oftentimes called the first lines of defense. As such, it is appropriate to consider the skin as a component of innate immunity. Similarly, the following bodily functions contribute to host defense and should be considered parts of innate immunity: the lysosomal enzymes found in salivary, lacrimal, and vaginal secretions, which have bacteriastatic properties; the cough reflex, which is an important mechanism to clear the bronchial passages of irritants and potential infectious microbes; and the fever response, which is an important reaction to an infection because of the limited temperature range for the growth of most bacteria.

More traditional components of the innate immune defense system include phagocytic cells such as neutrophils and macrophages, NK cells, and the soluble products, type I IFN and complement. Neutrophils and macrophages are the primary cells involved in inflammatory responses. Their contribution to innate immunity is based on their abilities to phagocytize (i.e., to engulf; literally the foreign particle becomes enclosed by the cell membrane of the phagocyte into a phagosome) and to kill bacteria. The latter mechanism is carried out either by the extracellular release of lysosomal enzymes, oxygen radicals, bactericidal proteins, and proteinases or by the intracellular fusion of phagosomes containing the microbes and lysosomes containing these destructive mediators. Phagocytic cells are attracted to the site of infection or inflammation, a process known as chemotaxis, by a number of factors including some complement components and some cytokines, which are discussed later. Moreover, microbial cells and other foreign particles are also capable of attracting the attention of phagocytic cells directly because of unique properties. For example, bacteria produce peptides with an unusual chemical structure beginning with formyl-methionine sequences that are produced in very small amounts by mammalian host tissue. Therefore, large amounts of formyl-methionine peptides will stimulate neutrophil chemotaxis and phagocytosis. A second example has been identified in macrophages which have receptors for sugars typically found on many microbial organisms, that is, mannose, L-fructose, and galactose. The destructive capabilities of both macrophages and neutrophils can also be modulated by cytokines, primarily lymphokines produced by antigen-specific T cells, as discussed later.

NK cells are leukocytes of lymphoid or myeloid origin with the ability to kill target cells without prior sensitization. NK cells require intimate contact with target cells before lysis can take place. One postulated mechanism for cytolysis involves the production and secretion of a cytolytic protein, perforin, which functions to produce transmembrane channels in the target cell and ultimately leads to porous membrane lesions and cell death. The attachment of NK cells to their target cells is accomplished through an as yet poorly understood chemical means by which these cells seem to recognize certain viral or tumor-associated markers. As with the macrophage, the killing capability of NK cells can be modulated by T-cell-derived lymphokines, most notably IFN-y.

In addition to their ability to produce and release destructive inflammatory mediators, neutrophils and monocytes/macrophages can contribute to the innate immune response by the production and release of cytokines. The cell sources, targets, and actions for a number of cytokines are summarized in Table 1. IL-1 has been the most studied interleukin because it was the first one to be discovered and because it triggers a wide variety of activities in several organ systems. For the purposes of this entry, the discussion will be limited to actions of IL-1 associated with the innate immune response which include the activation of neutrophils, macrophages, and NK cells; cytostatic and cytotoxic actions for some tumor cells; the induction of the fever response in the brain (IL-1 has been identified as the 'endogenous pyrogen'); and the stimulation of some acute phase-reactive proteins by the liver. IL-6 is produced by a number of different cell types and possesses a profile of activity similar to IL-1, including the following actions: an increase in the synthesis of the major acute phase-reactive proteins by the liver and pyrogenic activity in the brain. IL-1 and IL-6 are known to act synergistically.

IL-8 is produced by activated monocytes and macrophages and acts on neutrophils as a chemotactic factor and as a stimulus for enzyme release and an oxidative burst. IL-12 is also synthesized by monocytes/macrophages in response to bacteria or other parasites and acts on NK cells to activate them. TNF is produced predominantly by activated macrophages and it draws its name from the fact that it was originally isolated as a factor which was capable of triggering a hemorrhagic lesion in transplanted tumors. However, the effects of TNF are now known to extend well beyond that original definition. For example, TNF produces many of the same actions that were identified for IL-1, and these two cytokines can act synergistically. TNF is also known as cachectin because of its association with the wasting syndrome (cachexia) characteristic of chronic diseases, including some malignancies. It is important to emphasize that IL-1, IL-6, IL-12, and TNF also have actions on lymphocytes, and these immunoregulating properties are discussed later. The fact that certain cytokines can contribute to both innate and acquired immunity is an indication of their interdependency. The fact that certain soluble products and cells contribute to multiple components of the immune system is also an indication of the overlapping nature of host defense capabilities, a concept which is sometimes referred to as the redundancy of the immune system.

As noted previously, IFN exists as two types, and it is the type I or viral IFNs—IFN- α and IFN- β , which are produced in an antigen-independent fashion, that contribute to the innate immune response. As with the phagocytic cells, the trigger for the production of type I IFN is a unique feature of the genetic makeup of viruses. Viruses make much greater quantities of double-stranded RNA than do mammalian cells, and the presence of large amounts of double-stranded RNA stimulates the production of viral IFN. Although the sources of IFN- α and IFN- β are different (i.e., leukocytes and fibroblasts or epithelial cells, respectively), their effector functions are similar and include the following actions: stimulation of NK cells, induction of antiviral activity, and cytostasis of some tumor cells.

Complement is not a single soluble factor but a carefully regulated system of ~ 20 functionally linked proteins. The linkage results because many complement proteins have protease activity, and they interact in an ordered cascade, the so-called complement cascade. In several steps in the cascade, there is the cleavage of small-molecular-weight peptides, which possess most of the biological activities attributed to the complement system. A detailed description of this obviously complicated system is beyond the scope of this entry. Instead, two points will be emphasized, the mechanisms for activating the complement cascade and the biological activities of some of the products of the complement system. The latter point will be discussed as it relates to the relative contributions of innate versus acquired immunity to host defense.

The classical activation of the complement cascade is triggered by Ig complexes with antigen and will be discussed in the following section. The alternative pathway of the complement cascade is triggered by nonimmune-specific activators, most notably polysaccharides associated with the surface of some microbes. Although these two activational schemes differ in the way that nonself triggers the complement cascade, there are common mediators in the two pathways and the biological consequences of the complement components are similar. There are receptors for various components of complement (usually designated CR) located on both neutrophils and macrophages. Some complement peptides (most notably, activated C3b, C5a fragment, and the activated complex of C567) function as chemotactic factors for these inflammatory cells. Other complement peptides (most notably, activated C3b) will attach to the microbe and facilitate the adherence and subsequent phagocytosis of the microbe by neutrophils and macrophages, a process known as opsonization. Finally, the terminal product of the complement cascade, the activated complex of C6789, can form a lytic unit which can attack the cell membrane and directly kill the microorganisms by punching holes in their cell membrane. It is thought that most cells of the host are equipped with surface proteases that inactivate complement and protect them from cytolysis.

Humoral versus Cell-Mediated Immunity

Acquired immunity is antigen dependent and comprises all the specific immunological reactions associated with lymphocytes. In light of the existence of an antigen-specific defense system, a legitimate question arises as to why we have such an elaborate nonspecific immune system. One of the primary reasons may be that an acquired immune response takes time. For example, 5 days are needed to generate a primary antibody response, and the body must rely on the innate immune system to hold the infection in check during this time. As noted previously, acquired immunity can be subdivided into two effector arms, humoral immunity and cell-mediated immunity. The 'humor' (i.e., a bodily fluid) associated with humoral immunity is the secreted form of Ig in the blood. The 'cells' associated with cell-mediated immunity are the various subpopulations of T cells.

Because Ig is the primary effector for humoral immunity, this component of the immune system is associated with the activities of antigen-specific B cells. The steps involved in an acquired immune response by B cells and the regulation of B-cell activity are depicted in Figure 2 and described later. In this section, the emphasis will be on the specific contributions that Ig makes in the functioning of the immune system. The basic structure of the Ig or antibody molecule consists of four protein chains of two types – that is, two identical light chains and two identical heavy chains. These protein subunits are linked in a fixed and precise orientation to form a 'Y'shaped molecule. The 'forked' end of the antibody molecule contains two variable regions and is the site which recognizes and binds the specific antigen. To accommodate the many antigens that exist, these variable regions differ from one antibody molecule to another antibody molecule. Each type of antibody molecule is synthesized by a clone or family of identical antigen-specific B cells. The 'closed' end of the antibody molecule is nearly identical among all antibodies and is called the constant region. Although the constant region of the antibody molecule is not involved in the specific binding of antigen, this component of the molecule is critical to the effector functions of Ig, as described later. The remaining feature of the Ig molecular structure that needs to be emphasized is that the heavy chains can vary in type, which gives rise to the various major classes of Ig, namely, IgM, IgG (there are four subclasses of IgG), IgE, IgA (there are two subclasses of IgA), and IgD. The existence of multiple types of Ig adds to the repertoire with which the humoral arm of acquired immunity can add to host defense, as discussed later.

The different types of Ig also provide insights into the complexities associated with B-cell biology. Surface Ig is the hallmark feature of the B cell and is one of the principal phenotypic markers used to identify and enumerate B cells. Surface Ig is also a 'receptor' in the truest sense of the term. The ligand for this

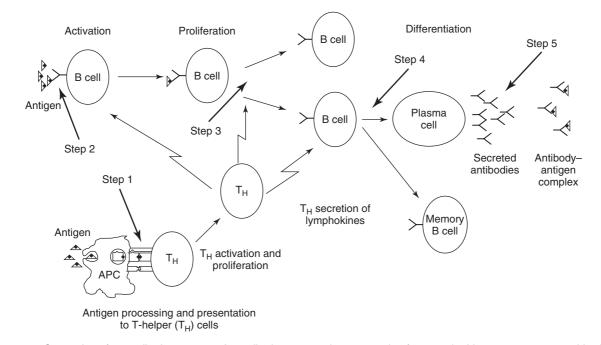


Figure 2 Generation of an antibody response. An antibody response is an example of an acquired immune response and is characterized by a five-step process. The production of memory cells is also depicted.

receptor is the antigen, and because of the molecular structure of Ig, this ligand-receptor (i.e., antigenantibody) interaction is the driving force for the tremendous specificity associated with humoral immunity. It is important to emphasize that the cytoplasmic region of surface Ig contains only three amino acid residues, which makes it difficult, if not impossible, to conclude signal transduction is being mediated via this domain. However, surface Ig can associate with type I transmembrane glycoproteins to form a B-cell receptor (BCR) complex, which can generate the necessary signal transduction pathways to trigger activation and proliferation in B cells in the presence of the appropriate ligand (antigen). Virgin B cells express either IgM or IgD or both on their surface. The function of IgD is limited to its role as a receptor on B cells, and it cannot mediate any of the effector functions associated with the other classes of Ig. IgM is the first class of Ig to be released by the B cell after antigen challenge during a primary antibody response. In the serum, IgM exists as a pentamer. Depending on the nature of the antigen, B cells can undergo a process known as class switching, which results in the generation of IgG, IgA, or IgE. As described later, the T cell plays a critical role in class switching through the release of a variety of lymphokines. Antigens triggering this type of interaction between B and T cells are therefore called T-dependent antigens. Some antigens can trigger an antibody response in a T-independent fashion and are generally limited to the production of IgM.

Once released, the various forms of Ig possess a number of effector functions to engage the antigen. It is important to emphasize that a given microbe, tumor cell, or foreign protein can express several types of antigens and multiple copies of these antigens. If the antigen to which an antibody is directed is associated with the toxic portion of a molecule, then the antibody can neutralize the toxin. The production of neutralizing antibodies is frequently a problem in the therapeutic application of recombinant proteins generated through biotechnology. This problem can become a rate-limiting step in the preclinical testing of human-derived proteins in animal models because they are perceived as nonself by their hosts. Another example of the ability of antibodies to neutralize occurs when the antibody is directed against an antigen on the surface of an infectious particle which serves as the cellular adhesion site. In the presence of the antibody, the infectious particle cannot attach to the cellular target to initiate infection. Although the neutralizing capabilities of antibodies are known, in actuality, they play a relatively minor role in the effector functions associated with humoral immunity in host defense. In particular, it is important to emphasize that antibody can only bind to an antigen and, in itself, cannot destroy anything.

Most of the effector functions of humoral immunity are mediated by processes activated by antibody. Moreover, the effector processes activated by antibody have already been described as key participants in innate immunity. For example, the classical activation of the complement cascade is triggered by antigen-antibody complexes and is specifically mediated by the constant region of the Ig molecule. Both IgM and IgG can activate the complement system in this manner, which results in all of the biologically active components identified previously, including the lytic unit, the chemotactic factors, and the complement peptides which opsonize the microbe to facilitate its phagocytosis. IgM and IgG can function to opsonize some microbes independent of complement activation in an antigen-dependent fashion because macrophages and neutrophils have receptors on their surface which recognize the constant region of Ig (Fc receptors; FcR). Fc receptors also play a major role in the ability of IgG to participate in a process known as antibody-dependent cellular cytotoxicity, whereby antigen-specific antibody attaches via FcR to certain types of cells, including NK cells, enabling these cells to attach intimately to the target cell and trigger cell death. Finally, Fc receptors are also the primary effector mechanism for IgE, which is the principal immune defense against certain types of parasitic infections (most notably, helminths) and is produced primarily by the external immune system along secretory surfaces. IgE binds to Fc receptors on the surfaces of mast cells and basophilic granulocytes. Once bound to FcR, IgE can serve as an antigen-specific receptor on the surface of these inflammatory cells to trigger the release of a variety of proinflammatory factors, including the vasoactive amines (histamine) and products of the arachidonic cascade (leucotrienes and prostaglandins).

IgA is the principal antibody present in a number of secretions and is the major antibody associated with the external immune system. IgA lacks the effector functions identified previously and acts mainly in immune exclusion (prevention of entry of potentially infectious entities into the body). As noted previously, there are differences in the immune effector mechanisms associated with the internal immune system or systemic immunity and the external immune system or local immunity. Systemic immunity is mediated by IgM and IgG, the latter is the major form of Ig found in the blood. Local immunity is mediated primarily by IgA and IgE. The contribution by the external immune system should not be underestimated because about half of the body's lymphocytes are associated with this system, and its capacity for Ig synthesis is ~ 1.5 times that of the internal immune system.

The other arm of the acquired immune system is cell-mediated immunity, for which antigen-specific T cells play the primary effector role. The antibody associated with humoral immunity is particularly effective against extracellular pathogens and it is a major constituent of serum. However, Igs are watersoluble proteins which cannot venture across the lipid membranes of cells. Therefore, cell-mediated immunity is needed to defend against intracellular pathogens such as protozoans, fungi (Candida), viruses, and certain bacteria (Mycobacteria and *Listeria*). Cells infected with these types of intracellular microbes are able to signal the body that they are infected by expressing pieces of the microbe on their surface. Cell-mediated immunity is also an important defense against certain types of malignancies. Central to this component of immune function, which is sometimes referred to as immunosurveillance, is the concept that tumor cells express antigens on their surface that are not found on normal tissue counterparts, so-called tumor-specific antigens.

As with the B cell, the antigen specificity of T cells is derived from a surface receptor. For T cells, the antigen receptor is a heterodimeric molecule (either the α,β heterodimer or the γ,δ heterodimer) which has a constant and a variable region, similar to that previously described for the Ig molecule. Moreover, as with surface Ig, the T-cell receptor (TCR) cannot in itself mediate transmembrane signal transduction, and it is coupled on the cell surface with the CD3 molecule, where 'CD' stands for cluster of differentiation, which has become the standard nomenclature to refer to a multitude of surface markers. CD3 consists of at least four invariant polypeptide chains and is thought to mediate the signal transduction associated with binding of the TCR to antigen. The expression of CD3 has become the hallmark feature of the T cell and is the principal phenotypic marker used to identify and enumerate T cells. Although there are similarities, there are also major differences between the TCR and its counterpart in the B cell. First, it is clear that there are structural differences between the TCR and BCR, and that T cells recognize different antigenic determinants than those recognized by B cells. Second, the TCR complex includes a number of different accessory molecules, namely CD4 and CD8, which play essential roles in the recognition of antigen by T cells. CD4 and CD8 also serve as important phenotypic markers for distinct subpopulations of T cells, T helper cells (T_H; Note: as indicated below, some CD4⁺ cells are classified as T_D cells by virtue of their primary role in mediating a delayed hypersensitivity response) and T

suppressor/T cytotoxic cells (T_s/T_c), respectively. The importance of these various subpopulations is described in greater detail below. Finally, unlike the B cell, in which the secreted form of Ig becomes the primary effector for humoral immunity, neither the TCR nor any component of its complex are secreted for effector function.

Antigen-specific T cells contribute to host defense capabilities by two basic mechanisms. One type of T cell, which is usually designated T_D and expresses the CD4 phenotype, orchestrates an inflammatory response called a delayed hypersensitivity response through the release of a variety of lymphokines. Some T_D-derived lymphokines are capable of causing cell lysis independently of a direct attachment between effector and target cell and are called lymphotoxins. As with the ability of antibody to activate effector functions in humoral immunity, other T_D-derived lymphokines act to markedly stimulate the destructive capabilities of inflammatory cells associated with innate immunity and include macrophage-activating factor, macrophage-inhibiting factor (inhibits the directed movement or chemotaxis of macrophages at the site of infection), and IFN-y, which stimulates both macrophages and NK cells. The second type of T cell is designated T_C for T cytotoxic and expresses the CD8 phenotype. As the name implies, T_{CS} are capable of killing target cells in an antigen-specific fashion through intimate contact. These T cells are thought to play the major role in the rejection of a foreign tissue graft or transplant. Although T_Cs and NK cells have many similarities, including enhanced activity in response to IFN-y and the postulated role for cytolytic proteins such as perforin, they recognize their targets differently. Only T_{C} s are antigen driven and require initial exposure to antigen to become active. Interestingly, T_C cells are capable of producing a protein which interacts with perforin and renders it lytically inactive, thereby providing these cells with some resistance to their killing capability.

Regulation of an Acquired Immune Response

As emphasized throughout this entry, antigen plays the critical role in providing the driving force and the specificity of an acquired immune response. This type of response can be regulated by several general mechanisms including cellular cooperativity, the cytokine network, and genetically determined regulation. Cellular cooperativity can be mediated by both direct cell-to-cell contact and the release of soluble factors, especially the lymphokines. The 'cytokine network' is a relatively recent term used to emphasize the fact that cytokines can both up- and downregulate lymphocyte activity. Genetically determined regulation is mediated by two major types of proteins encoded for by the MHC. In humans, MHC-derived proteins are called human lymphocyte antigens. MHC class I proteins are expressed on all cells of a given host and are critically involved in the designation of self versus nonself. These surface markers determine tissue compatibility and are the primary targets (i.e., antigens) in allograft or transplant rejection. MHC class II proteins are expressed on only certain types of immune cells and play a central role in the control of cellular interactions during an immune response.

All three of these mechanisms will be highlighted in the discussion of the five basic steps of an acquired immune response, which are illustrated below and which are depicted in Figure 2 for the generation of an antibody response. It is important to emphasize that these same five steps are involved in the generation of a cell-mediated immune response:

- Step 1: antigen recognition and presentation
- Step 2: lymphocyte activation
- Step 3: lymphocyte proliferation
- Step 4: lymphocyte differentiation
- Step 5: effector function

Step 1: Antigen Recognition and Presentation

Thus far, antigen has been depicted as the structures associated with a foreign substance that allow it to be recognized as nonself and to therefore provide the driving force for an acquired immune response. It has also been emphasized that both B and T cells are equipped with surface molecules that effectively function as the receptors for antigen, allowing these cells to recognize antigen in a highly specific manner. Generally speaking, the form of the antigen as it appears on the foreign substance when it is introduced into the host is not the form of the antigen which is recognized by the 'antigen receptors' on the surface of lymphocytes. The antigen must be taken up by specialized cells and 'processed' so that the most immunogenic components can be 'presented' to the lymphocytes. As depicted in Figure 3, cells carrying out this step of an acquired immune system are called antigen-presenting cells (APCs). In order to present antigen to T cells, the immunogenic components must be presented in the context of MHC molecules, that is, a T cell can only recognize antigen if the APC and the T cell share the same MHC type and antigen recognition by T cells is said to be MHC restricted. The role of MHC in antigen processing and presentation is shown in Figure 3. During antigen processing, the microbe or foreign particle is internalized within a vesicle and broken down. The most immunogenic components become associated with MHC-derived proteins, which are produced by the endoplasmic reticulum, and the antigen-MHC protein complex is returned to the surface of the APC. $T_{\rm H}$ cells, which express CD4 on their surface, can only recognize antigen in the context of MHC class II molecules, and all APCs which can present antigen to T_H cells must express MHC class II molecules. As noted previously, CD4 molecules play an accessory role in the recognition of antigen by T_H cells, and it is the CD4 molecule which recognizes the MHC class II antigens.

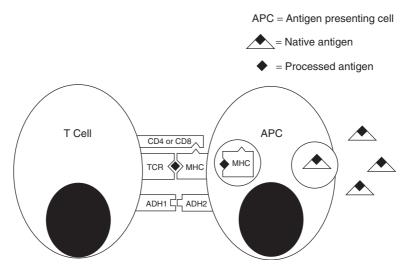


Figure 3 Antigen presentation to T cells. The roles of antigen processing and of MHC-derived proteins in antigen presentation, the first step in the five-step process to generate an acquired immune response, are depicted.

Some examples of APCs include macrophages in the spleen, dendritic cells in lymph nodes, and Langerhan cells in the skin. While it is true that many APCs can also be classified as phagocytic cells, antigen processing and presentation and phagocytosis are two distinct functions. This point can be readily appreciated by the fact that B cells are recognized as efficient APCs under conditions of low antigen concentration. The participation of B cells in antigen processing and presentation occurs by virtue of their ability to complex with antigen via their surface Ig and by the fact that B cells can express MHC class II molecules on their surface. Moreover, because B cells are able to recognize antigen via surface Ig, antigen presentation to B cells is not MHC restricted. Antigen presentation to T_S/T_C cells is also known to be MHC restricted. Because these cells express CD8 on their surface as an accessory molecule to the TCR complex, recognition of antigen can only take place in the context of MHC class I antigens. As noted previously, most cells of the body express MHC class I proteins. When a cell becomes infected, it alerts T cells of the infection by coexpressing some antigenic determinant of the microbe in a complex with MHC class I proteins. This situation makes sense when one considers that T_C cells are CD8⁺ and are the primary effector cells to engage certain types of virally infected cells and tumors (i.e., those tumors which express tumor-specific antigens) as well as allografts (i.e., a transplant of nonself, with different MHC class I proteins expressed). One important point about MHC proteins that needs to be emphasized is that their expression can be upregulated by certain cytokines, most notably the IFNs, as summarized in Table 1. Another point that needs to be emphasized about the process of antigen presentation is that other cell surface molecules, depicted in Figure 3 as adhesion molecules (ADH 1 and ADH 2), play important roles in the cell-to-cell contact.

Step 2: Lymphocyte Activation

Because both the BCR and TCR are effectively the receptors for the ligand, which is an antigen, once an antigen has been processed and presented to a specific B or T cell in the proper conformation, it can trigger a number of second messenger systems which ultimately initiate transcription. The cell becomes activated and begins to move into the cell cycle toward mitosis and cell division. An important part of this process is the upregulation of surface receptors for cytokines which facilitate the movement of these cells through the cell cycle to proliferate and subsequently to differentiate. Because these receptors are generally expressed in very low levels in a resting cell, only the antigen-activated cells become responsive. In general, as shown in **Figure 2**, antigen-specific T_H cells are the primary source of these lymphokines. The ability of B cells to respond to the majority of antigens is critically dependent on these T_H cellderived lymphokines, and these antigens are called Tdependent antigens. Other antigens, usually large polymeric molecules with repeating antigenic determinants, are able to trigger B-cell responses without requiring T_H cell-derived lymphokines, and these antigens are called T-independent antigens.

Step 3: Lymphocyte Proliferation

The third step in an acquired immune response is the proliferation of antigen-specific lymphocytes. This step is often referred to as 'clonal expansion', where a clone is a family of cells having an identical antigenic specificity. Therefore, a 'polyclonal activator' is a substance which can trigger the proliferation of several clones, generally not in an antigen-specific fashion. Mitogens, substances which can cause lymphocytes to enter mitosis, are examples of polyclonal activators. A 'monoclonal antibody' is a genetically engineered antibody which has a single antigenic specificity because it is produced by a homogenous clone of cells.

As depicted in Figure 2 and described previously, the movement of antigen-activated lymphocytes into proliferation is regulated by lymphokines produced predominately by antigen-specific T_H cells. The characteristics of many of these lymphokines are summarized in Table 1. IL-2, which was previously known as T-cell growth factor, is of particular importance to T cells, including $T_{\rm C}$ and $T_{\rm H}$ cells. In the latter cell type, this lymphokine functions as an autocrine growth factor (i.e., a factor which can stimulate proliferation in the same cells where it is produced). IL-2 can also promote the proliferation of B cells, as can the following T_H cell-derived lymphokines: IL-4, IL-5, IL-6, and IL-1, a cytokine produced by macrophages/monocytes. As indicated in Table 1, some cytokines act as negative regulators of cell growth. Of particular importance to both B and T cells is transforming growth factor- β (TGF- β).

Step 4: Lymphocyte Differentiation

The proliferation in step 3 can be repeated several times (i.e., 'clonal expansion'). Eventually, the lymphocytes will stop proliferating and will begin to differentiate into antigen-specific effector cells. As with proliferation, this step in an acquired immune response is under the control of lymphokines produced primarily by T_H cells. One of the critical steps in the differentiation of B cells is the 'switch' to the

specific type of Ig molecule that will ultimately be secreted. As indicated in Table 1, IFN- γ stimulates the production of IgG2- α , IL-4 stimulates the production of IgE and IgG1, IL-5 stimulates the production of IgA, and IL-9 can act synergistically with IL-4 to stimulate IgE and IgG. A fully mature antibody-secreting B cell is called a plasma cell. A number of cytokines can also increase the generation of T_C cells including all types of IFN, IL-2, IL-4 (this action is synergistic with IL-2), IL-10, and IL-12.

Step 5: Effector Function

The basis for the effector function by antigen-specific lymphocytes – especially antibody-secreting B cells, T_D cells, and T_C cells – and the participation of other cellular components of the immune system in acquired immunity – including NK cells, macrophages, and neutrophils – have already been emphasized in other sections of this volume. It has also been emphasized that T_H cells play an important effector role in acquired immunity as regulators in the growth and differentiation of B cells and T_C cells. In this regard, it is important to note that both populations of T cells whose participation in an acquired immune response is mediated by the secretion of lymphokines, that is, T_H and T_D cells, are characterized by the expression of CD4.

Recent evidence suggests that there are at least two subpopulations of T_H cells, designated T_{H1} and T_{H2}. To date, the distinction between these two populations has been operationally defined and is based on the respective profiles of lymphokines which are produced: whereas both T_{H1} and T_{H2} cells produce IL-3 and GM-CSF; only T_{H1} cells produce IL-2 and IFN- γ , and only T_{H2} cells produce IL-4, IL-5, IL-6, and IL-10. The importance of this phenomenon can be understood from the discussion of the primary effects of these lymphokines. By virtue of the production of IL-2 and IFN- γ , T_{H1} cells will facilitate the generation of a cell-mediated immune response. In contrast, T_{H2} cells will facilitate the generation of a humoral immune response by virtue of producing lymphokines which support B-cell function. Moreover, as indicated in Table 1, T_{H1} cells can downregulate the activity of T_{H2} cells via the production of IFN- γ , and T_{H2} cells can downregulate the activity of T_{H1} cells via the production of IL-4 and IL-10. The basis for this 'cross talk' between the two types of $T_{\rm H}$ cells has been called the cytokine network. An important outcome of this cytokine network is that under certain conditions, a cell type traditionally characterized as a 'helper' cell can actually 'suppress' an immune

functional component (i.e., T_{H1} cells can suppress T_{H2} cells via IFN- γ , and T_{H2} cells can suppress T_{H1} cells via IL-4 and IL-10).

Certain types of T cells expressing CD8 are known to function as antigen-specific T_S cells. T_S cells are activated under conditions designed to induce immunological tolerance, previously referred to as a state of anergy, and their importance can be confirmed in cell transfer experiments. The mode of action of T_S cells is uncertain and may involve the secretion of soluble factors as described for T_H cells. T-cell-derived suppressor factors have been shown to be associated with APCs, such as macrophages. The action of T_S cells is capable of being directed toward T_H cells and B cells in an antigen-specific fashion. Therefore, it becomes obvious that the relative balance between T_H cells and T_S cells can exert tremendous influence over the magnitude of a given immune response and can even dictate if an immune response occurs.

Memory Cells: the Goal of Vaccinations/ Immunizations

One final point about acquired immunity needs to be emphasized. During the five steps of an acquired immune response, some of the antigen-specific and activated lymphocytes will undergo proliferation but will not be stimulated to differentiate and become effector cells, which have a relatively short half-life during a primary immune response. As shown in Figure 2, these cells will instead be programmed with the same antigenic specificity and will be returned to the blood and/or lymph circulation as long-lived memory cells. Upon reinfection with the same microbe, the secondary immune response, which is initiated by these memory cells, will occur quicker and with greater intensity. In the case of a humoral immunity, IgM is the predominant Ig released during a primary immune response, whereas IgG is the predominant Ig released during a secondary immune response. The generation of memory cells is the goal of vaccinations and immunizations where the host is injected with nonpathogenic (i.e., inactivated or attenuated) forms of infectious microbes. As children, we are vaccinated to several infectious organisms, including measles, mumps, diphtheria, tuberculosis, rubella, and poliomyelitis. Just prior to the winter months throughout a good portion of the United States, many people elect to receive the latest influenza vaccines, the so-called 'flu shots'. These are all examples of vaccinations and the objective is to clonally expand antigen-specific memory cells. Often, the responses to childhood vaccines, sometimes called 'recall antigens', are checked as an assessment of human immunocompetence.

Consequences of Immunotoxicity

Having established an appreciation of the immune system, with an emphasis on its functional organization and capabilities, it is now possible to discuss the consequences of immunotoxicity beyond the generalized concept of a continuum, as depicted in Figure 1. However, prior to discussing more specific consequences of immunotoxicity, especially the immunosuppressive part of the continuum, the concepts of redundancy and immunological reserve need to be emphasized. While the immune system can be divided into innate and acquired immunity, it is important to emphasize that this classification is based on the role that antigen plays in triggering a response. As described in previous sections, the immune system contains several overlapping mechanisms to cope with a given opportunistic pathogen, regardless of how the response is triggered. Moreover, the same effector mechanisms that are involved in innate host defense capabilities are activated in acquired immunity, and the progression from innate to acquired immunity is generally associated with greater intensity (i.e., greater destructive capability). The concept of overlapping effector function is often called the redundancy of the immune system. For example, phagocytic inflammatory cells, complement-derived peptides, and antibodies are all involved in the defense against bacterial infections. The first two effectors are prime players in innate immunity and are markedly activated by antigen-specific antibody in acquired immunity. Similarly, macrophages, NK cells, IFNs, T_C cells, and T_D cell-derived inflammatory lymphokines are all involved in the defense against viral infections. Again, the first two effectors are prime players in innate immunity and are markedly activated by antigen-specific T cells in acquired immunity.

The importance of redundancy is that the consequences of suppression of a given component of the host defense capabilities are sometimes minimized because of these overlapping systems. This phenomenon is sometimes referred to as immunological reserve. The redundancy and reserve of the immune system are thought to have evolved because of the diverse nature of pathogens with which it must cope to protect self. Moreover, a point about opportunistic pathogens, which is too often ignored, is that they are generally not passive players in their assault on the host. A characteristic of most successful infectious organisms is the ability to elicit different mechanisms to evade detection and/or to minimize the full effects of the host's defense capabilities. Moreover, microbes also evolve rapidly, enabling them to devise new means to evade the inherited defenses of species that evolve much more slowly. Therefore, even if the manifestation of the suppression of a given functional component is not clearly associated with disease, this immunotoxic effect should still be considered adverse because it does represent a loss in functional capabilities.

When the immune system is suppressed, the most severe consequences will be an increase in the incidence and severity of infections and/or an increase in the incidence and progression of malignancies or cancer. The specific types of infections which are increased can provide some clues as to the specific components of the immune system which were suppressed. For example, suppression of humoral immunity (i.e., antibody production) and/or its associated effector systems, such as phagocytic cells (i.e., macrophages and neutrophils) and the complement cascade, will be characterized by infections mediated by extracellular pathogens, including some bacteria and parasites. In contrast, suppression of cell-mediated immunity and/or its associated effector systems, such as NK cells and the IFNs, will be characterized by infections mediated by intracellular pathogens, including protozoans, viruses, and some bacteria, as well as by increased tumor formation. That these are consequences of immunosuppression in man cannot be argued based on a multitude of studies in individuals with congenital or acquired immune deficiencies or in patients undergoing long-term treatment with immunosuppressive drugs subsequent to organ or tissue transplantation. That these are consequences of exposure to suspected immunotoxic (i.e., immunosuppressive) xenobiotics in animal models also cannot be argued, primarily because extensive dose-response characterizations can be conducted under wellcontrolled experimental conditions and because changes in various immune functional parameters can be correlated with changes in host resistance models. However, this is not the case in humans, where there are few clear-cut examples of, or convincing evidence for, xenobiotic-induced immunosuppression outside of a therapeutic context. What becomes clear (even in the animal studies) is that the effects of immunotoxic drugs and chemicals are generally much more subtle than either the immune dysfunctions associated with congenital conditions or those produced by drugs used for immunosuppressive therapy.

As indicated in Figure 1, the other side of the immunotoxicity continuum is manifested either as a hypersensitivity response or as an autoimmune disease. A hypersensitivity response is an acquired immune response (i.e., by definition, it is manifested on second contact with a particular antigen) which

occurs in an exaggerated or inappropriate form to cause tissue damage and which is a characteristic of the individual (i.e., there is a genetic predisposition).

Autoimmune disease occurs when the reactions of the immune system are directed against the body's own tissues and is also characterized by a genetic susceptibility. Examples of autoimmune diseases include myasthenia gravis, in which cholinergic receptors, especially those associated with neuromuscular junctions, are targeted; multiple sclerosis, in which myelin is targeted; and rheumatoid arthritis, in which connective tissue, especially the synovial lining of joints, is targeted. The terms 'hypersensitivity' and 'autoimmunity' are often confused and are certainly interrelated. Based on their definitions, a hypersensitivity response can be a mechanism by which an autoimmune disease is produced. In contrast to the situation regarding suppression, in which the immune system is by and large a passive target for xenobiotic-induced changes, exaggerated immune responses can be mediated by two entirely different types of interactions by the immune system with drugs and chemicals. First, the immune system can again be a passive target for the enhancing effects of drugs and chemicals, such as occurs when a xenobiotic mimics or causes the aberrant production of immunomodulatory cytokines or when a xenobiotic disrupts the regulatory mechanisms which serve to protect self (i.e., suppress a suppressor). Another way that xenobiotics can enhance immune function is by acting as an adjuvant, which is defined as any substance which nonspecifically enhances the immune response to an antigen. The classic adjuvant is complete Freund's adjuvant (CFA), which is a waterin-oil emulsion containing killed mycobacteria. The effectiveness of adjuvants in enhancing immune responses can be demonstrated by the fact that animals are often injected with CFA to increase the production of antigen-specific antibodies and by the desire to develop an adjuvant that is safe in man (i.e., CFA produces severe side effects) which could be used in conjunction with vaccines or immunotherapy. The specific mechanism(s) for the actions of an adjuvant, including CFA, is not known. Moreover, the existence of environmental adjuvants is controversial and/or poorly studied. Therefore, adjuvants will not be further discussed in this entry.

In the second type of interaction, the components of the immune system are active participants in that the xenobiotic or some fraction of a xenobiotic is recognized as nonself and therefore provides the driving force for the response. Drugs and chemicals which are capable of triggering an immune response are generally low-molecular-weight substances possessing some inherent reactivity. For the most part, the xenobiotic

cannot be considered an antigen, simply because in itself it is not capable of stimulating an immune response. Instead, these substances are called 'haptens', which are defined as small molecules that can act as antigenic determinants but which cannot stimulate an immune response by themselves. The immune response is triggered when the hapten binds to some tissue of the host, the so-called 'carrier'. This property is called the sensitizing potential of the hapten and is associated with its inherent reactivity. Hapten-specific immune responses are therefore triggered only in the presence of the hapten-carrier complex and can be mediated either by humoral immunity (i.e., antibody), as in an allergic response, or by cell-mediated immunity (i.e., specifically a delayed hypersensitivity response), as in contact dermatitis. The damage associated with either type of hypersensitivity response can be directed against the tissue which is bound by the hapten. Therefore, the morbidity associated with hypersensitivity responses can be manifested in a number of ways reflecting the target tissues, including contact dermatitis, rhinitis, allergy, and anaphylaxis. Animal models have been developed which can clearly demonstrate the sensitizing potential of xenobiotics. Moreover, hypersensitivity disease has become an important human health problem in industrialized societies. One striking example of the consequences of hypersensitivity disease in humans is occupational asthma, which is one of the most common occupational ailments in the Western world. However, it is important to emphasize that the establishment of a cause and effect relationship is more straightforward in the case of hypersensitivity than immune suppression. The onset of hypersensitivity is always a consequence of exposure to an exogenous agent. On the other hand, the repercussions of immune suppression following exposure to a xenobiotic, especially one that produces only modest changes in immunocompetence, in most cases will be subtle. These consequences will likely be manifested as a slightly greater susceptibility to common opportunistic infections, such as those responsible for the common cold or the flu.

Autoimmunity is much more complex than hypersensitivity. Animal models exist for many autoimmune conditions, and autoimmunity has been clearly demonstrated in humans, although it is a relatively infrequent occurrence. Therefore, the existence of autoimmune disease and the expected consequences cannot be denied. However, the ability of drugs and chemicals to exacerbate or trigger autoimmune disease in either animal models or humans is poorly understood. In fact, of all the possible consequences of immunotoxicity, autoimmunity is unquestionably the least understood. Primarily because of the strong genetic component in the susceptibility to autoimmunity, deciphering the exact role of xenobiotics in the induction of these conditions has proved to be very difficult.

Mechanisms of Immunotoxicity

As described previously, the immune system is a complex, widely distributed and tightly regulated series of overlapping effector functions designed to allow the discrimination between self and nonself. As such, the immune system is characterized by a number of features that make it vulnerable to being targeted by exposure to xenobiotics. Several of these features are briefly highlighted below.

Immune System Features Associated with Vulnerability to Xenobiotics

Many of the effector functions of the immune system are dependent on a multitude of cell types, which all share a common precursor, the pluripotent stem cell. Therefore, any damage to the stem cell would be expected to have devastating consequences, several of which would extend beyond the immune system, most notably involving the red blood cell. Fortunately, the stem cell is refractory to xenobiotic-induced perturbation and is only affected by high doses of radiation. However, subsequent steps of hematopoiesis are affected by exposure to chemicals, with benzene being a classic example. Acute toxicity to benzene is associated with pancytopenia, aplastic anemia, and, at high doses, immunosuppression and leukemia.

The generation of mature lymphocytes with the capability of being programmed to respond against nonself in an antigen-specific manner but without the risk of responding to self is dependent on a complex maturational process that takes place in primary lymphoid organs, such as the bone marrow and thymus. Therefore, xenobiotic-induced damage to these microenvironments can contribute to immunosuppression as well as problems with immune regulatory functions. In addition, the cells that make up the microenvironment within the primary lymphoid organs (e.g., bone marrow stromal cells and thymic epithelium) can also contribute to the mechanism by which xenobiotics alter the differentiation of leukocyte precursor cells. One example of such a mechanism has been the demonstration that the immunotoxic polyaromatic hydrocarbon (PAH), and 7,12-dimethylbenzanthracene (DMBA) induces apoptosis of pro/pre-B cells, precursors of the mature B cell, in a manner that is dependent on direct cellcell contact with bone marrow stromal cells for delivery of the 'death' signal. In addition to cell-cell contact, the involvement of an unidentified protein derived from the bone marrow stromal cells has been established in the DMBA-induced apoptosis of pro/pre-B cells, which is trypsin-sensitive, greater than 50 kDa in size and is likely a carrier of an immunotoxic DMBA metabolite formed in the stromal cells.

B and T lymphocytes are generally quiescent resting cells requiring appropriate stimulation by an antigen in order to elicit an effector function. Antigenic stimulation of lymphocytes activates multiple signal transduction cascades which alter the profile of gene expression to induce lymphocytes to first clonally expand, by undergoing numerous rounds of proliferation, and then to terminally differentiate into effector cells (e.g., antibody-secreting plasma cells, cytokine-secreting helper T cells or an armed cytotoxic T cell). Xenobiotics that interfere with the signal transduction cascades initiated at the antigen receptor are extremely potent immunosuppressive agents. Two examples of such agents are the transplantation drugs, cyclosporin A and FK506, both of which block T-cell activation by targeting critical components of the T-cell receptor-initiated signal transduction cascade and upregulation of the T-cell growth factor, interleukin-2 (IL-2).

Proliferation (i.e., the clonal expansion of reactive lymphoid cells) is a critical step in any acquired immune response. Therefore, any drug or chemical with antiproliferative properties has the potential to be immunosuppressive. Many types of immunosuppressive drugs are in fact antiproliferative, including cyclophosphamide, methotrexate, and azathioprine. Moreover, many types of anticancer drugs which target highly proliferating cells exhibit immunosuppression as an important side effect. An aspect centered around proliferation, which is rarely considered in immunotoxicology, is the malignant processes of immunocompetent cells. Leukemias and lymphomas are characterized by uncontrolled proliferation of T cells, B cells, or monocytes. However, because these cells are arrested in a specific stage of maturation or differentiation, even though there is tremendous proliferation, these conditions are generally associated with decreased functional capabilities and are manifested as a profile of activity comparable to that associated with exposure to antiproliferative drugs or chemicals (i.e., immunosuppression). As noted previously, benzene is an example of a chemical which can trigger leukemias, and ethylene oxide is an example of a chemical which has been associated with the development of lymphomas. Because these conditions are most appropriately categorized as types of cancers, they will not be further discussed in this entry.

Virtually all aspects of immunity are dependent on recognition of surface structures and can be regulated by a multitude of soluble products. Therefore, any drug or chemical that affects protein synthesis, gene expression, and/or receptor expression has the potential to disrupt immune function.

The immune system is characterized by a unique distribution, and many of its components are located close to the principal sites of absorption, including the gastrointestinal tract, the pulmonary tract, and the skin. Therefore, the immune system is in a position to be exposed to potentially high concentrations of drugs and chemicals. This phenomenon is especially important in hypersensitivity responses where the primary portal of entry of the hapten can determine the specific site of the reaction.

Some components of the immune system have metabolic capability including some forms of the cytochrome P450 system. Therefore, some chemicals, which are inert in their parent form but can be metabolized to reactive intermediates, can be activated by the immune system. However, it is important to emphasize that the metabolic capability of the immune system is minor when compared with other organ systems, most notably the liver, and that it possesses only a small repertoire of metabolizing enzymes. This point is discussed below as a major type of indirect mechanism of immunosuppression.

The immune system is not only regulated by its own products but is also exquisitely sensitive to products generated by other systems. Two examples, that will be discussed below as major types of indirect mechanisms of immunosuppression, are changes in neuroendocrine status and liver damage.

Direct and Indirect Effects of Xenobiotics

The remaining discussion of the mechanisms of immunotoxicity will be presented from the perspective of the following three general consequences of immunotoxicity: immune suppression, hypersensitivity, and autoimmunity.

One of the critical features of any discussion of the mechanisms of immune suppression must be the appreciation that robust changes in immune function can be mediated by either direct or indirect effects (or both) of a xenobiotic. Direct effects can be associated with distinct types of cells. Perhaps the best examples are cyclosporin A and related immunosuppressive drugs, such as rapamycin and FK-506, which specifically target T cells via an interaction with cytosolic and/or nuclear proteins to disrupt antigeninduced activation of transcription. To date, despite the tremendous evolution of the discipline of immunotoxicology, no other xenobiotic associated

with occupational or environmental exposure has been as well-characterized from a mechanistic perspective as cyclosporin A. Nonetheless, a few examples are worthy of mention.

Cannabinoids, a family of more than 60 structurally related molecules, which constitute the active ingredients of marijuana, selectively suppress T cell and macrophage function. The mechanism of action, although still superficially understood, appears to be mediated, in part, by specific cannabinoid receptors, termed CB1 and CB2. Both CB1 and CB2 belong to the G-protein coupled receptor superfamily. These receptors are anchored into the plasma membrane by seven transmembrane regions which make up a ligand-binding pore. Agonist binding to G-protein coupled receptors results in a confirmational change in the receptor which in turn induces interactions between the C terminus of the receptor and GTPbinding proteins to initiate signal transduction into the cell. Binding of cannabinoid ligands, such as δ -9-tetrahydrocannabinol, to cannabinoid receptors results in at least two proximal events which occur independently of each other. The first is the inhibition of adenylate cyclase, the enzyme that converts ATP to cAMP. The downstream consequence is an inhibition of the cAMP signaling cascade as evidenced by decreased protein kinase A activity, reduced DNA binding by cAMP response element-binding proteins, and reduced transcription of cAMP responsive genes. The second consequence is a rapid induction of intracellular calcium, through the opening of calcium channels. Elevation of intracellular calcium prior to antigenic stimulation renders T cells anergic (i.e., unresponsive to antigenic stimulation).

Some chemicals selectively affect macrophage function, including asbestos. The effects on macrophage function are frequently manifested as a deficit in antigen-presenting capabilities. The mechanism of action of asbestos is mediated by the fact that asbestos fibers are phagocytized by macrophages to the point where they appear to become engorged.

Perhaps the environmental chemical most studied with regard to effects on the immune system has been 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which is also known as TCDD or 'dioxin'. While it appears that dioxin may affect multiple cell types, it is apparent that the B cell is an especially sensitive target. The mechanism of action for the immunotoxic effects of dioxin remains poorly understood. It is well established that many of the actions of dioxin, including inhibition of the primary antibody response, are mediated by a specific cytosolic receptor, termed the aryl hydrocarbon receptor (AhR). AhR exhibits a profile of activity similar to steroid receptors in that the ligand-activated receptor undergoes a nuclear

translocation, and functions as a transcription factor to regulate the expression of a wide number of genes. Although changes in mRNA expression levels for a number of immunologically relevant genes correlate with dioxin treatment of leukocytes, direct regulation of specific genes via the ligand-activated AhR has been more difficult to establish. There is compelling experimental evidence that genes coding for IL-1, IL-2 and mu Ig heavy chain are regulated, at least in part, by the ligand-activated AhR complex through dioxin response elements present either in the promoter or enhancer regions of the aforementioned genes. In the case of IL-1 and IL-2, TCDD treatment increases expression of both genes. Conversely, TCDD treatment strongly decreases mu heavy chain expression in LPS-activated B cells which is correlated with a marked inhibition of IgM secretion and decreased activity of the Ig heavy chain 3'-alpha enhancer.

Another chemical which affects multiple cell types is the semiconductor, gallium arsenide. Arsenic is the primary immunosuppressive component and both macrophages and T cells are the targets. In macrophages, antigen processing and presentation is identified as a functional target. The effect on T cells manifests as an antiproliferative effect mediated through a decreased expression of surface molecules and could be reversed by the exogenous addition of IL-2, IL-5, and IL-6.

Finally, a group of chemicals which has been widely studied for effects on the immune system includes the PAHs, such as benzo(a) pyrene [B(a)P] and DMBA. PAHs represent a prototypical class of carcinogens, and this action is mediated by the generation of a reactive intermediate, which is capable of binding directly to macromolecules like DNA. The mechanism of immunotoxicity also appears to be dependent on the generation of the reactive intermediates, and the available evidence suggests that certain types of immunocompetent cells, most notably the macrophage, have enough metabolic capability to activate PAHs such as B(a)P and DMBA. The specific macromolecular target that is responsible for the immunosuppression is not known, although it appears that these chemicals can affect multiple cellular targets. Interestingly, there are reports that exposure to PAHs can disrupt the production of immunomodulatory cytokines. Specifically, B(a)P decreases the production of IL-1, and the suppression by DMBA can be reversed by the exogenous addition of IL-2. In addition, and as already discussed, DMBA can induce apoptosis in pro/pre-B cells through a mechanism that is dependent on cell-cell contact with bone marrow stromal cells. Present evidence suggests that the stromal cells metabolize DMBA to a

form that is then transferred to pro/pre-B cells via a protein carrier, which is greater than 50 kDa in size, that delivers the death signal.

A number of chemicals with demonstrable suppression of immune function produce this action via indirect effects. By and large, the approach that has been most frequently used to support an indirect mechanism of action is to show immune suppression after in vivo exposure but no immune suppression after in vitro exposure to relevant concentrations. One of the most often cited mechanisms for an indirect action is centered around the limited metabolic capabilities of immunocompetent cells and tissues. A number of chemicals have caused immune suppression when administered to animals but were essentially devoid of any potency when added directly to suspensions of lymphocytes and macrophages. Many of these chemicals are capable of being metabolized to reactive metabolites, including dimethylnitrosamine, aflatoxin B₁, and carbon tetrachloride. Interestingly, a similar profile of activity (i.e., suppression after in vivo exposure but no activity after in vitro exposure) has been demonstrated with the potent immunosuppressive drug cyclophosphamide. With the exception of the PAHs, few chemicals have been demonstrated to be metabolized when added directly to immunocompetent cells in culture. A primary role for a reactive intermediate in the immune suppression by dimethylnitrosamine, aflatoxin B₁, carbon tetrachloride, and cyclophosphamide has been confirmed in studies in which these xenobiotics were incubated with suspensions of immunocompetent cells in the presence of metabolic activation systems (MASs). Examples of MASs include primary hepatocytes, liver microsomes, and liver homogenates. In most cases, confirmation of a primary role for a reactive metabolite has been provided by *in vivo* studies in which the metabolic capability was either enhanced or suppressed by the administration of an enzyme inducer or a metabolic inhibitor, respectively.

While the demonstration that the immune suppression by a given chemical is mediated by a metabolite, and not the parent compound, is an important observation, it does not in itself account for the mechanism of action. One indirect mechanism of action, which is consistent with a role for metabolism, involves a primary consequence of the generation of many reactive intermediates, that is, liver damage. Among many different types of adaptive responses, the liver is capable of secreting a number of soluble factors. Some of these soluble factors are capable of affecting immune responses. Most notably, as pointed out in Table 1, TGF- β is capable of modulating both T- and B-cell effector functions. Both carbon tetrachloride and cocaine cause immune suppression and liver damage over a comparable dose range. Support for a role by a serum factor was obtained by studies in which serum from either carbon tetrachloride-treated or cocainetreated mice suppressed the function of immunocompetent cells from untreated mice. Confirmation for a role by TGF- β was obtained by demonstrating its presence in the serum of treated mice and by showing that the suppression by the serum could be reversed by a neutralizing antibody against TGF- β . Significant advances have been made during the past several years concerning the molecular mechanism by which TGF- β modulates B and T cells. TGF- β is one of the most potent immunoregulatory cytokines yet to be identified. It possesses bifunctional activity, enhancing as well as inhibiting a wide variety of immunological responses depending on the context within which immunocompetent cells encounter this cytokine strongly suggesting that its primary role is to maintain immune homeostasis. Concordant with this notion, mice in which the gene for TGF- β has been knocked out rapidly develop a systemic autoimmune response in virtually all the vital organs within the first several months after birth. TGF- β exerts its biological activity through a heterodimeric TGF- β receptor, which in turn propagates signals into the cell via the Smad protein signaling cascade to regulate gene expression.

The liver damage associated with exposure to cocaine is a relatively minor component of its profile of activity. Cocaine is an abused substance that has been studied most extensively for its effects on the brain and on behavior. Cocaine is capable of triggering robust changes in a number of neuroendocrine factors, some of which suppress immune function. In particular, cocaine is able to cause immune suppression at least in part through an elevation in plasma corticosterone as a consequence of the stimulation of the hypothalamic-pituitary-adrenal neuraxis. Ethanol (i.e., at least after acute exposure) and morphine are two other examples of xenobiotics which are suspected of producing their primary actions on the immune system via neuroendocrine effects. In most cases, the evidence is based on a measurable increase in serum corticosterone at doses capable of causing immunosuppression and on the ability of the glucocorticoid antagonist, RU-486, to reverse the suppression.

Hypersensitivity Reactions

Three points about hypersensitivity reactions have already been emphasized. First, the ability of a drug or chemical to trigger a hypersensitivity reaction is due to an inherent property of the xenobiotic (i.e., hapten), its sensitizing potential. Second, in a hypersensitivity reaction, the immune system plays an active role in mediating the response against the hapten. Third, both antigen-specific antibody (i.e., humoral immunity) and antigen-specific T cells (i.e., cell-mediated immunity) can be the effectors which are responsible for the tissue damage associated with hypersensitivity reactions. Traditionally, different types of hypersensitivity have been classified using a scheme originally proposed in 1963. Although the Coombs and Gell classification scheme is still widely used today, it is important to emphasize that the different types of hypersensitivity reactions rarely appear individually and are most often seen as mixed components. Nonetheless, this classification still represents one of the easiest ways to appreciate how the immune system is involved with the tissue damage-associated hypersensitivity reactions. The first three types of hypersensitivity reactions (I–III) are mediated by antigen-specific antibody, while the fourth type of hypersensitivity reaction (IV) is mediated by antigen-specific T cells.

Type I

A type I hypersensitivity reaction is the classic example of an allergic reaction and is also called an immediate hypersensitivity reaction because of its rapid appearance upon challenge in a sensitized individual. The primary mediator for a type I reaction is IgE, which is produced by antigen (hapten)specific B cells in a T-cell-dependent fashion and which binds by its constant region to the Fc receptors on the surface of mast cells and basophils. As such, hapten-specific IgE can rightfully be considered a true biomarker for exposure in sensitized individuals. Therefore, in the absence of antigen-specific IgE, it is inappropriate to label a condition as an allergic reaction. This situation is true for occupational allergy, where only the presence of antigen-specific IgE should be accepted as a criteria for exposure to a suspected respiratory sensitizer. Upon a second contact with the hapten, the mast cells and basophils will be stimulated to degranulate and release a variety of vasoactive substances, which are the mediators of the inflammatory response and the accompanying tissue damage. The target organs for a type I reaction include the gastrointestinal tract, skin, lungs, and vasculature. Anaphylaxis is a systemic type I reaction which can be life threatening. As noted previously, many hypersensitivity reactions have a genetic component, and atopy is a term used to describe a genetic predisposition toward the development of IgE-mediated reactions against common environmental antigens, such as pollen and dust.

In the section on the effector functions of antibody, it was emphasized that IgE was a primary player in the host defense against a variety of parasites. Therefore, it could be easily argued that individuals endowed with an ability to mount a robust IgE response possessed a distinct survival advantage over individuals without such a defense mechanism against parasitic infections. However, in most developed nations, parasitic infections have been all but eliminated. Interestingly, in most developing nations, while parasitic infections are still a problem, the incidence of allergy or chemically induced hypersensitivity reactions is very low. Taken together, these two observations have prompted the speculation that the incidence of allergy and chemically induced hypersensitivity reactions in industrialized societies is due, at least in part, to the fact that the components of the immune system associated with the production of IgE, in the absence of parasites, are now free to react to other nonparasitic substances in a counterproductive way.

Type II

A type II hypersensitivity reaction is also called a cytolytic reaction because the damage is mediated by hapten-specific antibodies which are capable of triggering cytotoxicity in the target cell. The antibodies involved in a type II reaction are both IgM and IgG, with the latter type predominating. The specific effectors which are responsible for the cell damage include both the complement system and phagocytic cells, and these effectors are activated exactly as described in the section on humoral immunity. The target organs for type II reactions include many cell types circulating in the blood. Examples of type II reactions include xenobiotic-induced hemolytic anemia or agranulocytosis.

Type III

A type III hypersensitivity reaction is also called immune complex disease. Examples of type III reactions include the Arthus reaction and serum sickness. The damage associated with type III reactions is mediated by the generation of hapten-specific antibody, primarily IgG. The basis for the damage associated with type III reactions is that soluble antigen–antibody complexes are deposited in key anatomical locations, such as small capillary beds in the skin or the glomerular regions of the kidney. As described in the section on humoral immunity, the classical activation of the complement cascade is mediated by antigen– antibody complexes. Therefore, the deposition of immune complexes can cause a very localized activation of the complement cascade resulting in the generation of chemotactic peptides as well as the lytic unit. The target organs for type III reactions include blood vessels in the skin, joints, and lungs and the glomerular regions of the kidney.

Type IV

A type IV hypersensitivity reaction is also called a delayed hypersensitivity reaction because of its delayed appearance (i.e., after 24-48 h) following challenge in a sensitized individual. This is the only type of hypersensitivity reaction which is not mediated by antibody and is instead dependent on the generation of hapten-specific T cells, specifically the T_D cells, which contribute to the inflammation and the accompanying tissue damage by the generation and release of a variety of lymphokines. Classic examples of type IV reactions include the response that some individuals have to poison ivy (i.e., again emphasizing the genetic component to hypersensitivity reactions) and contact dermatitis. Target organs besides the skin include the lungs (i.e., the target organ for the wellstudied tuberculin reaction), central nervous system (CNS), thyroid, and other organs.

Recent studies characterizing the basis for chemically induced hypersensitivity have uncovered an important interplay between type I hypersensitivity reactions, manifested primarily as respiratory sensitization, and type IV hypersensitivity reactions, manifested primarily as contact sensitization. The most important observation came from studies which showed that a predominantly respiratory sensitizer would still trigger an IgE response when applied topically. This observation can be accounted for by the cytokine network model which was described previously as important for cross talk between humoral immunity and cell-mediated immunity. Basically, a chemical with the capability of being a respiratory sensitizer will trigger an IgE response regardless of its route of exposure because it 'selects' or supports the development of a T_{H2}-dependent response, with the associated cytokine profile, IL-4, IL-5, and IL-10. In contrast, a chemical which lacks the capability of being a respiratory sensitizer; but which can still trigger contact dermititis, will select or support a T_{H1}-dependent response, with the associated cytokine profile, IL-2 and IFN-y.

Autoimmune Responses

If increased incidence and/or severity of infections represents the critical consequence of a suppressed immune response, then autoimmunity represents the antithesis for an exaggerated immune response. As noted previously, the characterization of the onset and progression of autoimmune conditions has been complicated by the critically important role that genetics plays in this process. While the exact association to drugs and chemicals as factors is poorly understood, several potential mechanisms have been proposed to account for this association. By definition, autoimmunity is the harmful consequence associated with an immune response which is mediated against self. Therefore, most of the mechanisms which have been proposed are centered around either a xenobiotic-induced change in the antigens associated with self or a xenobiotic-induced change in the recognition of self. Autoimmune responses that are driven by the first mechanism are associated with several possibilities which are identified as follows:

- Immune responses can be directed toward a foreign (i.e., nonself) antigen which has a similar chemical structure to an antigen which characterizes self.
- Immune responses can be directed toward a new nonself antigen that has become nonspecifically absorbed to a cell membrane. This mechanism is essentially the description of the sensitizing potential of a hapten and is one of the reasons why there is some overlap between hypersensitivity reactions and autoimmune conditions.
- Immune responses can be directed against a self antigen which is normally shielded or hidden but becomes available or expressed following exposure to a xenobiotic or during a disease process. Again, this is a potential area of overlap between hypersensitivity reactions and autoimmune disease. As described previously, hypersensitivity reactions can be mediated by the activation of immune effector processes which possess considerable destructive capability. Therefore, it is possible that one of the consequences of the tissue damage caused by a hypersensitivity reaction could be the expression of hidden antigens, which then sets the stage for the initiation of an autoimmune condition.

The second mechanism for autoimmunity is a change in the way that self is recognized. As described previously, the repertoire of antigen-specific immune effector mechanisms must develop with the capability of recognizing a tremendous number of nonself antigens while preserving the ability to recognize an equally vast number of self determinants. There are several regulatory mechanisms which are involved in this critical process that could be targeted to contribute to the onset and/or progression of autoimmune disease. First, T_S cells can play an important regulatory role in preventing an exaggerated immune

response. Therefore, the suppression of T_S cells could result in an inappropriate recognition of self antigens. Second, an important step in the maturation of both T and B cells is negative selection, whereby these cells are probed for their recognition of self determinants. Lymphocytes with antigen receptors that can recognize self determinants are either destroyed via the stimulation of apoptosis or these cells are rendered anergic. An important part of the probing process is centered around the transfer and presentation of self determinants of organs or tissues distal to the microenvironments of the thymus or bone marrow. Recent evidence has suggested that this process may be one of the more sensitive targets for triggering an autoimmune mechanism. As such, any xenobiotic-induced changes in the movement of self determinants to the primary lymphopoietic organs and/or any xenobiotic-induced changes in the primary lymphopoietic organs themselves can be a mechanism for autoimmunity.

Autoimmune diseases may be tissue specific, where the damage is associated with a specific type of tissue or a specific organ, or tissue nonspecific, where the signs and symptoms are associated with several organs and tissues. The primary sites of tissue damage in autoimmune disease are many and varied. The following organs, cells, and organelles have all been determined to be the site of autoimmune reactions: nuclei (specifically histones and/or single-stranded DNA - one of the hallmark indicators of certain types of autoimmune disease is the expression of anti-nuclear antibodies), red blood cells, lymphocytes, neutrophils, platelets, Igs (primarily IgG), striated muscle (cholinergic receptors), smooth muscle, mitochondria, skin (basement membranes), thyroid (thyoglobulin), kidney (glomerular and tubular basement membrane), CNS (myelin), connective tissue (synovial lining of joints), lung, and liver. Both cell-mediated immunity and humoral immunity can be involved as effector mechanisms in causing the damage in autoimmune conditions.

Emergence of Regulatory Immunotoxicology Guidelines

The maturity and acceptance of a subdiscipline of toxicology can frequently be directly correlated to the level of interest being demonstrated by the regulatory community. One of the key issues facing immunotoxicology which has scientific, political, and societal implications is the approach to immunotoxicity testing for regulatory purposes. The Office of Prevention, Pesticides and Toxic Substances (OPPTS) of the US Environmental Protection Agency (EPA)

published guidelines entitled, Biochemicals Test Guidelines: OPPTS 880.3550 Immunotoxicity in 1996. These guidelines described the preferred study design for an exceptionally thorough evaluation of the potential immunotoxicity of biochemical pest control agents. This guideline described a panel of tests that included standard toxicology tests as well as immune functional tests assessing both humoral and cell-mediated immunity. OPPTS 880.3550 clearly presented a very comprehensive approach to immunotoxicity: but a second document. Biochemicals Test Guidelines: OPPTS 880.3800 Immune Response, was needed to provide the rationale for when these studies should be conducted. The 880 series of immunotoxicity guidelines would arguably detect any type of immunotoxic potential by pesticides. However, the comprehensive nature of these guidelines rendered them prohibitively expensive and time consuming. The EPA released the Health Effects Test Guidelines: OPPTS 870.7800 Immunotoxicity in 1998. These guidelines described the approach to immunotoxicology testing for nonbiochemical agents regulated by the EPA. The testing approach in OPPTS 870.7800 reflected the continued evolution of the science of immunotoxicology and reflected a more limited, case-by-case approach than previously described by the earlier more comprehensive guidelines. The cornerstone of OPPTS 870-7800 was a functional test, the primary T-dependent antibody response (TDAR), which had been demonstrated in a number of intralaboratory studies to possess the greatest predictivity of known immunotoxicants. Subsequent tests, including the NK cell assay and the phenotypic quantitation of T and B cells, would be conducted on a case-by-case basis depending on the outcome of the TDAR.

The earliest immunotoxicity guidelines from the Food and Drug Administration (FDA) were centered on food additives as the Draft Redbook II in 1993. This document, although never finalized, contained an extensive description of immunotoxicology testing. In general, the Redbook guidelines reflected the 'tier' approach to immunotoxicology, as described in greater detail below. Specifically, the Redbook emphasized a step-wise approach that began with expanded studies utilizing data obtained in standard toxicology testing as initial indicators of immunotoxicity. Progressively more complicated immunological tests were prescribed using an approach that was very much case-by-case, with each new level of testing predicated on positive results in the preceding level. The FDA Center for Drug Evaluation and Research (CDER) released its document entitled, Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs, in 2002. This document is arguably the most comprehensive description of approaches to immunotoxicology. Not only does the FDA CDER *Guidance for Industry* describe the entire spectrum of adverse events associated with the immunotoxicology continuum, including immune suppression, immunogenicity, hypersensitivity, autoimmunity and adverse immune stimulation, this document also provides approaches at the level of specific methodology for evaluating each event. As with the earlier document from the FDA, the new *Guidance for Industry* advocates the use of information derived from standard repeat-dose toxicity studies to provide the earliest indicators of immunotoxicity.

Approaches to Immunotoxicology

As noted above, several US government agencies with regulatory responsibilities, including the FDA and the EPA, have drafted recommendations for guidelines for immunotoxicity testing strategies. All testing strategies to date recognize the complexity of the immune system as a target organ and recognize that no single immune parameter can be used with sufficient confidence to test for the hazard of immunotoxicity. Therefore, historically, immunotoxicity has been assessed by a battery of assays usually structured in a multitiered approach. However, recent validation studies, most notably studies conducted by the National Toxicology Program, have indicated that immunotoxicity can be assessed with a finite number of assays. Several concepts that have had an impact on the evolution of a testing strategy are highlighted below.

Although the concept of required immunotoxicity testing is a relatively recent development, the toxicity of the immune system has been an important part of routine toxicity testing for some time. The endpoints have included weights and histological evaluation of key immunocompetent organs, including the spleen and thymus, leukocyte counts and differentials, and some parameters in clinical chemistry, including globulin measurements. However, while it is acknowledged that these standard toxicology endpoints are important and can provide some indication of immunotoxicity, it is generally recognized that they are not sufficient as a predictor of immunotoxicity hazard. As many as 30% of known immunotoxic chemicals would be missed if only these endpoints were used. While these endpoints become better predictors when chemicals are assessed at high doses (i.e., at or above the maximum tolerated dose), these types of exposures will also increase the likelihood that indirect mechanisms of immunotoxicity

(i.e., such as neuroendocrine changes, liver damage, or effects on other organ systems) will be involved.

Most experts in the field of immunotoxicology recognize that immunotoxicity can only be measured if the immune system is asked to perform its function. Therefore, a specific functional parameter is now recognized as a critically important component of the first tier of a testing strategy. One of the most sensitive indicators of immune suppression in most animal models has been the primary response to an antigen. An especially sensitive parameter is the antibody response to a T-cell-dependent antigen. The sensitivity of this type of assay is consistent with the description of this response as being dependent on the cooperativity of multiple cell types, including the B cell as the primary effector cell, T cells as important regulatory cells, and APCs. Regardless of the specific immune parameters included in a testing strategy, the interpretation regarding immunotoxicity can only be made in the context of a well-designed study from the perspective of the dose-response relationship.

Because an increase in the incidence and/or severity of infection has been consistently identified as one of the hallmark indicators of immune suppression, a great deal of effort has been put forth into the design and characterization of host resistance models. One of the key features of these characterizations has been the correlation of changes in various host resistance models with changes in specific immune parameters. These results have consistently indicated that changes in specific immune functional parameters are associated with the predicted/anticipated changes in host resistance models. It is now generally accepted that host resistance models are not a feasible choice as an initial predictor of immunotoxicity because of their complexity and cost and that these models are best positioned in the second tier of a testing strategy.

Because immunotoxicity exists in a continuum, it is important to measure xenobiotic-induced changes in immune function in both directions. By and large, more effort has been invested in the validation of studies to address the immunosuppressive part of the continuum. However, recent validation studies have been conducted to address the sensitizing potential of chemicals, including a major effort by the National Toxicology Program.

One of the obvious and most important goals of an experimental immunotoxicity testing strategy is to enable the best extrapolations between the results generated in the animal models and the potential risk of immunotoxicity in humans. One of the recent fallouts of this goal has been the recognition that the historic approaches that have been used in clinical immunology may not have much use in human immunotoxicology. While these end points are sufficient to detect immunodeficiencies associated with either congenital disorders or immunosuppressive drug therapy, they do not possess the necessary sensitivity to detect the more subtle consequences of xenobiotic-induced immunotoxicity. Specifically, many of these end points, including mitogen-induced lymphoproliferation, the analysis of lymphocyte surface markers, and the response to recall antigens, lack appreciable sensitivity in most animal studies. As a result, several recent proposals have been put forth to reevaluate the way that we measure immune function in humans. Most of these testing strategies have incorporated plans to measure the primary response to a new antigen, and several of these testing strategies have recommended using newly developed vaccines as the new antigen.

The immune system has unquestionably been identified as a potential target organ for drugs and chemicals. Therefore, the hazard exists. The assessment of the risk associated with xenobiotic-induced immunotoxicity represents one of the key challenges for this discipline in the immediate future.

See also: Biomarkers, Human Health; Blood; Molecular Toxicology–Recombinant DNA Technology; Multiple Chemical Sensitivities; Polycyclic Aromatic Hydrocarbons (PAHs); Psychological Indices of Toxicity; Resistance to Toxicants; Sensitivity Analysis; Skin.

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Implant Studies

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Implant or implantation tests are designed to assess the localized effects of a biomaterial or device designed for therapeutic use inside the human or animal body. These tests are performed to evaluate and establish the safety of a new biomaterial or device. and to meet regulatory requirements. Implantation testing methods essentially attempt to imitate the intended use and likely misuse conditions. Although different tests use various animal species, the rabbit is the species of choice, with implantation performed in the paravertebral muscle. Implantation can be either surgical or nonsurgical: the surgical method involves the creation of a pouch in the muscle into which the implant is placed, while the nonsurgical method uses a cannula and stylet to insert a cylinder-shaped implant. A macroscopic examination may be supplemented with microscopic analysis, and the degree of tissue reaction in the test site is evaluated as a measure of biocompatibility. The implant may be maintained for from 7 days to a year for the study.

The principal regulatory guidelines for implant studies come from the ISO (International Organization for Standardization) and the Ministry of Health, Labour, and Welfare in Japan (MHW). The biological evaluation of medical devices has become more globally harmonized in recent years, concurrently with the publication of the ISO 10993 standard for the testing of medical devices. Some countries still require their national guidelines to be met, but most will accept ISO 10993 as a parallel alternative to their own regulations. Examples of some differences between the ISO and MHW sets of guidelines are summarized below. It should be noted that all guidelines, whether accepted or under preparation, should be regarded as more of a dynamic process than a rigid framework since the various standards are subjected to continuous revision and evaluation. The impact on this will come from authorities, notified bodies, and from national and international expert and working groups (e.g., see the European Centre for Validation of Alternative Methods (ECVAM) working group activity below). Further, the recommended tests must be conducted with consideration for the information available from other sources, with knowledge of the type of material a device is made from, and with awareness of its planned end use and likely misuse (Table 1).

The interactions between intact organisms and implanted devices or biomaterials are complex. Not

Table 1	Differences in ISO 10993 and the MHW guidelines for		
assessing the effects of device or material implantation			

ISO 10993	MHW 1995
Time point(s) of assessment: sufficient to achieve steady state; e.g., 2, 4, 6, and 12 weeks	7 days and 4 weeks
Number of animals: at least three per time period of assessment	At least four per time period
Number of samples of evaluation: at least eight per time period for test and control	No minimum number specified
Evaluation criteria: comparative evaluation of responses to test and control materials	If more than two of the four test sites in each animal exhibit a significant response compared to control sites, the test is considered positive

only do implanted materials affect the organism, but the organism can affect the implanted materials. Most longer-term implant studies incorporate retrieval of the implant at the end of the study to allow evaluation of this latter aspect.

Effects of the implant on its host almost always include evoking a foreign bodily response. The extent of this and other responses are assessed both quantitatively and semiquantitatively, with the latter being done with reference to established grading scales.

Bollen and Harling (see Further Reading section) provide an overview of how to conduct a risk assessment for a medical device and discuss the use of ISO 14971, one of the newest standards in this area, for risk management. This publication also covers ISO 10993, which (as discussed above) covers the biological evaluation of medical devices. A hypothetical risk evaluation of a medical device is also described.

A working group under the auspices of the European Centre for Validation of Alternative Methods has recommended alternative methods that can be used for safer testing of medical devices. These include two *in vitro* tests as potential substitutes for the *in vivo* assays for skin and eye irritation.

Finally, there is a special class of tumorgenesis that is induced by subcutaneously implanted devices only in rodents (induction of fibrosarcomas by what is called the Oppenheimer effect).

See also: Biocompatibility; Foreign Body Response.

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Import and Export of Chemicals See Hazardous Chemicals, Import/Export of.

In Vitro Test

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In vitro test systems do not employ intact higher organisms as models, and include a large set of alternative models addressed extensively under their own entries in this book. These *in vitro* test systems tend to have the benefit of being low in cost to use and to have well-known mechanisms of action. They can be used for assessing or predicting the toxic effects of chemicals and for elucidating the mechanisms of action. The systems include the use of cell or tissue cultures, isolated cells, tissue slices, subcellular fractions, transgenic cell cultures, and cells from transgenic organisms.

The systems also include *in silico* modeling. For example, *in vitro* methods have been and are being developed for the prediction of toxic effects based on the data from traditional toxicity studies combined with structure–activity comparisons and knowledge

Table 1 Types of in vitro models for toxicity testing and research

Level/examples	Advantages	Disadvantages
Lower organisms (earthworms, fish)	Range of integrated organismic responses	Frequently lack responses typical of higher organisms
Isolated organs	Intact yet isolated tissue and vascular system	Donor organism still required Time consuming and expensive
	Controlled environmental and exposure conditions	No intact organismic responses Limited duration of viability
Cultured cells (such as the hERG assay)	No intact animals directly involved Ability to carefully manipulate system Low cost	Instability of system Limited enzymatic capabilities and viability of system
	Ability to study a wide range of variables	No (or limited) integrated multicell and/or organismic responses
Chemical/biochemical systems	No donor organism problems Low cost Long-term stability of preparation Ability to study a wide range of variables Specificity of response	No <i>de facto</i> correlation to <i>in vivo</i> system Limited to investigation of a single defined mechanism
Computer simulations (also known as <i>in silico</i> modeling)	No animal welfare concerns Speed and low per-evaluation cost	May not have predictive value beyond a possibly narrow range of chemical structures Expensive to establish

of toxicologically important chemical structures. Additional *in vitro* test systems are being developed for use in high-throughput toxicology and pharmacology for the understanding of mechanisms of toxic action and for genomics, transcriptomics, and proteomics applications.

The most famous *in vitro* test system is the Ames mutagenicity assay and, indeed, most mutagenicity tests employ *in vitro* systems. *In vitro* systems are also widely employed for assessing pyrogenicity and cytotoxicity in the medical device industry. Another example is the *hERG* assay, a test required for new pharmaceuticals under ICH S7B to identify drugs with a potential to cause changes in heart electrical function. The term *hERG* is an acronym for human Ether-a- Go-Go Related Gene. This gene encodes the pore-forming unit of one of the cardiac membrane channel that conducts potassium current.

Various *in vitro* test systems are described in Table 1.

See also: Ames Test; Analytical Toxicology; hERG (Human Ether-a-Go-Go Related Gene); In Vivo Test;

Microtox; QT Interval; Toxicity Testing, Alternatives; Toxicity Testing, Irritation; Toxicity Testing, Modeling; Toxicity Testing, Mutagenicity; Toxicity Testing, Validation.

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In Vivo Test

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Toxicologists are taught to consider and use various methods, including tests on animals, as part of their effort to conduct (as the Society of Toxicology states) "toxicological research to ensure and enhance the quality of human and animal health and the environment." They are also taught about the effective and humane use of laboratory animals in research, and about the ongoing development of valid alternatives to animal testing. The types of animal models and the alternatives to animal testing approaches that are being used in toxicology are typically those that have been accepted by the scientific community and recognized by regulatory bodies.

The major benefit of using an *in vivo* test is that it provides an intact biological system including exposure route-specific absorption, distribution, metabolism, and excretion responses, as well as tissuespecific responses to the parent compound and any metabolite(s) that reach the various tissues. Many of the factors affecting these processes in chemical toxicity are not yet well defined, but an *in vivo* test system provides a tool for evaluating the chemical toxicity in an intact biological system without knowing every aspect of these processes. The tests on animals (also called *in vivo* studies) used by toxicologists are generally considered to be only those performed in intact higher organisms (most commonly, mammals). Eight different species are currently used with any frequency in toxicological research. These are, in approximate numbers of animals utilized (from most to least), rat, mouse, rabbit, guinea pig, hamster, dog, ferret, and monkey. The cat and the frog, while common biological models, have not been used in toxicity testing for some time.

In many cases as the toxicology profile of a test agent begins to be developed, humans are assumed for risk assessment purposes to be at least as sensitive to the effects as the most sensitive species used in evaluating the effects of the chemical or other material, and at least as sensitive as the most sensitive alternatives to animal testing approaches being used. Such assumptions could change as the toxicology profile is developed and understanding is gained about the metabolism, target organs, toxic responses, etc. The key rationale for using *in vivo* test systems can be summarized as follows:

- They provide evaluation of actions/effects on intact animal and organ/tissue interactions.
- Either neat (i.e., undiluted) chemicals or complete formulated products (complex mixtures) can be evaluated.
- Either concentrated or diluted products can be tested.
- They yield data on the recovery and healing processes.
- They are the required statutory tests for agencies under such laws as the (US) Federal Hazardous Substances Act (unless data are already available), (US) Toxic Substances Control Act, (US) Federal Insecticides, Fungicides, and Rodenticides Act, Organization for Economic Co-operation and Development, and the (US) Food and Drug Administration laws.
- Quantitative and qualitative tests with scoring system are generally capable of ranking materials as to relative hazards.
- They are amenable to modifications to meet the requirements of special situations (such as multiple dosing or exposure schedules).
- They have an extensive available database and cross-reference capability for evaluation of relevance to human situation.
- They involve the case of performance and relative low capital costs in many cases.
- Tests are generally both conservative and broad in scope, providing for maximum protection by erring on the side of overprediction of hazard to humans.
- Tests can be either single endpoint (such as lethality, corrosion, etc.) or multiple endpoint, including such test systems as a 13 week oral toxicity study.

Limitations of *in vivo* testing systems that serve as a basis for seeking *in vitro* alternatives for toxicity tests include the following:

- The complexity of the *in vivo* system and the types of toxic effects (e.g., an effect could be the result of quite different toxic mechanisms) can make it a challenge to difficult to understand what has happened and its relevance to human risk assessment. For example, there could be potential confounding or masking of the findings in an *in vivo* test system.
- *In vivo* systems might be intended to only assess the local effects at the site of application or the immediate structural alterations produced by an

agent (however, this may be a purposeful test system limitation).

- Toxicologist and technician training and monitoring are critical (particularly due to the subjective nature of evaluation).
- In vivo tests do not always predict results in humans if the objective is to exclude or identify agents creating a high degree of toxicity, for example, skin corrosion.
- Structural and biochemical differences between test animals and humans make extrapolation from one to the other difficult.
- Lack of standardization of some *in vivo* systems.
- Possible variability in correlating the results with those from human exposures.
- Possible considerable biological variability between individual animals.
- Depending on the animal models and toxicity endpoints of interest, the results might be in large, diverse, and fragmented databases that are not readily comparable.

Some test protocols and toxic endpoints are particularly liable to produce misleading or difficult to judge results. Several examples of possibly confusing results obtained in carcinogenesis bioassays are useful in discussing this point:

- Perhaps the first to be recognized in toxicology concerned the induction of sarcomas following the local injection of chemicals subcutaneously in rats. Although there can be little doubt that the injection of small quantities of chemicals such as 7,12-dimethylbenz(*a*)anthracene actually induces these tumors, overloading the tissues with dye-stuffs may well lead to cancer because of a mechanism dependent on factors other than the specific interactions of the test chemical.
- Bladder stone formation can lead to bladder cancer in rats and mice, thus making it difficult to be certain whether a chemical that leads to bladder stone formation and turnorigenesis is or is not a true animal and/or human carcinogen.
- Another example is renal toxicity resulting from the accumulation of a protein, α -2u-globulin, in male rat kidney proximal tubule lysosomes. This protein is synthesized exclusively by adult male rats, and the nephrotoxicity of agents such as D-limonene in male rats is attributed to its ability to bind to α -2u-globulin. Other species, including humans, synthesize proteins that share significant homology with α -2u-globulin; however, none of these proteins, including the mouse equivalent of α -2u-globulin, can produce this toxicity. The tumorigenic activity of D-limonene in male rats

has been concluded to be nonrelevant to humans because of (1) the male rat specificity of the nephrotoxicity and carcinogenicity, (2) the role that α -2u-globulin plays in the toxicity, as evidenced by the complete lack of toxicity in other species despite the presence of structurally similar proteins, and (3) the lack of genotoxicity of both D-limonene and D-limonene-1,2-oxide, supporting the concept of a nongenotoxic mechanism, that is, sustained renal cell proliferation. Both D-limonene and *cis*-D-limonene-1.2-oxide (the major metabolite involved in this toxicity) are negative in in vitro mutagenicity screens. Therefore, the toxicity-related renal cell proliferation is believed to be integrally involved in the carcinogenicity of D-limonene as persistent elevations in renal cell proliferation may increase fixation of spontaneously altered DNA, or serve to promote spontaneously initiated cells.

Perhaps the greatest cause of confusion in the interpretation of carcinogenicity bioassays occurs when a substantial background incidence of tumors is enhanced. It should be asked whether the test chemical is inducing such tumors or merely enhancing their incidence. Although this problem is clearly recognized with chemicals that enhance the already high incidence of pulmonary tumors in strain A mice, there has been little discussion of the confounding effects of naturally occurring tumors that demonstrate a lower but still appreciable incidence. The B6C3F1 male mouse used in the National Cancer Institute, National Toxicology Program (NCI/NTP) Bioassay Program in the United States demonstrates a 15-60% incidence of hepatic cell tumors by two years of age. However, whether this confounds the interpretation of a bioassay or whether enhancement of the yield of such tumors, as opposed to their direct induction, is relevant to the effects of the chemical in humans is not asked; instead, these chemicals are usually uncritically accepted as carcinogens and generally regulated as such.

There are many tumors that have a naturally high incidence, such as tumors of the endocrine tissues in certain strains of rats. In each case, it is necessary to consider the overall evidence that agents increasing the yield of such tumors may or may not induce cancer in humans. Such considerations require in-depth knowledge of biological and biochemical mechanisms of carcinogenesis and development of new and testable ideas. Increased emphasis on how agents exert their effect, rather than on which agents exert an effect, will move toxicology to the forefront of integrated biological science.

One further problem needs to be addressed regarding human and animal reactions to toxic agents. Although it is possible to control the exposure of a test animal quite precisely in a well-run experiment, humans are exposed to an ever-changing multitude of chemicals because of the food they eat, the drugs they take, or the lifestyles they have chosen. Therefore, single-substance toxicological tests may either overemphasize or underemphasize the significance of the potential hazard to humans, except possibly in the case of massive exposures. There is very limited laboratory evidence on the effects of chemical mixtures because a single chemical assay is so expensive that the assay of mixtures becomes prohibitively costly. However, the coadministration of a carcinogen and a promoting agent may lead to far more tumors, than either agent alone. By contrast, two carcinogens, such as 4-dimethylaminoazobenzene and 3-methylcholanthrene, may fail to produce tumors when given together, yet they do so when given separately. More information on chemical interactions continues to be needed, if animal tests to humans even with qualitative accuracy are extrapolated.

See also: Analytical Toxicology; Animal Models; Toxicity Testing, Alternatives; Toxicity Testing, Modeling; Toxicity Testing, Validation.

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Indole

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 120-72-9
- SYNONYMS: 2,3-Benzopyrole; 1-Benzazole
- CHEMICAL FORMULA: C₈H₇N
- CHEMICAL STRUCTURE:



Uses

Indole is used as a chemical intermediate, a perfume fixative, and as a synthetic flavor. In addition, it is possibly a kairomone (a volatile chemical released by a plant to attract phytophagus insects). Indole is also a component of tobacco smoke, and occurs naturally in coal tar, jasmine oil, and orange-blossom oil. It is also a bacterial decomposition product of tryptophan in the gut.

Background Information

The indole nucleus is found in a large number of naturally occurring compounds; the compound constitutes $\sim 2.5\%$ of jasmine oil and 0.1% of orangeblossom oil; in both cases, it contributes to their fragrances. Although synthetic methods have been described for the manufacture of indole, extraction from the 240–260°C coal-tar distillate fraction is the only commercial source. Interesting, given its use in fragrances, is that indole has an intense fecal odor, presumably at higher concentrations.

Exposure Routes and Pathways

The general population may be exposed via inhalation of ambient air or tobacco smoke, ingestion of food, and dermal contact with vapors, food, perfumes, and other products containing indole. Occupational exposure may occur through inhalation or dermal contact at workplaces where indole is produced or used. Indole is one of the odorous components found in sewage and animal wastes, including human feces, and occurs in animal tissues where putrefactive processes have occurred, presumably by the decomposition of tryptophan.

Toxicokinetics

Indole absorbed from the gut is hydroxylated to form indoxyl, which conjugates with sulfate to produce indican (indoxylsulfuric acid) in the liver. Indoxyl and indican are found in human plasma and urine. The daily urinary excretion of indoxylsulfate in normal adults was reported to average 200 mg (range 140–250 mg). Indole was not detected in the blood of rabbits exposed at 10 mg m^{-3} for 3 h.

Mechanism of Toxicity

Indole causes oxidative damage to membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat oral LD_{50} is 1 g kg^{-1} ; rabbit dermal LD_{50} is 790 mg kg⁻¹; mouse oral LD_{50} is 1070 mg kg⁻¹; mouse intraperitoneal LD_{50} is 117 mg kg⁻¹. Indole is a severe (primary) eye and skin irritant.

Human

Indole at a few parts per million has an unpleasant odor and can elicit toxic symptoms, such as nausea. The consistent toxicological property of indole, an aromatic amine, observed in animal studies is its ability to cause the formation of Heinz bodies, which are known to be produced by other aromatic amine compounds, such as aniline.

Chronic Toxicity (or Exposure)

Animal

Rhesus monkeys, rats, and mice were exposed continuously to indole at a concentration of 10.5 ppm (50 mg m^{-3}) for 90 days. Hematological examination of the exposed rodents revealed that numerous Heinz bodies were present in the blood. Pathological studies on some of the exposed mice revealed that 95% of the animals had pigment in the renal tubular cells. However, renal abnormalities were not found in any exposed monkeys or rats that were examined. Pathological examination showed no brain damage in the monkeys, and histopathological studies of the heart, lung, liver, and kidney from the exposed monkeys

revealed no statistical difference from that of control monkeys. In another study, indole at 1.6% in the diet did not induce bladder or liver cancer in hamsters; however, no other organs were examined. Chronic studies by the subcutaneous route have shown that indole might have a weak leukemogenic activity in mice, but not in hamsters. Indole has enhanced the incidence of bladder cancer in some bioassays, for example, addition of indole to a diet containing 2-acetylaminofluorene produced an increased incidence of bladder cancer in hamsters. Other indole compounds have been studied for their carcinogenic and anticarcinogenic effects, for example, indole-3carbinol, a minor cruciferous vegetable component, inhibited dimethylbenzanthracene (DMBA)-induced mammary tumors in rats.

Human

Indole is a possible carcinogen.

Clinical Management

On exposure, skin should be washed with soap and copious amounts of water.

Environmental Fate

Indole will mainly exist in the vapor phase and is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals, nitrate radicals, and ozone with estimated half-lives of $\sim 2-3$ h, < 1 min, and 6 h, respectively. Indole is expected to readily biodegrade under both aerobic and anaerobic conditions in soil and water. Bioconcentration in aquatic organisms should be low, given an estimated bioconcentration factor value of 25.

Ecotoxicology

Indole is moderately toxic to zooplankton.

Exposure Standards and Guidelines

Indole was granted GRAS (generally recognized as safe) status by the Flavor and Extract Manufacturer's Association (FEMA) in 1965 (FEMA GRAS No. 2593). It was approved by the Council of Europe in 1970 to be included in the list of admissible artificial flavoring materials. For indole, there is no American Conference of Governmental Industrial Hygienists threshold limit value and no US Occupational Safety and Health Administration permissible exposure limit for indole.

See also: Carcinogenesis; Generally Recognized as Safe (GRAS); Phenol.

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Indoor Air Pollution See Pollution, Air Indoor.

Industrial Hygiene

Andrew Maier

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Industrial hygiene is a professional field with responsibility related to protecting the health of workers, and protecting communities that may be impacted by work-related exposures. Historically, industrial hygiene has been described as the anticipation, recognition, evaluation, and control of workplace health hazards. Industrial hygiene has a broad scope, with practitioners often having responsibility for the prevention of work-related health risks from chemical as well as diverse physical agent exposures (noise, temperature extremes, ergonomic considerations, nonionizing radiation, etc.).

The American Industrial Hygiene Association (AIHA) has developed informational brochures to educate the public on common roles and responsibilities of a professional industrial hygienist. Typical roles as presented by the AIHA include (with the addition of specific examples of such activities):

- Investigating and examining the workplace for hazards and potential dangers such as conducting exposure assessments for a chemical or physical agent.
- Making recommendations on improving the safety of workers and the surrounding community – such as developing ventilation system requirements, emission abatement strategies, and developing protective equipment guidelines.
- Conducting scientific research to provide data on possible harmful conditions in the workplace such as developing new sampling techniques or data evaluation tools.
- Developing techniques to anticipate and control potentially dangerous situations in the workplace and the community – such as development of preventive assessment strategies for newly introduced chemicals or processes or participating in community emergency response planning committees.
- Training and educating the community about jobrelated risks.
- Advising government officials and participating in the development of regulations to ensure the health and safety of workers and their families.
- Ensuring that workers are properly following health and safety procedures.

Industrial hygienists typically have formal training in disciplines related to engineering, chemistry, physics, biology, or a related physical science from an accredited college or university. Certification is viewed as an important milestone for ensuring the continuing professionalism of the occupation. Through the American Board of Industrial Hygiene a practicing industrial hygienist can become a Diplomate of the American Academy of Industrial Hygiene, which entitles him/her to the designation of Certified Industrial Hygienist. Similar certifications are used to ensure a professional level of practice in other countries. For example, a professional designation as Registered Occupational Hygienist is available to qualified professionals in Canada.

Some Organizations Relevant to Industrial Hygiene

There are a number of professional and regulatory organizations that provide resources on occupational health topics. Key organizations include the American Conference of Governmental Industrial Hygienists, American Industrial Hygiene Association, Canadian Centre for Occupational Safety and Health, the European Union's European Agency for Safety and Health at Work, International Labor Organization, (US) National Institute for Occupational Safety and Health, and the (US) Occupational Safety and Health Administration.

See also: American Conference of Governmental Industrial Hygienists; American Industrial Hygiene Association; Exposure Criteria; International Labour Organization (ILO); National Institute for Occupational Safety and Health; Occupational Toxicology; Occupational Exposure Limits.

Further Reading

- DiNardi SR (ed.) (2003) The Occupational Environment: Its Evaluation and Control. Fairfax, VA: AIHA.
- Harris RL (ed.) (2000) *Patty's Industrial Hygiene*, 5th edn. New York: Wiley.

Relevant Websites

- http://www.acgih.org American Conference of Governmental Industrial Hygienists.
- http://www.aiha.org American Industrial Hygiene Association.
- http://www.ccohs.ca Canadian Centre for Occupational Safety and Health.
- http://europe.osha.eu.int European Union, European Agency for Safety and Health at Work.
- http://www.ilo.org International Labor Organization.
- http://www.cdc.gov (US) National Institute for Occupational Safety and Health.
- http://www.osha.gov (US) Occupational Safety and Health Administration.

Inert Ingredients (in Pesticides)*

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An inert ingredient is a chemical that has been intentionally added to a pesticidal product for reasons other than directly affecting the target pest. Inert ingredients can be contrasted with active ingredients, that is, those that have pesticidal activity. The active and inert ingredients are combined to formulate the final product. Inert ingredients are included in formulations for many reasons. These are generally related to enhancing product performance by, for example, making it easier to apply, helping it to dissolve in water, assisting in pesticide dispersion/ adhesion, or stabilizing the product for longer shelf life.

Whether a chemical is defined as 'inert' or 'active' is a function of the product. The term inert in this context does not necessarily mean harmless, nontoxic, or not biologically active. Inert ingredients can range from extremely toxic to compounds of minimal concern. In recognition of this, the US Environmental Protection Agency (EPA) has requested registrants to voluntarily substitute the term 'other ingredients' in lieu of the term 'inert ingredients' to help dispel the impression that inert is equivalent to harmless.

For example, xylene is considered to be an inert ingredient in some formulations, when used as a solvent. Xylene is not harmless; exposure can result in severe health effects ranging from skin irritation to reproductive and nervous system toxicity. Another example is piperonyl butoxide, used in insecticidal formulations. Piperonyl butoxide has clear biological activity; it acts to inhibit metabolic enzymes. It does not, however, directly kill or immobilize insect pests. Piperonyl butoxide potentiates the active ingredient in insecticidal formulations by blocking detoxification pathways in the insect.

The US law (Federal Insecticide, Fungicide, and Rodenticide Act) requires that product labels for pesticides must list active ingredients and their percentages by weight. Similar labeling is not required for inert ingredients except for those defined as being of toxicological concern (see below). In 1987, the US EPA instituted a policy (52 FR 13305, Inert Ingredients in Pesticide Products) to reduce the potential for adverse effects from pesticide products containing toxic inert ingredients. This policy established data requirements for new inert ingredients and categorized inert ingredients into four lists (Table 1).

- List 1: Inert ingredients of toxicological concern. The criteria for placing a chemical on List 1 include carcinogenicity, adverse reproductive effects, neurotoxicity, or other chronic effects, or developmental toxicity (birth defects). These effects must be demonstrated in laboratory or human studies and the data subject to peer review. The criteria also include documented ecological effects and the potential for bioaccumulation. List 1 originally contained ~ 50 chemicals, but many of these were removed from the list because they were no longer used in pesticidal formulations. Future use of de-listed chemicals requires data submission to the US EPA and the guarantee that use of the inert ingredients will not pose an unreasonable risk to human health or the environment. There are 7 compounds currently on this list (Table 1). Any formulation containing any of these compounds must list them on the label.
- *List 2*: Potentially toxic inerts, with high priority for testing. These compounds may be structurally similar to chemicals known to be toxic

Table 1 Examples of inert ingredients and category

List 1: Inert ingredients of toxicological concern	Adipic acid, bis(2- ethylhexyl) ester Hydroquinone Nonylphenol Phthalic acid, bis(2- ethylhexyl) ester	Ethylene glycol monoethyl ether Isophorone Phenol
List 2: Potentially toxic inert ingredients/high priority for testing	1,1,1- Acetonitrile Fuel oil, no. 2 Paraffins Trichloroethane	Cresol Methyl isobutyl ketone Toluene Xylene
List 3: Inert ingredients of unknown toxicity	Acetone Camphor Piperonyl butoxide Tea tree oil	Boric acid Hydrogen peroxide Salicylic acid Zinc chloride
List 4: Inert ingredients of minimal concern	Beeswax Maltodextrin Wintergreen oil	Ethanol Nitric acid Sodium fluoride

^{*} This document has been reviewed in accordance with the US environmental Protection Agency policy and approved for publication. Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

and/or may have data suggesting a basis for concern.

- *List 3*: Inerts of unknown toxicity: Inert ingredients on this list have not yet been adequately evaluated. These substances will be assessed to determine if they merit reclassification to List 1, 2, or 4.
- *List 4*: Inerts of minimal concern, further broken down into List 4A: minimal risk inerts, including all commonly consumed foods; and List 4B: inerts which have sufficient data to substantiate that they can be used safely in pesticide products.

See also: Pesticides.

Relevant Websites

- www.epa.gov US Environmental Protection Agency, Inert Ingredients in Pesticide Products. This website gives links to regulations, to tables of inert ingredients categorized by list. Last updated January, 2004.
- www.oag.state.ny.us Office of the NY State Attorney General, 1996. The Secret Hazards of Pesticides. This report includes percentages of inert ingredients in some pesticide products, and identifies adverse health effects of some compounds listed as inert ingredients.

Information Resources in Toxicology

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Introduction

The availability of toxicological information dramatically increased during the twentieth century, spurred by an increased awareness of how chemicals and other environmental factors influence biological systems. While during the first half of the twentieth century toxicology was considered a subset of pharmacology, it became a focused discipline largely through public awareness of toxicological issues. Recognition of the need for protection from hazardous chemical exposure, and environmental impact, resulted in legislation designed to safeguard the consumer. This legislation mandated continuing research on the effects of chemicals on humans and the environment. In the past 30 years the major legislation in the United States dealing with controls on medicinals, environmental contaminants, and energy have all included provision for the collection and dissemination of data. Spurred on by these types of regulatory requirements and worldwide concern for the safety of the planet industry, research organizations, government agencies, academic centers, and international groups have all contributed to a huge body of toxicology information.

This entry provides a selection of printed and electronic information resources of use in identifying toxicological information. Technological advances and ways to communicate on a global scale have provided great opportunities for sharing knowledge. It is estimated that scientific information doubles every 4 years; in the vast toxicological arena, this figure is greatly accelerated. Today there are literally hundreds of public databases and databanks that could be consulted for toxicology-related information, thousands of journal articles published each year on the effects of xenobiotics on humans and the environment, and more than a score of organizations whose primary activities are the creation of banks of available files of data and the production of research reports in specialized areas of toxicology.

This huge amount of information, scattered throughout the literature of the scientific disciplines chemistry, biology, medicine - and present in many forms - raw data, technical reports, articles, monographs, statutes, and regulations - presents an enormous challenge to identify and retrieve relevant information. In the past decades technological developments have provided computerized systems capable of storing and providing access to information on a scale unimagined less than 30 years ago. Some systems provide access to data itself, and some are designed to provide information on sources of data. Some systems determine hazard assessment by applying sophisticated algorithms and use of mathematical modeling, and some systems are designed as interactive, multimedia instructional tools. The development of hypertext and other sophisticated software systems for navigating databases, electronic superhighways such as the Internet linking investigators to potentially valuable electronic repositories of nformation, the creation of local storage and retrieval systems such as those based on CD and DVD technology, and the developments in electronic communication systems provide opportunities, and frustrations, for the investigator.

Although we have access to an enormous amount of information, the quality and reproducibility of data varies considerably. Governmental and regulatory influences have not provided any guarantees as to the accuracy of data related to xenobiotics and the researcher needs to understand the purpose and mandates behind an information resource to fully evaluate the data contained within. For example, the National Institute for Occupational Safety and Health (NIOSH) Registry of Toxic Effects of Chemical Substances (RTECS) criteria for selection of data is not based on the reproducibility of that data. Other sources, such as the National Library of Medicine's Hazardous Substances Databank (HSDB), present information compiled from a wide variety of source materials that are extensively reviewed by experts.

The professional is usually aware of the major texts, journals, organizations, and other standard resources in their own field. However, because of the interdisciplinary nature of toxicology, and the need to access information that may reside in the resources of other scientific areas of study, the user is frequently at a loss. Some types of sources are difficult to find. The so-called 'gray literature' - for example, manufacturer's brochures, internal agency/company reports, conference proceedings, or translations, all excellent sources of potentially useful information can be extremely difficult to identify. Other obstacles include those related to technology. Hardware limitations, varying search software and operating systems, and telecommunications problems can limit the investigator's ability to identify needed resources. There are also the common problems of interpretation of data-language barriers or varying scientific research conventions.

Valuable assistance in finding information can be obtained by consulting information intermediaries with subject expertise who can identify and access information resources unknown or unavailable locally. These individuals may be in local information centers or may be brokers who specialize in individual information services or creation of compilations of data for a fee. An example of this type of service is the Comprehensive Health and Environmental Monographs (CHEMS) division of Health and Environment International. This service creates detailed reports on the health and environmental effects of a chemical. Professional organizations, database producers, and government agencies can also be sources of experts as well as providers of direct information. An example of the former type of service is UNEP-Infoterra, an international referral and research organization of the United Nations Environmental Program (UNEP). UNEP-Infoterra provides access to an extensive range of information sources, as well as expert consultants, worldwide. The US Environmental Protection Agency (EPA) is the National Focal Point for this organization in the United States, one of over 175 sites around the globe.

Keeping Current

Columns and review sections in journals or newsletters can help the professional keep abreast of new resources or provide comparative information on established resources in selected areas. Collections of reviews are also available online in such sources as Comprehensive Core Medical Library (CCML) by Ovid Technologies, Inc., Book Review Index (online and in print) by Wilson, and the National Library of Medicine's TOXLINE database. Reviews of popular works dealing with consumer health or environmental concerns can also be found in newspapers, popular magazines, and consumer organization newsletters. Summaries and reviews of online databases and databanks can be found in the information science journals, directories of online resources, and producer/vendor documentation.

In addition to journals, texts, and newsletters, resource information can be identified by examining the program reports of government agencies, scientific organizations, or research institutions. Names of experts, identification of programs of interest, and organs for dissemination of information can all be identified in this way. An example of this type of resource is Access EPA, which was published by the Information Access Branch, Information Management and Services, Division, US Environmental Protection Agency (US Government Printing Office, Washington, DC) (ISBN 0-160483301). While no longer in print, the information content of this outstanding pathfinder to the resources of the US EPA can be found in more comprehensive form in the EPA's 'Information Sources' website, http://www. epa.gov/epahome/resource.htm, which provides ready access to the EPA clearinghouses, special information dockets, hotlines, records, databases, newsletters, email discussion lists, libraries, publications, directories, and other information resources supported by the US EPA.

Guides to the Literature

There are few guides to the literature of toxicology, but many excellent guides exist in narrower areas. The following is a selection of available tools published within the past several years: Snow B (1999) Drug Information: A Guide to Current Resources, 2nd edn., Lanham, MD: Scarecrow Press (ISBN 0-810833204).

Designed as a self-study guide as well as a reference tool, this volume is considered the best work in the field. Two chapters of particular interest in this context deal with side effects of therapeutic agents, including adverse reactions, poisonings, and drug interactions.

The Toxics Directory (2000). California Environmental Protection Agency, Environmental Health Hazard Assessment, Pesticide and Environmental Toxicology Section, Oakland, CA.

This directory lists agencies and organizations that provide information about or have authority over toxic substances and their health effects. The organizations range from local to national, with emphasis on California-based resources.

Webster JK (1987) *Toxic and Hazardous Materials:* A Sourcebook and Guide to Information Sources. Westport, CT: Greenwood (ISBN 0-313245754).

Although dated, this book still lists useful resources published prior to 1987. It is organized into separately authored chapters, by subject (e.g., radioactive materials, laws and regulations, and transportation), and lists more than 1600 sources (e.g., literature, organizations, audiovisuals, databases, agencies, research centers, and libraries).

Wexler P (2000) Information Resources in Toxicology, 3rd edn., San Diego, CA: Academic Press (ISBN 0-127447709).

This is probably the best, most current literature guide available today. Separate sections deal with the US and foreign information resources. Printed and online sources, professional organizations, government agencies, regulations, education centers, and testing laboratories are covered for the United States and for selected countries. A history of toxicology and toxicology information systems developments are also included.

Wexler P (ed.) (2001) Digital Information and Tools. Part I. *Toxicology* 157(1–2).

Wexler P (ed.) (2002) Digital Information and Tools. Part II. Web Resources in Special Toxicology Topics. *Toxicology* 173(1–2).

Wexler P (ed.) (2003) Digital Information and Tools. Part III. Global Web Resources. *Toxicology* 190(1–2).

These three special issues of the journal *Toxicology* provide a comprehensive overview of Web-based toxicology resources that are available to the public. Part I focuses on government resources, including those of the US EPA and various other federal agencies, subnational toxicology information resources (state, territorial, tribal, municipal, and community),

resources available from professional toxicology societies and citizen groups, and information on tools available for searching the Web. Part II focuses on information available in specialized areas of toxicology, including alternatives to animal testing, cancer information, food, drug, and pesticide toxicology, developmental toxicity, environmental toxicology, forensic, genetic and veterinary toxicology, and others. Part III looks at information sources provided by agencies from other countries, most notably Canada, Finland, Germany, Italy, Russia, Sweden, and the United Kingdom. All issues are extremely helpful guides to finding toxicology information on the Web.

Texts

This section provides a select list of classic or standard texts, dictionaries, thesauri, glossaries, directories, handbooks, encyclopedias, databooks, and some new works that provide useful toxicology information. Online versions of some of these works are now being offered via site licensing agreements. The primary focus is on works that have been published or revised within the last several years. For information on texts published prior to 1995, consult Wexler's *Information Resources in Toxicology* (listed in the previous section).

Klaassen CD (ed.) (2001) Casarett & Doull's Toxicology: The Basic Science of Poisons, 6th edn., New York: McGraw-Hill (ISBN 0-071347216).

A classic, well-documented and detailed text on the general principles, toxic responses by body system, toxic effects of major toxicant classes, and major discipline applications of toxicology. This latest edition features an expanded coverage of risk assessment, contains sections on molecular biology and pharmacogenomics, and includes online references in addition to traditional print journal and review articles.

Ballantyne B, Marrs TC, and Syversen T (eds.) (2000) *General & Applied Toxicology*, 2nd edn., London: Macmillan (ISBN 0-333698681).

This encyclopedic, three-volume set provides an excellent in-depth review of the science of toxicology, its specializations, and practice. This latest edition has been extensively revised and contains 35 new chapters.

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn., nine vols. New York: Wiley (set, ISBN 0-471319430; Vol. I, ISBN 0-471319325; Vol. II, ISBN 0-471319333; Vol. III, ISBN 0-471319341; Vol. IV, ISBN 0-47131935X; Vol. V, ISBN 0-471319368; Vol. VI, ISBN 0-471319392; Vol. VII, ISBN 0-471319406; Vol. VIII, ISBN 0-471319414; Vol. IX, ISBN 0-471319422, cumulative index).

The latest edition of the classic giant compendium, *Patty's Industrial Hygiene and Toxicology*, has now been divided into separate four- and nine-volume sets that cover industrial hygiene and toxicology, respectively. It is one of the most complete in the area of occupational, industrial and general toxicological information. Online versions of both sets are available through licensing agreements with the publisher.

Burczynski ME (ed.) (2003) An Introduction to Toxicogenomics. Boca Raton, FL: CRC Press (ISBN 0-849313341).

This text is an excellent primary source of information for students, scientists, and clinicians interested in the new discipline of toxicogenomics. In six sections, this text covers the fundamentals of gene expression profiling analysis, the use of expression profiling in toxicological testing and research, model systems used in toxicogenomic studies, and the areas of mechanistic and predictive toxicogenomics. The last section addresses the future of toxicogenomics, including its use in risk assessment studies and the complex ethical issues that surround the use of toxicogenomics data.

Derelanko MJ and Hollinger MA (eds.) (2002) Handbook of Toxicology, 2nd edn., Boca Raton, FL: CRC Press (ISBN 0-849303702).

This is an excellent compendium of practical reference information for the toxicologist. The focus is on providing normal values, reproductive indices, physiological parameters, regulatory requirements, procedures, values, endpoints, recommended sources, animal care, and tables/graphs of use to the practicing toxicologist. Information is presented in a loose chapter arrangement with detailed tables of contents for each.

D'Mello JPF (ed.) (2003) Food Safety: Contaminants and Toxins. Cambridge, MA: CABI Publishing (ISBN 0-851996078).

This new text addresses the major toxins and contaminants found in plant and animal products that constitute the staple diets of the world. In four parts: part 1 addresses plant and microbial toxins; part 2 deals with contaminants arising from anthropogenic sources; part 3 addresses contemporary issues, including prion diseases, genetically modified foods, and radionuclides in foods; and part 4 addresses ongoing concerns related to information from the earlier sections.

Dreisbach RH and True B (2002) Handbook of Poisoning: Prevention, Diagnosis, and Treatment, 13th edn., Boca Raton, FL: Parthenon Publishing Group (ISBN 1-850700389).

This handbook for the clinical toxicologist is a standard in emergency situations, covering all types of poison situations – agricultural, medical, industrial, and household. The work is organized to facilitate ease of use in emergency situations and as a reference source. Background chapters are on prevention, identification/diagnosis, management, and legal/medical responsibilities.

Duffus JH and Worth HG (eds.) (1996) Fundamental Toxicology for Chemists. Cambridge, UK: Royal Society of Chemistry.

This text provides a unique perspective of toxicology specifically for chemists. It addresses general principles as well as emerging issues in reproductive toxicology, ecotoxicology, and behavioral toxicology.

Ellenhorn MJ and Barceloux DG (1997) Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning, 2nd edn., Baltimore, MD: Williams and Wilkins (ISBN 0-683300318).

This text provides an excellent reference for clinicians in emergency and occupational health settings. Medical toxicology is defined here as poisoning by overdose of medication and exposure to chemicals and toxins not ordinarily used therapeutically. This is a good companion work to Dreisbach. Also available on CD-ROM and via online subscription.

Genium's Handbook of Safety, Health, and Environmental Data for Common Hazardous Substances. Genium Publishing Corporation (1999). Schenectady, NY: McGraw-Hill (ISBN 0-078531152).

This handbook provides chemical profiles on over 4500 materials. Each profile contains comprehensive listings of physical data, health and toxicity effects, disposal, and regulatory information. Also available on CD-ROM.

Gilman AG, Goodman LS, Hardman JG, and Limbird LE (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edn., New York: McGraw-Hill (ISBN 0-071354697).

This gigantic textbook is the standard in clinical pharmacology and contains an enormous amount of information of use to the clinician and researcher. It is strong in drug interactions and mechanism of action. It is updated every 5 years. Available on CD-ROM.

Goldfrank LR, Flomenbaum NE, Lewin NA, *et al.* (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn., New York: McGraw-Hill (ISBN 0-071360018).

This is a unique text with strength as both a training manual and in clinical management settings (second in popularity to Ellenhorn and Barceloux).

Haddad LM, Winchester JF, and Shannon MW (eds.) (1998) Clinical Management of Poisoning and

Drug Overdose, 3rd edn., Philadelphia, PA: Saunders (ISBN 0-721664091).

This book provides in-depth information on the clinical management of chemical poisoning and drug overdose. Chapters provide background, pharmacology, pathophysiology, diagnosis, and clinical management information for each compound listed.

Hamilton A, Hardy HL, and Harbison RD (1998) Hamilton and Hardy's Industrial Toxicology, 5th edn., St. Louis, MO: Mosby (ISBN 0-815141815).

This classic provides historical and current reviews on the toxic effects of industrial chemicals, including metalloids, chemical compounds, organic polymers, pesticides, physical agents, dusts, and special topics in industrial toxicology.

Hayes AW (ed.) (2001) *Principles and Methods of Toxicology*, 4th edn., Philadelphia, PA: Taylor and Francis (ISBN 1-560328142).

A textbook and standard reference designed to provide a thorough introduction to toxicology in the broadest sense. Strengths include coverage of methods, techniques, procedures, interpretation of data, and examination of controversial areas.

Herbicide Handbook, 8th edn. (2002) Champaign, IL: Weed Science Society of America.

This work, sponsored by the Weed Science Society of America, contains a wealth of physical, chemical, and toxicological data on more than 150 herbicides. Extensive data are presented on each chemical. Included are producer information and references (\$65.00; Weed Science Society of America, PO Box 7050, Lawrence, KS 66044-8897).

Hodgson E and Smart RC (eds.) (2001) *Introduction to Biochemical Toxicology*, 3rd edn., New York: Wiley (ISBN 0-471333344).

Designed as an advanced toxicology textbook and general reference source. Contains well-organized chapters on mechanisms of action, organ systems, interactions, and specific pathways.

Hodgson E, Mailman RB, and Chambers JE (1998) *Dictionary of Toxicology*, 2nd edn., London: Macmillan Reference (ISBN 0333547004).

A condensed and informative guide to the fundamentals and applications of toxicology, this text is suitable for both the novice as well as the expert scientist. The 2nd edition contains ~ 4000 concise, informative entries on a variety of terms and chemicals in toxicology. Coverage includes biochemical and mechanistic toxicology, environmental and regulatory toxicology, chemical carcinogenesis, risk assessment and risk management, analytical chemistry, and molecular biology. Chemical entries include chemical structures and CAS numbers.

Koren G (ed.) (2001) Maternal – Fetal Toxicology: A Clinician's Guide, 3rd edn., New York: Dekker (ISBN 0-824703782) (ISBN 0-585404283 electronic book).

This text contains a series of reviews on the toxic effects of drugs and other compounds, viruses, radiation, and occupational hazards. A chapter on drugs of choice in pregnancy and a section on diagnosis of fetal malformations are included. An online version is available.

Krieger RI (ed.) (2001) Handbook of Pesticide Toxicology, 2nd edn., San Diego, CA: Academic Press (Set, ISBN 0-124262600; Vol. I, ISBN 0-124262619; Vol. II, ISBN 0-124262627).

In two volumes, this comprehensive and timely compendium of scientific knowledge covers the toxic effects or pesticides in humans and animals. The 1st edition, edited by Hayes WJ and Laws ER, consists of three volumes that were updated and expanded versions of *Toxicology of Pesticides* (1975) and *Pesticides Studied in Man* (1982), both of which are out of print. Includes information on the diagnosis and treatment of pesticide poisonings. A true classic.

Lewis RJ, Sr. (ed.) (2000) Sax's Dangerous Properties of Industrial Materials, 10th edn. (three vols. New York: Wiley (ISBN 0-471354074).

This huge compendium provides properties, toxicity data, and regulatory status (United States) for over 20000 chemical substances. Extensively indexed by CAS registry number and synonyms. Online and CD-ROM versions are available. Lewis has published a more moderately sized book that contains over 5000 selected entries from this text: Lewis RJ Sr. (2002) *Hazardous Chemicals Desk Reference*, 5th edn. (ISBN 0-471441651).

Lewis RA (1998) *Lewis' Dictionary of Toxicology*. Boca Raton, FL: Lewis (ISBN 1-566702232).

This is an extensively cross-referenced text that covers a broad array of terms commonly used in toxicology and related fields.

Mackay D, Wan YS, and Kuo CM Illustrated Handbook of Physical – Chemical Properties and Environmental Fate for Organic Chemicals. Boca Raton, FL: CRC Press.

Volume I: Monoaromatics, Chlorobenzenes and PCBs (1991) (ISBN 0-873715136).

Volume II: Polycyclic Aromatics, Dioxins, Dibenzofurans and Phenols (1992). (ISBN 0-837315837).

Volume III: Volatile Organic Chemicals (1993). (ISBN 0-873719735).

Volume IV: Oxygen, Nitrogen and Sulfur Containing Compounds (1995) (ISBN 0-1566700353).

Volume V: Pesticide Chemicals (1997) (ISBN 1-566702550).

This collection of volumes provides physical – chemical data on compounds likely to impact the

environment. The emphasis is on structure – activity relationships and prediction of environmental chemodynamics. Calculations are included and explained.

Massaro EJ (ed.) (1997) Handbook of Human Toxicology. Boca Raton: FL: CRC Press (ISBN 0-84934493X).

This is a highly detailed compendium of information on a few selected topics in human toxicology: metals toxicology; nutrition and toxicology, inhalation toxicology; immunotoxicology; and reproductive and developmental toxicology. Each section includes state-of-the-art methodology, topics of current interest, difficult to locate data, and complete references. An annual serial version, titled *Human Toxicology Handbook*, is available on CD-ROM through CRC Press.

O'Neil MJ et al. (eds.) (2001) Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 11th edn., Rahway, NJ: Merck & Co. (ISBN 0-911910131).

A compendium of quick information that contains a diverse collection of more than 10 000 monographs organized alphabetically. Approximately 4000 monographs cover pharmaceuticals and drugs, 2000 cover naturally occurring substances and plants, 1000 focus on elements and inorganic chemicals, and almost 1000 pertain to compounds of agricultural significance. Includes nomenclature, physical/chemical properties, patents, uses, literature references, structure, toxicity, and related data. Available online on several public vendor systems and on CD-ROM.

Proctor NH and Hughes JP (1996) In: Hathaway GJ (ed.) *Proctor and Hughes' Chemical Hazards of the Workplace*, 4th edn., New York: Van Nostrand Reinhold (ISBN 0-442020503).

This book contains concise summaries of over 600 of the most common substances found in the industrial setting. Includes CAS numbers, chemical formulas, synonyms, physical properties, exposure sources and routes, and signs and symptoms of acute and chronic exposures.

Rom WN (ed.) (1998) *Environmental and Occupational Medicine*, 3rd edn., Philadelphia, PA: Lippincott-Raven (ISBN 0-316755788).

This text covers basic information on occupational medicine and related areas. This text is suitable for occupational health practitioners and is readable enough for practitioners in other medical disciplines.

Rossoff IS (2002) Encyclopedia of Clinical Toxicology: A Comprehensive Guide and Reference. Boca Raton, FL: Parthenon Publishing Group (ISBN 1-842141015).

An excellent and comprehensive guide, this text provides concise information on the clinical toxicology of prescription and over-ther-counter (OTC) drugs, chemicals, herbals, plants, fungi, marine life, reptiles and insect venoms, food ingredients, clothing, and environmental toxins. Organized alphabetically, this book features an extensive appendix that cross-references each compound for alternative nomenclature.

Schardein JL (2000) Chemically Induced Birth Defects, Drug and Chemical Toxicology Series, 3rd edn., revised and expanded. New York: Dekker (ISBN 0-824702654) (ISBN 0-585383693 electronic book).

This current and readable text is a comprehensive literature review that addresses mammalian (including human) exposure during organogenesis. Also available as an electronic book online.

Shepard TH (2001) Catalog of Teratogenic Agents, 10th edn., Baltimore, MD: Johns Hopkins University Press (ISBN 1-801867223).

A standard text listing the teratogenic potential of hundreds of substances; provides indexes, summaries of literature, and references to source materials.

Sipes IG, McQueen CA, and Gandolfi AJ (eds.) (1997–2002) *Comprehensive Toxicology*, 14 vols. New York: Pergamon (ISBN 0-080423019 set) (Vol. 1, ISBN 0-080429661; Vol. 2, ISBN 0-08042967X; Vol. 3, ISBN 0-080429688; Vol. 4, ISBN 0-080429696; Vol. 5, ISBN 0-08042970X; Vol. 6, ISBN 0-080429718; Vol. 7, ISBN 0-080429726; Vol. 8, ISBN 0-080429734; Vol. 9, ISBN 0-080429742; Vol. 10, ISBN 0-080429750; Vol. 11, ISBN 0-080429769; Vol. 12, ISBN 0-080429777; Vol. 13, ISBN 0-080429785; Vol. 14, ISBN 0-444508686).

This encyclopedic work of 14 volumes addresses general toxicology principles, toxicological testing and evaluation, biotransformation, target organ toxicology, as well as behavioral toxicology, chemical carcinogens, and anticarcinogens. Also available on CD-ROM.

Sittig M and Pohanish RP (2002) Sittig's Handbook of Toxic & Hazardous Chemicals and Carcinogens, 4th edn., Norwich, NY: Noyes Publications (ISBN 0-81551459X) (ISBN 1-591243262 electronic book).

This easy to use two-volume handbook presents chemical safety and health information on nearly 1500 toxic and hazardous chemicals. The utility of the 4th edition has been enhanced by addition of eight appendices: five cross index chemicals by CAS number, molecular formula, synonyms and trade names, DOT ID, and RTECS; one is a glossary of terms used; one lists oxidant chemicals; and one contains a list of confirmed and suspected carcinogens. References are included. Also available online.

Spencer PS and Schaumburg HH (eds.) (2000) Experimental and Clinical Neurotoxicology, 2nd edn., New York: Oxford University Press (ISBN 0-195084772).

This comprehensive and useful text provides a broad overview of the neurobiological basis of neurotoxic phenomena in human and veterinary medicine, as well as an alphabetical compendium that describes the neurotoxic properties of over 450 chemicals, drugs, and mixtures, including plants and venoms. It replaces the 1980 edition (available online at http://www.ohsu.edu/research/croet/faculty/spencer/book/first_ed.html), which featured micrographs depicting the neuropathology of neurotoxic agents.

Target Organ Toxicology Series. New York/ Washington, DC: Raven Press/Taylor and Francis.

Wallace KB (ed.) (1997) Free Radical Toxicology. Dixon RL (ed.) (1995) Reproductive Toxicology, 2nd edn.

Dean JH et al. (eds.) (1994) Immunotoxicology and Immunopharmacology, 2nd edn.

Kimmel CA and Buelke-Sam J (eds.) (1994) Developmental Toxicology, 2nd edn.

Kotsonis FN, Mackey M, and Hjelle JJ (eds.) (1994) Nutritional Toxicology.

Plaa GL and Hewitt WR (eds.) (1994) Toxicology of the Liver, 2nd edn.

Waalkes MP and Ward JM (eds.) (1994) Carcinogenesis.

Gardner DE, Crapo JD, and McClellan RO (eds.) (1993) *Toxicology of the Lung*, 2nd edn.

Hook JB and Goldstein RS (eds.) (1993). Toxicology of the Kidney, 2nd edn.

Acosta D, Jr. (ed.) (1992) Cardiovascular Toxicology, 2nd edn.

Chiou GCY (ed.) (1992) *Ophthalmic Toxicology*. Tilson HA and Mitchell CL (eds.) (1992) *Neurotoxicology*.

Hayes AW (ed.) (1985) Toxicology of the Eye, Ear, and Other Special Senses.

Irons RD (ed.) (1985) *Toxicology of the Blood and Bone Marrow*.

Thomas JA, Korach KS, and McLachlan JA (eds.) (1985) *Endocrine Toxicology*.

Drill VA and Lazar P (eds.) (1984) Cutaneous Toxicity.

Schiller CM (ed.) (1984) Intestinal Toxicology.

This excellent, though expensive, series of works focuses on target organ toxicity and disease states. Some of the earlier volumes are out of print.

2004 TLVs and BEIs (2004) American Conference of Governmental Industrial Hygienists (ACGIH), Cincinnati, OH (ISBN 1-882417542).

This pocket-sized handbook provides threshold limit values (TLVs) established by the ACGIH as guidelines for good practices. Main sections provide data for chemical and physical agents. Also includes sections that explain background and tests, biological exposure indices, and the role of the organization. A companion work titled *Documentation of the Threshold Limit Values and Biological Exposure Indices* (available in paper and on CD-ROM) provides the data and references used in developing the TLVs and BEIs.

Tomlin C (2000) *The Pesticide Manual: A World Compendium*, 12th edn., Farnham, UK: British Crop Protection Council (ISBN 1-901396126).

Provides chemical, physical, analytical, use, and toxicity data for nearly 1200 pesticides, herbicides, and other agricultural chemicals. Contains *The Agrochemicals Handbook* from the Royal Society of Chemistry. Environmental fate/transport, resistance information, and lists of manufacturers are also included. A companion tool from the Royal Society of Chemistry is the 3rd edition of the *World Directory of Pesticide Control Organizations* (ISBN 0-85404437X), which gives sources of contacts in over 160 organizations worldwide involved in the control of pesticides.

Tu AT Handbook of Natural Toxins. New York: Dekker.

Volume I: Keeler RF and Tu AT (eds.) (1983) *Plant and Fungal Toxins* (ISBN 0-824718933).

Volume II: Tu AT (ed.) (1984) Insect Poisons, Allergens, and Other Invertebrate Venoms (ISBN 0-824772075).

Volume III: Tu AT (ed.) (1988) Marine Toxins and Venoms (ISBN 0-824776674).

Volume IV: Hardegree MC and Tu AT (eds.) (1988) *Bacterial Toxins* (ISBN 0-824778405).

Volume V: Tu AT (ed.) (1991) *Reptile Venoms and Toxins* (ISBN 0-82478376X); electronic web version available (ISBN: 0585357161).

Volume VI: Keeler RF and Tu AT (eds.) (1991) *Toxicology of Plant and Fungal Compounds* (ISBN 0-824783751); electronic web version available (ISBN: 0585360588).

Volume VII: Tu AT (ed.) (1992) Food Poisoning (ISBN 0-824786521).

Volume VIII: Moss J (ed.) (1995) *Bacterial Toxins* and Virulence Factors in Disease (ISBN 0-824793811); electronic web version available (ISBN: 0585345031).

This series of publications describes all aspects of toxins and the consequences of exposure to them. It includes descriptions and categorization of toxins, symptomology with exposure, treatment, and prevention of contact. All volumes in this series are still in print or available as electronic versions on the Web.

Verschueren K (2001) Handbook of Environmental Data on Organic Chemicals, 4th edn., New York: Wiley (ISBN 0-471374903) (ISBN 1-59124482X electronic book).

Provides information on the properties, air pollution factors, water pollution factors, and biological effects of thousands of chemicals. All information is referenced, and the introduction contains a mini review of the ecotoxicologic relevance and determination techniques for the data presented in each monograph. Also available by online subscription.

Wexler P (2005) *Encyclopedia of Toxicology*, 2nd edn., San Diego, CA: Academic Press.

This text is a comprehensive collection of concise and readable explanations of basic principles in toxicology and the potential hazards of chemicals. It contains more than 1000 entries, including entries related to research and clinical toxicology, risk assessment, ecotoxicology, epidemiology, radiation, noise, information resources, organizations, and education. As with the Ist edition, this volume is extensively cross-referenced, contains a detailed index, and provides numerous references to primary and secondary literature.

Periodicals

During the past 30 years, there has been a steady increase in the number of journals transmitting information on toxicity, hazard, and risk. As knowledge has grown, more specialized titles have appeared reflecting the expanding literature of narrower disciplines, that is, cellular toxicology, aquatic toxicology, food toxicology, contact dermatitis, risk analysis, drug/nutrition interactions, molecular toxicology, genomics, and target organ/system toxicity. Also, because of the cross-disciplinary nature of toxicological concerns, relevant information appears not only in the primary toxicology journals but also in those of related disciplines – medicine, epidemiology, food, biology, agriculture, and so on. New journal titles will frequently be distributed directly to professionals and announcements will appear in the review sections of professional journals. Additional titles of journals and series publications can be found in Ulrich's International Periodicals Directory (Bowker) or The Serials Directory (Ebsco). These directories are available in print and on CD-ROM, as well as online through major vendors.

Generally, information published in a research journal is well known in the scientific community long before it appears in print. The research journal is not an effective rapid communication device but one for quality control, claiming of priority, and as a mechanism of archiving research information. Because the potential sources of toxicological information are so widespread, typical hand scanning issues of journals for newly published information can be overwhelming for the investigator. Also overwhelming is the increasing cost of journal subscriptions. The average cost of a journal has increased $\sim 10\%$ each year since 1990, forcing local collections to judiciously examine journal renewals.

The use of tertiary indexing and abstracting sources can provide an effective alternative to the timeconsuming scanning of journal issues and limited subscription resources. These services provide regularly updated title, author, and subject access to the contents of thousands of journal titles. Enlisting the power of computerized systems can automate this process. Profiles of user interest areas are applied against large databases of journal references and results delivered to users on a recurring basis. In some services the user not only can browse the source information (and frequently an abstract) of a newly published article but can also request a full text copy of the original. An example of this type of service geared to rapid communication of journal contents is the Current Contents service from the Institute for Scientific Information, Inc., available in both print and electronic form.

Increasingly, scientific journals are now providing direct access to full-text articles online, and many libraries are carrying online subscriptions to journals, either exclusively or in addition to print versions. Many of the larger publishers also provide special services to access their online holdings. An example of this type of service is Elsevier's *ScienceDirect* digital library. Moreover, bibliographic databases, such as CAS's *SciFinder* and *SciFinder Scholar*, ISI *Web of Knowledge* and *MEDLINE*, are now providing full-text access to articles retrieved in bibliographic searches. In a few cases (e.g. MEDLINE via OVID Technologies), links to full-text articles are made directly from the References Cited portion of a journal article search.

Listed below is a sampling of the core journal titles in toxicology. They have been organized into simple subject categories. Sample copies of journals are quite easily obtained from publishers. Addresses and phone numbers for these publishers may be found in *Ulrich's* or *The Serials Directory*.

General

Advances in Modern Environmental Toxicology (Princeton Scientific)

Annual Review of Pharmacology and Toxicology (Annual Reviews)

Archives of Toxicology (Springer)

The Banbury Report (Cold Spring Harbor Laboratory Press)

Chemical Research in Toxicology (American Chemical Society) Critical Reviews in Toxicology (CRC Press) Drug and Chemical Toxicology (Dekker) Food and Chemical Toxicology (Pergamon) Human and Experimental Toxicology (Macmillan) Journal of Analytical Toxicology (Preston) Journal of Applied Toxicology (Wiley) Journal of Biochemical and Molecular Toxicology (Wiley) *Neurotoxicology and Teratology* (Elsevier) Pharmacology and Toxicology (Munksgaard) Regulatory Toxicology and Pharmacology (Academic Press) Toxicity Review (HMSO, London) Toxicologic Pathology (Society of Toxicologic Pathologists) Toxicological and Environmental Chemistry (Gordon and Breach Science) Toxicological Sciences (formerly Fundamental and Applied Toxicology) (Academic Press) Toxicology (Elsevier Science) Toxicology and Applied Pharmacology (Academic Press) Toxicology and Industrial Health (Princeton Scientific) Toxicology in vitro (Pergamon) Toxicology Letters (Elsevier Science) Toxicology Mechanisms and Methods (Taylor and Francis) Veterinary and Human Toxicology (American Academy of Veterinary and Comparative Toxicology) **Alternative Toxicology Testing** Alternatives to Animal Testing and Experimentation: AATEX (Japanese Society of Alternatives to Animal Experimentation) Alternative Methods in Toxicology (Liebert)

Alternatives to Laboratory Animals: ATLA (Fund for the Replacement of Animals in Medical Experiments)

ILAR News (Institute of Laboratory Animal Resources)

Toxicology in Vitro (Elsevier)

Cancer and Carcinogenesis

Cancer Epidemiology Biomarkers & Prevention(American Association for Cancer Research)

Cancer Research (HighWire Press)

Carcinogenesis: A Comprehensive Survey (Raven Press)

Clinical

Adverse Drug Reactions and Toxicological Reviews (Oxford University Press) Clinically Important Adverse Drug Interactions (Elsevier Science) Drug and Chemical Toxicology (Dekker) Emergency Medical Clinics of North America (WB Saunders) Emergency Medicine (Cahners Publishing) Human Toxicology (Macmillan) Journal of the Association of Food and Drug Officials (Association of Food and Drug Officials) Journal of Toxicology. Clinical Toxicology (Dekker) Reactions Weekly (ADIS International)

Toxicon (Pergamon)

Environmental

Ambio (Royal Swedish Academy of Sciences) Aquatic Toxicology (Elsevier) Archives of Environmental Contamination and *Toxicology* (Springer) Archives of Environmental Health (Heldref) Bulletin of Environmental Contamination and *Toxicology* (Springer) Chemosphere (Pergamon) Developments in Toxicology and Environmental Science (Elsevier Science) Ecotoxicity and Environmental Safety (Academic Press) Environmental Health Perspectives (NIEHS) Environmental and Molecular Mutagenesis (Wiley) Environmental Science Research (Plenum) Environmental Toxicology and Chemistry (Pergamon) International Journal of Environmental Analytical Chemistry (Gordon and Breach Science) Journal of Environmental Health (National Environmental Health Association) Journal of Environmental Pathology, Toxicology, and Oncology (Begell House) Journal of Environmental Science and Health (Dekker) Journal of Hazardous Materials (Elsevier Science) Journal of the Air and Waste Management Association (AWMA) Journal of Toxicology and Environmental Health (Taylor and Francis) Pesticide and Toxic Chemical News (Food Chemical News) Reviews of Environmental Contamination and

Toxicology (Springer) Toxicological and Environmental Chemistry (Gordon and Breach Science)

Genomics, Proteomics, and Bioinformatics

American Journal of Pharmacogenomics (Adis) Bioinformatics (Oxford) Environmental Health Perspectives – Toxicogenomics (NIEHS) Genomics (Academic Press) Journal of Proteome Research (ACS Pubs) Molecular Genetics and Genomics (Springer) Pharmacogenomics Journal (Nature Publishing Group) Pharmacogenetics (Williams and Wilkins)

Mutagenesis

Chemical Mutagens: Principles and Methods for Their Detection (Plenum)

Environmental and Molecular Mutagenesis (Wiley) *Mutagenesis* (IRL Press) *Mutation Research* (Elsevier Science)

Occupational and Industrial

American Industrial Hygiene Association Journal (American Industrial Hygiene Association)

American Journal of Industrial Medicine (Wiley-Liss)

International Archives of Occupational and Environmental Health (Springer)

Journal of Occupational Medicine (Williams and Wilkins)

Occupational and Environmental Medicine (BMJ)

Radiation

Advances in Radiation Biology (Academic Press) Annals of the ICRP (Pergamon) International Journal of Radiation Oncology Biology Physics (Elsevier) Radiation Research (Academic Press)

Reproduction and Teratology

Advances in the Study of Birth Defects (University Park Press)

Birth Defects Research (Wiley-Liss) Issues and Reviews in Teratology (Plenum) Neurotoxicology and Teratology (Pergamon) Teratology (Wiley) Reproductive Toxicology (Pergamon)

Series

A type of publication related to the periodical is the report series. While many of the journals listed previously provide monographic reviews (i.e., Advances in Modern Environmental Toxicity, CRC Review Series, Methods in Toxicology, and Reviews of Environmental Contamination and Toxicology) the report type of publication issues from government agencies, scientific organizations, or research institutes. They vary considerably in scope and purpose but typically provide excellent summary information compiled by panels of experts. Many of these series are irregularly produced but issue consecutively within a volume/issue framework. Identification of series entries may be found by consulting catalogs such as the National Library of Medicine's LOCATORplus. LOCATORplus, which replaces NLM's previous online catalogs (CATLINE, SERLINE, AVLINE, and Locator), is available from the NLM homepage at http://www.nlm.nih.gov. Another useful source is the OCLC Online Union Catalog, which contains entries for materials contained in thousands of libraries in North America and the United Kingdom.

Many government agencies are now offering technical reports, journals, newsletters and other series for free online, and in full-text format. Examples include resources from the Agency for Toxic Substances Disease Registry (ATSDR) and Centers for Disease Control and Prevention (CDC). A brief, very select list of major sources of these series and their producers include:

Agency for Toxic Substances and Disease Registry, US Government (ATSDR)

1600 Clinton Road Atlanta, GA 30333 Tel.: +1-404-498-0110 + 1-888-42-ATSDR (Toll-free) URL: http://www.atsdr.cdc.gov ATSDR Toxicological Profiles US Centers for Disease Control and Prevention (CDC) 1600 Clifton Road Atlanta, GA 30333 Tel.: +1-404-639-3311 +1-800-311-3435 (Toll-free) URL: http://www.cdc.gov Morbidity and Mortality Weekly Report and many others European Commission Headquarters 200 rue de la Loi **B-1049** Brussels Belgium Tel.: + 32 2 299 11 11 URL: http://europa.eu.int EUR Report Series, Reports of the Scientific Committee on Cosmetology, Reports of the Scientific Committee for Food

Food and Agriculture Organization of the United Nations (FAO)

Publishing Management Service Viale delle Terme di Caracalla

00100 Rome Italy URL: http://www.fao.org Email: Publications-Sales@fao.org (series include those of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)) FAO Food and Nutrition Papers, FAO Plant Production and Protection Papers, JECFA Monographs on Toxicological Evaluation of Food Additives, Reports of the FAO Panel of Experts on Pesticide Residues in Food and the Environment International Agency for Research on Cancer (IARC) (part of the World Health Organization) 150 Cours Albert Thomas 69372 Lyon Cedex 08 France Tel.: +33 (0)4 72 73 84 85 URL: http://www.iarc.fr IARC Monographs (and supplements) on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Scientific Publications British Industrial Biological Research Association (BIBRA) Woodmansterne Road Carshalton, Surrey SM5 4DS United Kingdom Tel.: +44 (0)20 8652 1024 URL: http://www.bibra.co.uk/ Email: help@bibra.co.uk Toxicity Profiles Environmental Protection Agency, US Government (EPA) Ariel Rios Building 1200 Pennsylvania Avenue, N.W. Washington, DC 20460 Tel.: +1-202-272-0167 URL: http://www.epa.gov/ Too numerous to list - see EPA's 'Information Sources' website, http://www.epa.gov/epahome/resource.htm World Health Organization Avenue Appia 20 CH-1211 Geneva 27 Switzerland Tel.: +41-22-791-2111 URL: http://www.who.int/en/ Email: inf@who.int WHO Technical Report Series, Environmental Health Criteria, Health Aspects of Chemical Safety, Food Additives Series, Weekly Epidemiological Record, and others National Academy of Sciences/National Research Council (NRC)

500 Fifth Street N.W.

Washington, DC 20001 Tel.: +1-202-334-2000 URL: http://www.nas.edu Medical and Biologic Effects of Environmental Pollutants, Biologic Markers, Drinking Water and Health National Center for Toxicological Research (NCTR) 3900 NCTR Road Jefferson, AR 72079 Tel.: +1-870-543-7130 URL: http://www.fda.gov/nctr/ NCTR Quarter, FDA Consumer National Institute of Environmental Health Sciences (NIEHS) P.O. Box 12233 Research Triangle Park, NC 27709 Tel.: +1-919-541-3345 URL: http://www.niehs.nih.gov Email: webcenter@niehs.nih.gov Environmental Health Perspectives

Newsletters

Newsletters can provide timely reporting of 'hot' information – research findings, regulatory updating, society/organization news – all in brief reports. Most professional organizations support some kind of newsletter to communicate to its membership, but the ones of most interest here focus on current news used to inform and mobilize. Some government and commercial titles in this area include:

BNA Toxics Law Reporter/Daily (Bureau of National Affairs)

Environmental Reporter (Bureau of National Affairs)

Environmental Toxicology Newsletter (UC Davis) Hazardous Substances and Public Health (ATSDR) Occupational Safety and Health Reporter/Daily (Bureau of National Affairs)

Pollution Prevention News (P2 News2!, US EPA) Toxic Materials News (Business Publishers)

Electronic access to newsletters is provided via a number of services. These services vary in scope and how often they are updated. Examples include the *PTS Promt Newsletter* database (IAC) with a focus on business and industry and the *Newsletters in Print* database (Gale Research) available in print and online via several vendor systems. The *McGraw-Hill Publications Online* database carries approximately 60 leading publications on-line in full-text form, many of them newsletters and bulletins.

The need for extremely rapid reporting of information can be filled by specialized, and expensive, electronic 'clipping services' that offer daily feeds of news items from dozens of newspapers, newsletters,

newswires, and other types of information resources directly to the user's workstation or by fax. Examples of these are NewsDesk from PR Newswire, Dow Jones Interactive, NewsEdge from The Thomson Corp., and AccuClip Express from CyberAlert, Inc. The Internet has become a very popular mechanism for disseminating timely information via newsgroups and mailing lists (listservs). At least in the United States, an increasing number of associations and interest groups are communicating directly through use of the Internet. Government agencies, commercial producers, and research organizations are using this network as well. It is estimated that more than 18 000 newsgroups and hundreds of thousands of electronic newsletters are available over the Internet, and this figure is growing daily. One such recent effort of interest to the toxicology community is P2 News2!, a monthly newsletter that covers pollution prevention activities at the EPA, produced and distributed over the Internet by the Office of Pollution Prevention and Toxics of the US EPA. The US FDA also supports a publicly available server on the Internet covering staff activities, recent regulatory actions, and administrative news. The National Institute of Standards and Technology at the US Department of Commerce sponsors the Fedworld system, which provides access to federal government bulletin boards, government files, newsletters, and links to other government computerized systems.

Computerized Information

In the mid-1960s, the first publicly available systems for accessing machine-readable databases appeared. With the explosion of information in the sciences, coupled with the vast number of resources (journals, reports, newspapers, and monographs) in which this information was appearing, the use of computers to store and retrieve information has become an effective means to handle the increasing flow. As a result, computer use has increased exponentially, as evidenced by the growth in number of host computers that store data for use on the Internet. In 1969, four computers held all the information available to users of the nascent 'World Wide Web', whereas today it is estimated that over 132 million host computers now serve in this capacity.

Information files can be accessed through communication with host computers, where data are stored by producers or vendors, or by loading them locally, such as through CD-ROM and DVD storage. No matter what medium is chosen, it is important to recognize the disadvantages of each route: Online may mean subscriber and use charges, but it is convenient and universally available via telecommunications systems; CD-ROM and DVD storage can be very cost-effective for unlimited use, but production invariably necessitates a delay in currency over online. The information science literature contains several articles providing criteria, and checklists help in determining the most appropriate medium for each situation. Transparent bridges between online and CD, rewriteable CD/DVD technology, easier to use interfaces, mass market media platforms, and other developments are increasing the investigator's ability to locate, identify, and retrieve useful information.

Besides the development of data and text information systems, software programs are being designed to provide assistance in predicting the toxicity of substances based on structure - activity relationships, such as the TOPKAT program by Health Designs, Inc., or by an expert system, such as the CASE system by Case Western Reserve University. Computer-assisted instruction programs, incorporating text, audio, graphic, and full-motion video technologies, are an exciting use of computer-assisted instruction. Examples of this last type of CD-ROM product include the environmental health, industrial hygiene, and occupational health and safety titles of the ACTIV Series from ITC (Herndon, VA) and the hazardous materials series training program from Interactive Media Communications (Waltham, MA).

Directories of information on computerized sources, such as the *Gale Directory of Databases* (Gale Research Inc.) and the Mekler directory *CD-ROMs in Print*, give identification, coverage, content, and availability information for publicly available electronic files, as do many of the literature guides listed in the introduction to this entry. An example of a resource tool more limited in scope is the *Environmental Software Directory* (Donley Technologies). Articles and columns appearing in the information science literature (*Online, Searcher, Database, CD-ROM Professional*, and *Information Today*) can provide excellent reviews of new files and comparative studies on resources in specific subject areas.

As the availability of new information technologies has grown, so has the promise of free and equitable access to sources of data and information. Many schools as well as municipal and university libraries now offer free access to computers that are connected to the Internet, and governmental bibliographic and nonbibliographic databases, such as the suite of resources from the National Library of Medicine, are available free online. In addition to these national databases (which are listed later in this entry), a variety of regional, state, tribal, county, and municipal governmental and private sector toxicology information resources have become available on line. These sub-national resources often provide environmental quality and public/environmental health-related information (or links to information) on subjects such as lead poisoning prevention, fish and wildlife consumption advisories, asthma, air and water pollution and other information that is specific to localized geographic areas. The following are some examples of region-specific online toxicology information sources or guides to available resources (A more comprehensive list of subnational sources of toxicology information can be found in Stoss FW (2001) *Toxicology* 157:51 – 65.)

Federal and private sector regional resources:

US EPA regional directory (www.epa.gov/epahome/locate2.htm)

EnviroMapper (http://www.epa.gov/enviro/html/ em/)

SurfYourWatershed (http://www.epa.gov/surf) EnviroFacts (http://www.epa.gov/enviro/)

Environmental Defense Scorecard (http:// www.scorecard.org/)

Mapcruzin (http://www.mapcruzin.com/)

State, county, and local resources:

Environmental Health and Safety Online (http:// www.ehso.com/)

Environmental Organization Web Directory (http://www.webdirectory.com/government/states/)

Library of Congress State and Local Government Directory (http://www.loc.gov/global/state/stategov.html)

GovLinks' (http://governing.com/govlinks/ glinks.htm)

Local and regional topic-specific sites:

Lead poisoning prevention (http://www.cdc.gov/ nceh/lead/lead.htm)

Cancer registries (http://www.cdc.gov/cancer/npcr/)

Radon, indoor air quality (http://www.ehso.com/ ehshome/radon.htm)

Toxic chemical release inventories by state (http:// www.epa.gov/tri/statecon.htm)

Emergency planning-state and local (http://rtk.net/ lepc/webpage/hotlinks.html and http://www.fema.gov/library/diz02.shtm)

Poison control centers (http://www.aapcc.org/findyour.htm)

Brownfield programs (http://www.epa.gov/swerosps/bf/glossary.htm#brow)

Fish consumption advisories (http://www.epa.gov/ ost/fish/)

Occupational safety and health (http://www.croetweb.com and http://www.osha-slc.gov/fso/osp/)

Selection and Use of Electronic Sources

It is estimated that in 2001 almost 100 million people nationwide, or 75% of adults in the United States,

looked for health-related (including toxicological) information online. But anyone can put information on the web. There is no governing body, and information is not screened or standardized in any way to verify its accuracy or usefulness. With the unregulated flow of new sources on the Internet, as well as the thousands of databases available through public and subscription vendors, it becomes increasingly important to evaluate each resource as regards quality of information content. The following are criteria that one can use to determine the value of a resource:

Author Is it clear who writes or is responsible for the material on the site?

Are the author's credentials provided?

Is there a sponsoring institution and, if so, how credible and well known is it?

Is a third party supporting or sponsoring the site? Is contact information given for the author or sponsoring institution?

Purpose of the Site Is the purpose or mission of the website or sponsoring organization stated?

Is the purpose to inform, persuade, sell, present a viewpoint, or create or change an attitude or belief?

Is there advertising on the site and is it clearly differentiated from the informational content?

Date Is it clear when the site was last updated?

Content Does the site exhibit good grammar, spelling and literary composition?

Does the information consist of documented facts or personal opinion?

Are the sources of factual information provided so they can be verified?

Is there comprehensive coverage of the subject matter?

Are there external links to other sources of information?

Does an editorial board or healthcare professional review the content?

What criteria are used for selecting information displayed on the site?

Using these criteria, there can be a variety of reasons to be skeptical about information that is presented on a website. Lack of authorship or date, vague or sweeping generalizations, overstated significance and extreme tone or language – all mark the information presented as suspect. The credibility of information is also diminished by the absence of source documentation, personal testimonials as the only information sources, and purported 'miracle cures' recommended in lieu of well-established scientific or medical protocols. One other clue that can provide some information about the source of a website is its uniform resource locator (URL), or web address. Education-related URLs end in '.edu', whereas commercial sites usually contain '.com' in the URL. The URLs of nonprofit organizations usually end in '.org', and government and military organizations end with '.gov' and '.mil', respectively. Despite these guidelines, it is important to note that anyone can register a .com, .org or .net website for any use (including personal).

The following web resources provide good tips for evaluating or presenting information on the web:

Healthfinder: Your Guide to Reliable Health Information (US Federal Govt.)

http://www.healthfinder.gov/(English version)

http://www.healthfinder.gov/espanol (Spanish version)

Usability.gov: National Cancer Institute site for improving the communication of cancer research

http://usability.gov

Tips for Searching the Web

Use More Than One Search Engine There are advantages and disadvantages associated with the method a given search engine uses to locate information. Search engines can be classified into three types: Web Crawlers (or Web Spiders) automatically collect and catalog web pages by looking at the full text on a page, collecting all relevant information, and then following links on the page to locate other relevant sites. Examples include Google (www.google.com) and AllTheWeb (www.alltheweb.com). Web crawlers can potentially access a large portion of the Internet. Directories are search engines that contain only information that web managers have submitted. A website will not appear in a directory unless the web manager has submitted its URL under the most appropriate heading. Examples of directories include Yahoo (www.yahoo.com), MSN Search (www.msnsearch.com) and Open Directory (www.dmoz.org). Meta-search engines automatically submit a keyword search to several other search tools and retrieve results from all their databases. They are convenient time-savers for relatively simple keyword searches of one or two words or phrases in quotation marks. Examples include Profusion (www.profusion.com) and Dogpile (www.dogpile. com).

Use Lower Case Letters Most search tools are not case sensitive or only respond to initial capitals, as in proper names. Thus, it is safe to use all lower case letters, inasmuch as lower case always retrieves upper case letters.

Use Tools Offered by the Search Engine This includes using the Help button. Also, it is helpful to try the 'more like this', 'related searches', or 'narrow your search' options offered by some search engines. Advanced search options often help to eliminate sites that are unlikely to provide the desired information. For example, the advanced search option on Google allows selection or exclusion of specific types of URL, such as '.com' sites.

Use Quotation Marks Around a Search Phrase Without quotation marks, search engines return pages that contain the search terms somewhere in the page, but not necessarily near each other. With quotation marks, most search engines will only provide results in the exact order and way you specify them.

Use Search Engine Math (+/-) Search engine math can increase the specificity of a search, and works for nearly all major search engines. It allows the inclusion or exclusion of documents containing certain words through the use of the + and - symbols. For example, to find information about radiation but not nuclear radiation, type '+radiation nuclear'.

More information about search engines and searching tips can be found at Search Engine Watch: www.searchenginewatch.com (click on 'Web Searching Tips')

The following is an illustrative, selected list of electronic files containing publicly available toxicology information. Represented here are databases which cover toxicology under a larger discipline medicine, biology, and chemistry; those devoted to specific areas of toxicological concerns - occupational health, reproductive toxicology, environmental health, hazard assessment; industry-specific files such as those for specific industries - petroleum, nuclear power, agricultural, and engineering; and those that cover specific types of data - regulations, legislation, newsletter, industry or technical reports, and books. In many cases electronic databases are the counterparts to printed indexing, abstracting, or full-text source materials. Increasingly, electronic files are being developed that have no print counterparts, such as the Hazardous Substances Databank. In this list, the host system(s) is noted for each file, as is the availability of tape or CD-ROM formats. Many of these databases may also be available through the Internet:

Aquatic Information Retrieval (AQUIRE):

Contains over 220 000 records, which cover acute, chronic, bioaccumulative, and sublethal effects data from experimental assays performed with more than 7000 chemicals on 3700 fresh and saltwater aquatic

species. Produced by the US EPA, ECOTOX Support, Mid-Continent Ecology Division, Duluth, MN (STN, CIS; online at EPA: http://www.epa.gov/ecotox)

Agricultural OnLine Access (Agricola):

This comprehensive bibliographic database contains more than 3.7 million literature citations for journal articles, monographs, proceedings, theses, patents, translations, audiovisual materials, computer software, and technical reports pertaining to all aspects of agriculture. Produced by the US Department of Agriculture (USDA) National Agricultural Library. (OVID, DIALOG, STN; on CD-ROM from Silver Platter; online at http://agricola.nal.usda.gov/) BioBusiness:

Contains over one million records covering the worldwide periodical literature on business applications of biological and biomedical research. Includes occupational health, biotechnology, bioremediation, pesticides, toxicology, and energy as well as chemical names and CAS Registry numbers. Produced by Biological Abstracts, Inc. (BIOSIS). (Data-Star, DIA-LOG, STN)

BIOSIS Previews:

Contains more than 14 million citations to the worldwide literature in the life sciences, environmental sciences, and experimental medicine dating back to 1969. One of the largest of the science databases. Produced by BIOSIS. Selected citations contained in a subset of TOXLINE. (Data-Star, CISTI, DIALOG, DIMDI, OVID, STN)

CA File (CAS):

The CA File is a bibliographic database available from CAS (Chemical Abstracts Service). It contains more than 22 million references to the worldwide chemistry literature, including journals, patents, patent families, technical reports, books, conference proceedings, and dissertations from all areas of chemistry, biochemistry, chemical engineering, and related sciences from 1907 to the present. There are also over 600 records for journal articles dated before 1907. A companion Registry File holds records for all the substances cited in the CAS Registry System. The CAS registry number is a widely used identifier for unique chemical substances. (CAN/OLE, Data-Star, DIALOG, ORBIT; collective indexes available from Silver Platter).

CAB Abstracts (CABA):

Contains more than four million records to worldwide literature in the agricultural sciences and related areas of applied biology since 1973. The records in this file contain bibliographic information, abstracts and indexing information, including CAS Registry Numbers[®], from the 46 journals published by CAB International, its producer. (CAN/OLE, DIALOG, DIMDI, OVID, STN) **CERCLIS** Database of Hazardous Waste:

Contains information on each hazardous waste disposal or spill site nominated or selected for the EPA National Priorities List for cleanup under Superfund (CERCLA) or SARA amendments. Over 44 000 to date. (CIS, WESTLAW)

Chemical Carcinogenesis Research Information System (CCRIS):

This is a scientifically evaluated and fully referenced data bank, developed and maintained by the National Cancer Institute (NCI). It contains over 8000 chemical records with carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results. Data are derived from studies cited in primary journals, current awareness tools, NCI reports, and other special sources. Test results have been reviewed by experts in carcinogenesis and mutagenesis. (CIS, DIMDI, TOXNET)

Chemical Evaluation Search and Retrieval System (CESARS):

This database contains toxicological data on over 850 chemicals of particular interest to the United States Great Lakes basin. Each record provides descriptive data on up to 23 topic areas, including physical and chemical properties, toxicity, and environmental fate. Produced by the Michigan Department of Natural Resources and the Ontario Ministry of the Environment. Fully evaluated and referenced data. (CCINFOline; contained in CCINFOdisc; CHEMpendium series from CCOHS) Chemical Safety. Neurobase (CSNR):

Chemical Safety Newsbase (CSNB):

Contains over 67 000 records of information on occupational hazards in the chemical industry – identification, storage, handling, transportation, emergency planning, regulations and legislation, standards and practices, and waste management. Only new information on well-known hazards is included. Produced by the Royal Society of Chemistry. (Data-Star, DIALOG, STN, ORBIT/QUESTEL, Data-Star)

ChemIDplus:

This is a free, Web-based search system that provides access to structure and nomenclature authority files used for the identification of chemical substances cited in National Library of Medicine (NLM) databases. It also provides structure searching and direct links to many biomedical resources at NLM and on the Internet for chemicals of interest. The database contains over 367 000 chemical records, of which over 142 000 include chemical structures, and is searchable by Name, Synonym, CAS Registry Number, Molecular Formula, Classification Code, Locator Code, and Structure. (online from NLM at http://chem.sis.nlm.nig.gov/chemidplus).

CHEMLIST (Regulated Chemicals Listing):

Contains information on over 230 000 chemicals subject to legislative and regulatory control. Covers

all US EPA TSCA (Toxic Substances Control Act) and EINECS (European Inventory of Existing Commercial Chemical Substances). Provides citations to regulations, ITC recommendations, rule violations, safety, and health studies, superfund actions, and citizen's petitions. Produced by the CAS. (STN, STN Easy, SciFinder)

DATALOG:

DATALOG was developed through the collaborative efforts of EPA's Office of Toxic Substances and the Syracuse Research Corporation (SRC). It includes bibliographic references to published journal articles on the environmental fate and physical-chemical properties of chemicals released into the environment. References to 18 environmental fate properties (e.g., water solubility, photolysis, hydrolysis, biodegradation, and more) are included for more than 16 000 chemical substances in over 320 000 records. (CIS).

Developmental and Reproductive Toxicology Database/Environmental Teratology Information Center Backfile (DART/ETICBACK):

Together, these databases contain over 75 000 references to the worldwide literature of teratology and some coverage of developmental and reproductive toxicology. DART covers 1989 to the present and ETICBACK covers 1950–1989. Over half the references are scanned into DART from MEDLINE and it includes coverage of technical reports and conference papers. Produced by the National Library of Medicine (both available through TOXNET).

DERWENT Drug File (RINGDOC):

Contains over 1.5 million citations dating from 1964 to the present with lengthy, quantitative abstracts to the worldwide journal literature of drugs and pharmaceuticals. Ability to search by structureactivity is unique to Derwent files. Covers fewer source journals than MEDLINE or EMBASE (1150) but provides more in-depth on-line information through indexing and abstracts. Other Derwent files include Derwent Veterinary Drug File (VETDOC) and Derwent Crop Protection File (PESTDOC) providing similar in-depth analysis of the literature in these areas. The Derwent Drug Registry is the companion nomenclature file giving names, therapeutic class, and structures for chemical compounds. Formerly a subscription service, selected files are now publicly available. (Data-Star, DIALOG, OVID, STN)

ECOTOX:

Provides chemical toxicity information for aquatic life, terrestrial plants, and wildlife. Information is derived from peer-reviewed literature sources. ECOTOX was created and is maintained by the US EPA, Office of Research and Development (ORD), and the National Health and Environmental Effects Research Laboratory's (NHEERL's) Mid-Continent Ecology Division. (available on the EPA website: http://www.epa.gov/ecotox).

EMBASE:

Contains more than 15 million records covering the world's biomedical literature related to human health and medicine. Environmental pollution and health, occupational health, and clinical toxicology are strong areas. Corresponds in part to 46 specialty abstract journals and two literature indexes produced under the *Excerpta Medica* specialty series titles by Elsevier Science. (CDP, Data-Star, DIALOG, DIMDI, OVID, STN; entire database and sections in composite discs available from Silver Platter and DIALOG; online at http://www.embase.com).

Enviroline:

This bibliographic database provides indexing and abstracting coverage of more than 1000 international primary and secondary publications reporting on all aspects of the environment. These publications highlight such fields as management, technology, planning, law, political science, economics, geology, biology, and chemistry as they relate to environmental issues. Enviroline corresponds to the print *Environment Abstracts* and contains over 300 000 records. Produced by the Congressional Information Service (DIALOG, DIMDI; available on CD through Bowker).

ENVIROFATE – Environmental Fate Database

Provides access to information on the environmental fate or behavior of chemicals released into the environment. Data on environmental transformation rates and on physical-chemical properties are included. Records are drawn from papers published around the world involving chemicals that are produced in excess of one million pounds annually. Over 15 400 records cover 1833 different chemicals, predominantly organic compounds. Developed through the collaborative efforts of EPA's Office of Toxic Substances and the Syracuse Research Corporation (SRC) (CIS).

Environmental Mutagen Information Center Data Base (EMIC/EMICBACK):

This database is no longer being updated, but does provide access to the bibliographic information of chemical, biological, and physical agents which have been tested for genotoxic activity, most of which were published since 1950. Contains bibliographic details and keywords of chemicals tested, CAS Registry Numbers, organisms studied, and assay systems used. Users can search by CAS Registry Number, subject terms, title words, and author. Produced by the US Oak Ridge National Laboratory. (CIS, TOX-NET; available on CD as a subfile in PolTox I and Toxline, both by Silver Platter). Food Science and Technology Abstracts:

Covers the worldwide literature of food science and technology. Includes information on occupational toxicology in the food handling and processing areas, toxicology of foods and packaging, and additives information. Produced by the International Food Information Service (IFIS). (Data-Star, DIA-LOG, DIMDI, ORBIT, STN; available on CD only or with several combined files from a number of producers).

GENE-TOX (Genetic Toxicology):

This database is no longer being updated. Assembled by expert panels at the US EPA Office of Toxic Substances with the cooperation of the NIEHS and the EMIC program at Oak Ridge National Laboratories, this file provides mutagenicity data on over 3200 chemicals from 39 assays systems. The Gene-Tox program was established to select assay systems for evaluation, review data in the scientific literature, and recommend proper testing protocols and evaluation procedures for these systems (CIS, TOX-NET).

Hazardous Substances Databank (HSDB):

This huge data file covers over 4500 potentially hazardous chemical substances. It contains information on human exposure, industrial hygiene, emergency-handling procedures, environmental fate, regulatory requirements, and related areas. All data are referenced and derived from a core set of books, government documents, technical reports and selected primary journal literature. HSDB is peer-reviewed by the Scientific Review Panel (SRP), a committee of experts in the major subject areas within the data bank's scope. (Data-Star, DIMDI, STN Easy, TOXNET; available as part of TOMES PLUS by Micromedex). Household Products Database:

This database links over 4000 consumer brands to health effects from Material Safety Data Sheets (MSDS) provided by the manufacturers. Information on specific products includes chemical ingredients and their percentages, which products contain specific chemical ingredients, manufacturers of specific brands and their contact information, and acute and chronic health effects information. Available from the National Library of Medicine at http://householdproducts.nlm.nih.gov

HSELINE:

Contains over 180 000 citations to the worldwide literature on occupational health and safety. Includes physical, chemical, and medical hazards. Covers all UK Health and Safety Commission and Health and Safety Executive publications and a wide range of journals, conference papers, reports, and legislation (United Kingdom). Produced by the Health and Safety Executive, United Kingdom (Data-Star, ORBIT; available on CD from Silver Platter; available on CD as part of several products).

International Pharmaceutical Abstracts (IPA):

Contains citations to the worldwide pharmaceutical and pharmacy literature from 1970 to the present, including drug therapy, toxicity, and pharmacy practice as well as legislation, regulation, technology, utilization, biopharmaceutics, information processing, education, economics, ethics and other topics. Covers more than 800 journal sources. Produced by the American Society of Hospital Pharmacists (Data-Star, DIALOG, DIMDI, OVID, STN; available on CD-ROM from Silver Platter).

Integrated Risk Information System (IRIS):

Contains information on hazard identification and dose – response assessment of over 600 hazardous substances. Covers toxicity, carcinogenicity, chemical and physical properties, and applicable regulations. Includes the reference dose as defined by US EPA, unit risk of exposure by oral and inhalation routes. Produced by the US EPA. (CIS, TOXNET; available on CD as part of TOMES Plus by Micromedex and on the EPA Internet website).

International Toxicity Estimates for Risk (ITER):

ITER is a free Internet database of human health risk values for over 600 chemicals of environmental concern from several organizations worldwide. The data are presented in table format with side-by-side comparisons of risk values from the various organizations, below which are synopses that provide explanations for differences among risk values as well as links to more detailed information. ITER currently contains data from the Agency for Toxic Substances Disease Registry (ATSDR), Health Canada, National Institute of Public Health and the Environment, The Netherlands (RIVM), US EPA, and independent parties whose risk values have undergone peer review. Produced by Toxicology Excellence for Risk Assessment (TERA). (TERA, http://www.tera.org/iter; TOXNET, http://toxnet.nlm.nih.gov).

Martindale Online:

This is a full-text electronic version of this standard directory of pharmaceuticals and ancillary substances (Martindale: The Extra Pharmacopoea). Contains reviews, physical/chemical properties, adverse reactions, toxicity, uses, actions, dosages, pharmaceutical properties, contraindications, interactions, and trade and generic nomenclature. Produced by the Royal Pharmaceutical Society of Great Britain. (Data-Star; available on CD-ROM from RPSGB.)

MEDLINE (Medical Literature, Analysis, and Retrieval System Online):

This is one of the largest and most popular international biomedical databases in the world. Strengths include coverage of pre-clinical and clinical aspects of biomedicine, drug and pharmaceuticals, human and veterinary toxicology, and the practice of medicine. It contains over 12 million references to the journal literature from 1966 to present. Produced by the US National Library of Medicine (CIS, Data-Star, DIA-LOG, DIMDI, EPIC, OVID, STN; available on CD from a number of producers).

MSDS-CCOHS:

This database contains the complete text of over 130,000 Material Safety Data Sheets compiled by the Canadian Center for Occupational Health and Safety (CCOHS). This information was gathered from over 500 manufacturers and suppliers in the United States and Canada. Each record covers one chemical substance and provides trade and supplier name, description, chemical/physical properties, reactivity, health hazards, storage and disposal, personal protection, cleanup and disposal, and emergency first aid (CCINFOLINE, STN; available on CD from CCOHS).

MSDS-OHS:

This collection contains full text Material Safety Data Sheets, Summary Sheets, and Label Data for more than 59 000 substances, including pure substances and mixtures, 92–96% of which are the most heavily used chemicals in industry. The database originated with Occupational Health Services, Inc. (OHS). The records include occupational, environmental, and regulatory data, as well as names, CAS Registry Numbers, and regulatory list numbers. The OHS online system provides a full file, a summary information file, and a file composed of records to chemicals used in the manufacture of pesticide and other agricultural chemical products (OHS, STN; available on CD from OHS).

NIOSH Technical Information Center (NIOSH-TIC):

This bibliographic database contains more than 200 000 references to the literature of occupational health and safety from monographs, journals, and reports. Produced by the US National Institute for Occupational Safety and Health (NIOSH). As of 1997, NIOSH will only add NIOSH publications and articles by NIOSH authors to the database (CCINFOLINE, CIS, DIALOG, ORBIT/QUESTEL, STN, TOXLINE).

Pollution Abstracts:

Contains references to worldwide technical and nontechnical literature on all aspects of pollution, solid waste management, and environmental quality. Covers journals, books, technical reports, conference papers, and government documents. Produced by Cambridge Scientific Abstracts (STN; CD-ROM available from Silver Platter and National Information Services Corporation). Registry of Toxic Effects of Chemical Substances (RTECS):

Contains toxic effects data (with citations) on over 157 000 chemicals identified by NIOSH and mandated by the Occupational Safety and Health Act of 1970. Acute and chronic effects, selected regulatory information, IARC reviews, TSCA status, GENE-TOX data, and NTP documents cited. Data selectively included, not comprehensive. This database is compiled, maintained, and updated by MDL Information Systems, Inc., under the authority of the US government (CIS, Data-Star, DIALOG, DIMDI, STN; CD version contained in CCINFOdisc: Core Series C2 from CCOHS and CHEMBANK from Silver Platter).

REPROTOX:

Provides reviewed and summarized information on the reproductive risk of hundreds of chemical substances. Includes coverage of industrial and environmental chemicals, drugs, nutritional agents, and radiation. Effects noted on fertility (male and female), pregnancy, development, and lactation. Produced by the Reproductive Toxicology Center (RTC) of the Columbia Hospital for Women, Washington, DC (available by direct access to the RTC).

SEDBASE:

This database contains current, full-text information from the last 12 years of *Meyler's Side Effects of Drugs*, published every 4 years; *Side Effects of Drugs Annual*, published every year in between; and *Marler's Pharmacological & Chemical Synonyms*. It also contains citations and abstracts from EMBASE (Files 72, 73) that have been referenced by the Meyler's texts. Produced by Elsevier Science (Data-Star, DIA-LOG, DIMDI; available on CD-ROM from Elsevier).

Toxic Substances Control Act Test Submissions (TSCATS):

Provides over 64 000 citations to unpublished health and safety studies, chemical tests, and substantial risk data on over 8400 chemical substances submitted to the US EPA under the Toxic Substances Control Act (TSCA). Copies of the original submissions are available from the US EPA (CIS, subset of TOXLINE; available on CD-ROM in PolTox I and Toxline from Silver Platter).

Toxics Release Inventory (TRI):

Consists of more than 1 150 000 records containing information on the annual estimated releases of toxic chemicals to the environment. This information is gathered by the US EPA through manufacturers/ importers/users of chemicals under the provisions of the SARA amendments of CERCLA. Records contain information on the storage, discharge, waste treatment, and waste transfer of approximately 650 chemicals. RTD Net: The Right to Know Network, a companion database, contains information from the state of New Jersey's Hazardous Substance Fact sheets collected under New Jersey Right-to-Know Act. This provides information on the safety and ecological effects of most TRI chemicals. Produced by the US National Library of Medicine and the US EPA (CIS, TOXNET; EPA website, RTK Net website).

TOXLINE (National Library of Medicine, Toxicology Information Program):

One of the largest online bibliographic sources for toxicology information, these databases contain over 3 million citations to all areas of toxicology. TOX-LINE references are drawn from various sources grouped into two parts – TOXLINE Core and TOX-LINE Special. A standard search of TOXLINE retrieves records from both subsets. Users can also limit retrieval to only one. Both files are available on the NLM MEDLARS system (DIALOG; on CD through Silver Platter).

Nomenclature and Locator Files

Many database producers provide controlled vocabulary systems, which assist the user in obtaining specific and comprehensive retrieval. Registry or nomenclature files can be useful in identifying the controlled vocabulary for a compound used in a specific file or to obtain a collection of synonyms identifying that compound (lab code, generic name, chemical name, trade names, government agency control numbers, etc.) to be used in searching other resources. The CAS Registry file provides structure information, provides synonyms, and a chemical identifier code (CAS registry name) used extensively worldwide. Chemical Abstracts started its identification system in the mid-1960s and has established records for over 12.7 million compounds. Derwent, producer of the Derwent Drug File and the World Patents Index provides a Drug Registry file and establishes its own unique registry number for chemical compounds referenced in its products. Several online files carry extensive nomenclature information embedded in substance records, such as the Merck Index and RTECS, for example. Some files also provide locator information for chemical substance information on files mounted on a particular vendor system along with the nomenclature of the compound. The Chemical Identification File (ChemIdplus), for example, provides nomenclature and locator information for the PubMed and TOXNET systems.

Vendors

Listed below is a selected list of online vendors that focus on providing toxicology information files. The directories listed in the beginning of this section also give contact information for online vendors.

- BIOSIS
 - 2001 Market Street, Suite 700 Philadelphia, PA 19103-7095 Tel.: +1-215-231-7500 (worldwide) +1-800-523-4806 (United States and Canada) URL: http://www.biosis.org Email: info@biosis.org
- CCINFOWeb (Canadian Centre for Occupational Health and Safety)
 135 Hunter Street East Hamilton, ON, Canada L8N 1M5 Tel.: + 1-905-570-8094
 1-800-668-4284 (toll free in Canada and the United States)
 URL: http://www.ccohs.ca
 Email: clientservices@ccohs.ca
- Chemical Information System (CIS) National Information Services Corporation (NISC USA) Wyman Towers, 3100 St. Paul Street, Baltimore, MD 21218 Tel.: +1-410-243-0797 URL: http://www.nisc.com Email: info@nisc.com
- Data-Star c/o DIALOG Corporation DIALOG Corporation 11000 Regency Parkway, Suite 10 Cary, NC 27511 Tel.: +1-919-462-8600 1-800-3-DIALOG (North America) URL: http://www.dialog.com Email: customer@dialog.com
- DIMDI (Deutsche Institut fur Medizinische Dokumentation und Information) Waisenhausgasse 36-38a 50676 Cologne, Germany Tel.: +49 (0) 221-47-241 URL: http://www.dimdi.de Email: posteingang@dimdi.de
- European Space Agency Information Retrieval System (ESA-IRS) ESA/ESRIN Via Galileo Galilei Casella Postale 64 00044 Frascati (RM), Italy Tel.: + 39-06-9418-0951 URL: http://www.esa.int Email: franca.morgia@esa.int
- Thomson MICROMEDEX 6200 S. Syracuse Way, Suite 300 Greenwood Village, CO 80111-4740

Tel.: + 1-800-525-9083 URL: http://www.micromedex.com Email: mdx.info@thomson.com

- National Library of Medicine Specialized Information Services
 2 Democracy Plaza, Suite 510
 6707 Democracy Blvd., MSC 5467
 Bethesda, MD 20892-5467
 Tel.: +1-301-496-1131 (local and international)
 +1-888-FINDNLM (toll free)
 URL: http://sis.nlm.nih.gov
 Email: tehip@teh.nlm.nih.gov
- ORBIT/QUESTEL 7925 Jones Branch Drive McLean, VA 22012, USA Tel.: + 1-703-873-4700 1-800-456-7248 (Toll-free) URL: http://www.questel.orbit.com Email: help@questel.orbit.com
- OVID 333 7th Avenue 20th Floor New York, NY 10001 Tel.: + 1-646-674-6300 + 1-800-950-2035 (Toll-free in United States) URL: http://www.ovid.com
- Email: sales@ovid.com
 SilverPlatter Information, Inc. 100 River Ridge Drive Norwood, MA 02062-5043 Tel.: +1-781-769-2599 1-800-343-0064 (Toll-free in the United States) URL: http://www.silverplatter.com Email: us customerrelations@ovid.com
- Royal Pharmaceutical Society of Great Britain (RPSGB)
 1 Lamberth High Street London SE1 7JN, England Tel.: +44-020-7735-9141

URL: http://www.rpsgb.org.uk Email: enquiries@rpsgb.org

STN International Europe Help Desk Postfach 2465 D-76012 Karlruhe 1, Germany Tel.: +49-7247-808-555 URL: http://www.stn-international.de US address: c/o Chemical Abstracts Service 2540 Olentangy River Road PO Box 3012 Columbus, OH 43210-0012 Tel.: +1-614-447-3600 URL: http://www.cas.org Email: help@cas.org

CD-ROMS

Computer disc technology has grown to become one of the most popular media for local electronic storage and retrieval. CD recordable technology is widespread, and CDs are now as easy to create as floppy disks. While the most common type of CD-ROM product for bibliographic retrieval is still the single source product, producers are using this powerful storage medium to mix material into subject collection discs, combining various types of resources – handbooks, journal articles, regulations, and directories. Below are examples of these composite CD-ROM products/series of interest to the toxicology professional:

CCINFOdisc Products (CCOHS):

This collection of several CD-ROM products provides a wealth of legislative, regulatory, handbook, directory, numeric, and bibliographic information focusing on occupational health, workplace safety, environmental hazards, regulatory information, and safety topics. There are 12 titles currently available. International in scope. Vendor: Canadian Centre for Occupational Health and Safety.

Environmental/Safety Library:

A comprehensive collection of EPA, OSHA and DOT regulations and state regulations. Contains titles from the CFR (all of Title 40 and portions of 29, 42, and 49), *Federal Register* notices from 1990 to present, and industry standards from OSHA and EPA. Updated monthly. Produced by Information Handling Service, Inc. Comarketed by MICROMEDEX, Inc

ENVIRONMENT ABSTRACTS:

Provides access to journal articles, conference papers and proceedings, and other materials on the environment. Covers air, water, and noise pollution; solid and toxic wastes; radiological contamination; toxicological effects; control technologies; resource management; population; endangered species; and geophysical and climatic change. Produced by the Congressional Information Service; updated quarterly. OSH-ROM:

Combines six databases with references to the world's literature on occupational health and safety and environmental medicine. Consists of CISDOC (CIS Abstracts by the International Labour Office), *HSELine* (from the Health and Safety Executive in the UK), *MHIDAS* (from the United Kingdom Atomic Energy Authority), *NIOSHTIC and NIOSHTIC-2* (from the US National Institute for Occupational Safety and Health), RILOSH Index (from the Ryerson Polytechnic University Library, Canada) and MEDLINE-OEM (occupational and environmental medicine subset from the National Library of Medicine, US). Available from Silver Platter Information, Inc.; updated quarterly.

PolTox:

The PolTox series from Silver Platter, Inc., provides information on pollution and toxicology. PolTox I contains *Aquatic Sciences and Fisheries Abstracts, Ecology Abstracts, Food Science and Technology Abstracts, Health and Safety Science Abstracts, Pollution Abstracts, RISKLINE, Toxicology Abstracts,* and all of *TOXLINE.* PolTox II contains information derived from EMBASE and PolTox III information from the CAB Abstracts database.

REPRORISK:

Contains a collection of reproductive risk information resources, including REROTEXT, a collection of reviews (with hazard ratings) of the health effects of industrial chemicals; REPROTOX from the Reproductive Toxicology Center at Columbia Hospital for Women; the text of Shepard's *Catalog of Teratogenic Agents*; and TERIS, the Teratogen Information System from the University of Washington. Produced by Micromedex, Inc.

TOMES Plus:

This title contains information on toxicology, occupational health, and environmental information with a focus on emergency situations of exposure and hazard control. It also addresses ergonomics and human health risk assessment. It contains bibliographic, full text, and numeric information. Consists of eleven files of information from various government sources – US EPA, OSHA, DOT, Coast Guard, NIOSH, and others. Updated quarterly. Produced by Micromedex, Inc.

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Inhalation See Respiratory Tract.

Inhalation Testing See Toxicity Testing, Inhalation.

Insect See Hymenoptera.

Insecticides See Permethrin.

Interactions See Interactive Toxicity.

Interactive Toxicity

S Satheesh Anand and Harihara M Mehendale

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Definition

Interactive toxicity is defined as effects of mixtures deviating from the additive toxic response expected based on the dose–response relationships obtained from individual components.

Background

Humans are exposed to a myriad of chemicals throughout their lifetime in the work place, the environment, and the home. Hence, the human exposure to chemicals is mixtures rather than single chemicals. The real world exposure scenarios are complex in terms of type of chemicals, the exposure situation (duration, routes, rates, and magnitudes), and the existence of confounding socioeconomic factors (ethnicity, diet, alcohol, smoke, age, area of

residence, etc.), which can affect the likelihood or course of toxicity. Approximately, 80 000 chemicals are in use today with more than 1000 added each year worldwide. Toxicity of many of these compounds has not been adequately tested. Some mixtures are intentional (such as pesticide formulations, gasoline, or laundry detergents) and other mixtures are generated (smelting, disinfection by-products, polycyclic aromatic hydrocarbons from fuel combustion). Cigarette smoking generates mixture of large number of chemicals during combustion of tobacco. Many natural chemicals are present in the foods that we eat. The number of chemicals produced by the chemical and pharmaceutical industries in the twentieth century has vastly increased human exposure to chemicals. Unlike the general public, the people living in the vicinity of hazardous waste sites are often subjected to complex and high amounts of chemical exposures. As estimated, 40 million people live within a 4 mile radius of waste sites.

The potential for unusual health effects of chemical mixtures due to the interaction of chemicals or their metabolites (e.g., metabolites of trichloroethylene and benzene) in or with the biosystem constitutes a real issue in the public health arena. However, toxicity testing to predict effects on humans has traditionally studied one chemical at a time for various reasons: convenient to handle, physiochemical properties readily defined, dosage could easily be controlled, biologic fate could easily be measured, and relevant data were often available from human occupational exposures. Chemicals are known to cause disease: for example, arsenic and skin cancer, asbestos and lung cancer, lead and decrements of IQ, and hepatitis B predisposes to aflatoxin-induced liver cancer but the link between the extent of human exposure to even well-defined chemical mixtures and disease formation remains relatively unexplored, but of paramount importance to public health.

Public concern and the interest of scientists and regulators regarding exposure to chemical mixtures have increased. Because of the heightened awareness of public about the unusual toxicity from the mixtures, the regulatory agencies such as the Environmental Protection Agency (EPA), the Agency for Toxic Substances and Disease Registry (ATSDR), and the National Institute of Occupational Safety and Health (NIOSH) are striving to predict the cumulative health risk of mixtures from multiple sources.

Basic Concepts of Interactive Toxicity

Three basic concepts for the description of toxicological action of mixture have been defined.

Simple Similar Action

It is also called as dose addition. This is a noninteractive process, that is, the chemicals in the mixtures do not affect the toxicity of one another. Each of the toxicants in the mixture contributes to the toxicity in proportion to its dose. All chemicals in the mixture act in the same way, by the same mechanisms and differ only in their potencies.

Simple Dissimilar Action

It is also called as response addition. In this case also the components do not interact with each other. However, the mode of action and possibly the nature and site of action differ among constituents. Response addition is referred to if each individual has a threshold and will only exhibit response beyond the threshold. By definition, response addition is determined by summing the responses of each toxicant in a mixture.

Interaction

Compounds may interact with one another, modifying the magnitude and the nature of toxic effect. The interaction may lead to higher (exaggeration) or lower (antagonism) toxicity as compared to the effects of individual compounds.

Exaggeration: This type is further divided as synergism – when the effect of mixture is higher than additivity based on the dose–response relationships of the individual compounds (e.g., asbestos workers who smoke have higher mortality ratio for lung cancer than only smokers or asbestos workers), and potentiation (PB + CCl₄ causes increased liver injury, but not resulting in death) and amplification (kepone + CCl₄ causes higher hepatotoxicity and death) – when a component that does not have an effect increases the effect of the other components.

Antagonism: The effect of mixture is less than that estimated for additivity on the basis of the toxicities of the components. The protection against mercury toxicity by selenium is a typical example of this category. This group is further classified into inhibition – a component does not have effect and decreases the effect of another component or other components and masking – one component overrides the effect of other.

Knowledge on the type of interaction is of profound importance. While the knowledge on synergistic interaction is of paramount public health importance, the knowledge on antagonistic interaction is necessary to reduce the unnecessary utilization of resources for cleanups. Because of the potential unexpected toxicity due to interaction of chemicals, toxicology of mixtures is an active area of scientific investigation.

Mechanisms of Interaction

The mechanisms involved in interactive toxicity have been shown to bring changes in the toxicokinetics and/or toxicodynamics of one chemical by another. Toxicokinetic changes affect the absorption, distribution, metabolism, and/or excretion of a chemical and can have profound effects on dose-response relationships. Changes at the toxicodynamic level might involve a competition between chemicals for binding to a target site, such as a receptor or changes in signal transduction pathways and cell cycle control. Presence of one or more chemicals may also interfere with the complex toxicodynamics of tissue repair, thereby permitting continuous escalation of toxic injury, culminating in organ failure, and death. These mechanisms affect the internal concentrations of the toxicants or their active forms and/or the tissue's response to the toxic insult. Information on these aspects is a prerequisite for predicting the toxic effect of mixture. The examples of various interactions and the mechanisms of interactions are presented in Table 1.

Challenges in Mixture Risk Assessment

Because many of the components of complex mixtures and their concentrations are unknown, determining the risk such mixtures may pose to human population is a daunting task. The effects of a chemical mixture are extremely complex and may differ for each mixture depending on the chemical composition. Some contaminants may induce differential effects depending on the route of exposure. Simple mixtures may contain two or three chemicals whereas complex mixtures, such as those found at hazardous waste sites, contain hundreds of chemicals, with varying degrees of toxicity and different modes of toxic action. Concurrent exposure to chemicals such as welding fumes, indoor air pollutants, tobacco smoke, alcohol, and drugs makes the health assessment of chemical mixture a more complex task. Clearly, toxicological evaluation of these complex mixtures is difficult but important for hazard assessment and assessment of risk to human health.

Most of the data that do exist on mixtures come from acute or chronic studies of simple and defined

Basis of interaction	Examples of interactive toxicities	
	Synergism	Antagonism
Pharmacokinetic		
Absorption	Neurotoxicity of <i>o</i> -ethyl- <i>o</i> -4-nitrophenyl phenylphosphonothioate enhanced by aliphatic hydrocarbons due in part to increased dermal absorption	Dietary zinc inhibits some aspects of lead toxicity in part by decreasing lead absorption
Distribution	Increased neurotoxicity from increased lead levels in brain after treatment with disulfiram, due to formation of complex that readily distributes lead to brain	Se protects against Cd toxicity by decreasing the concentration of Cd in liver and kidney and by redistributing Cd in the testis from low to high molecular weight Cd-binding proteins
Excretion	Decreased renal excretion of penicillin when coadministered with probenecid, potentiating its therapeutic effect	As antagonizes the effects of Se in part by enhancing the biliary excretion
Metabolism	Ops (profenofos, sulprofos, DEF) potentiate the toxicity of fenvalerate and malathion by inhibiting esterase, which detoxifies many pyrethroid insecticides and malathion	Se inhibits 2-acetylamino-fluorene-induced hepatic damage and tumorogeneis in part by shifting metabolism towards detoxification
Pharmacodynamic		
Interaction at same receptor site or target molecule	Priming doses of a toxicant activates transactivational mechanisms of tissue repair	Atropine antagonizes OP poisoning by blocking acetylcholine receptor sites
Interaction at different sites on same molecule	Tiazofurin and selenazofurin metabolites bind to different sites on ionosine monophosphate dehydrogenase to synergistically inhibit its activity	Antagonism of Cu binding to DNA by other divalent cations
Interaction among different receptor sites or targets	Amplification of hepatotoxicity of chlordecone + CCl ₄ by inhibition of hepatocellular repair due to calcium flooding and depletion of cellular energy	Opposing effects of histamine and norepinephrine on vasodilation and blood pressure (functional antagonism)

Table 1 Examples of various interactions and the mechanisms of interactions

Source: Adapted from ATSDR (2002) Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures.

mixtures at doses higher than those normally associated in the environment and studies of human occupational exposures. Majority of biologically significant interactions observed at high doses are not detectable at the low doses to which humans are exposed environmentally. Dose is important because interactive effects depend heavily on dose; therefore, characterizing interactions that occur at high dose such as those used in a rodent bioassay is likely to provide very little information about interactions at very low doses generally encountered in the environment. Minimum number of cancer and noncancer studies has been performed on real-world mixtures such as diesel engine emissions, recycled drinking water, urban air samples, tobacco smoke, and incinerator emissions.

Unfortunately, not only is there a lack of knowledge concerning the characterization of real-life mixtures based on human exposure but there are limited experimental strategies available also that focus on understanding the mechanisms of action of chemical mixtures as it relates to human health. As a consequence, one has limited abilities to predict how chemicals in a mixture interact with each other or with biological systems, leading to toxic effects or disease.

The major challenges in the mixture risk assessment are: complex exposure situation, uncharacterized mixtures, extrapolation from high to low doses and animals to humans. Resolving these issues is key in predicting and preventing the risk from mixtures to humans. Testing of all mixtures existing in the real world is virtually impossible. Even with well-studied individual compounds, immense problems exist in extrapolating the findings obtained at high doses in laboratory animals to humans being exposed to lower doses. Individual variability and impact of life style on the toxic outcome further complicate the issue.

Assessing Risk from Chemical Interaction

Mixture risk assessment usually involves substantial uncertainties ranging from inexact descriptions of exposure to inadequate toxicity information. In addition, there are other confounding factors such as life style, exposure to other contaminants, interindividual variation, etc. Because of the uncertainties, many fudge factors are incorporated and there is no single approach for mixture risk assessment. Most risk assessments evaluate the toxicity of individual chemicals and then combine them by simple addition to estimate risk related to chemical mixtures. However, adding risks ignores potential synergistic or antagonistic interactions that could lead to underestimation or overestimation of total risk, respectively. The National Institute of Environmental Health Sciences, EPA, ATSDR, NIOSH, Occupational Safety and Health Administration (OSHA), and American Conference of Governmental Industrial Hygienists (ACGIH) share the common goal of promoting research that will ultimately reduce the extent of adverse human health effects occurring as a consequence of exposure to mixtures of environmental agents. Recently, the Food Quality Protection Act and Safe Drinking Water Act Amendments were passed, raising awareness of chemical mixtures health issues. By and large, regulatory actions and industrial practices are based on use of the default assumption, additivity. The experimental evidence that can be used to infer effects at low doses appears to support the assumption that low-dose additivity does not underestimate, and in most cases probably overestimates, risk. While the simultaneous administration of chemicals of dissimilar mode of action caused no more than additive effects, the chemicals of similar mode of action causes antagonistic effects at no-observed-adverse-effect levels (NOAELs) of individual components.

EPA recommends three approaches: (1) if the toxicity data on mixture of concern are available, the quantitative risk assessment is done directly form these preferred data; (2) when toxicity data are not available for the mixture of concern, data of a sufficiently similar mixture can be used to derive quantitative risk assessment for mixture of concern: and (3) if the data are not available for both mixture of concern and the similar mixture, mixture effects can be evaluated from the toxicity data of components. According to EPA, the dose-additive models reasonably predict the systemic toxicity of mixtures composed of similar (dose addition) and dissimilar (response addition) compounds. Therefore, the potential health risk of a mixture can be estimated using a 'hazard index' (HI) derived by summation of the ratios of the actual human exposure level to estimated maximum acceptable level of each toxicant. A HI near to unity is suggestive of concern for public health. This approach will hold true for the mixtures that do not deviate from additivity and do not consider the mode of action of chemicals. Modifications of the standard HI approach are being developed to take account of the data on interactions.

The ATSDR has established a mixtures program that consists of three components: trend analysis to identify combinations of chemicals of concern, experimental studies to identify data that would be useful in the development and implementation of predictive decision, support methodologies, and development of assessment methodologies and guidance to provide health assessors with the tools to incorporate the evaluation of multiple-chemical exposure into site assessments. ATSDR suggests the weight of evidence (WOE), which estimates the joint actions (additivity, antagonism and exaggeration) for binary mixtures of chemicals based on the information on individual components. Several factors such as mechanistic interaction, uncertainty factors, route of exposure etc. are taken into account. The better the data set on the individual chemicals is, the more precise the joint action can be predicted. The draw back is the high- to low-dose extrapolation as most of the individual toxicity information is developed at high doses. According to WOE evaluations, considering common mechanisms for simple chemical mixtures can lead to better estimates of the observed toxic responses than the default assumption of dose additivity.

Future Directions

Although progress has been made in recent years by establishing fair risk assessment methodologies and safe concentrations for many individual compounds, related information for chemical mixtures is largely unavailable. No standard methods are yet in place to incorporate interactions because of the lack of understanding of the modes of action, toxicokinetics, and toxicodynamics. Additional research is needed to resolve many unknown and uncertainties concerning toxicity of chemical mixtures. A fuller understanding of biological effects induced by chemical mixtures is essential to the accurate prediction of the hazards and risks for humans and the ecosystem.

Considering the $\sim 80\,000$ chemicals in commerce, the task of testing these chemicals on individual basis, let alone as a mixture, is not feasible. Two options seem possible: directly investigate the effects of high priority mixtures and develop extrapolation models for remainder. EPA, ATSDR, and NIOSH have organized the Mixed Exposures Research Group, composed of almost 20 federal and state agencies to develop and share regulatory approaches. Systematic toxicity testing of mixtures, using conventional toxicology and carcinogenicity testing methodologies is highly impractical because of the numbers of chemicals and the limited scientific resources. Therefore, the development of unconventional, efficient, and predictive toxicology methods is imperative. These approaches may greatly reduce animal usage, personnel, resources, and time required to evaluate the carcinogenicity of chemicals and chemical mixtures. Using computational technology, mathematical and statistical modeling, mechanistically based short-term toxicology studies, and cellular and molecular biology techniques would greatly enhance the predictability of the methodologies. Significant advances have been made in alternative toxicologic testing methods such as in vitro

testing, physiologically-based pharmacokinetic/pharmacodynamic modeling, biologically based doseresponse modeling, and quantitative structure-activity relationships modeling, and the 'omics' technologies (transcriptomics, proteomics, metabonomics). The genomics evaluates the gene expression, proteomics elicits resulting protein synthesis and metabonomics captures the change in metabolism following toxic insult. The use of 'omics' would map early toxicityrelated alterations in cells, tissues, or animals exposed to chemicals, and thus will lead to insights into numerous toxicologically relevant cellular processes simultaneously. These tools can find utility in the decision-making process and the performance of risk characterization. These alternate methods would aid in understanding the mechanistic basis for interactions at a quantitative level and provide realistic risk assessments for chemical mixtures.

Chemical Interaction and Susceptible Population

There is a strong concern and some evidence that sensitive population such as smokers, alcoholics, children, genetically predisposed, etc. are vulnerable to toxicity. Children are found to be more sensitive than adults to some chemicals because of the immature development of the defense system. Recent studies show association between low-level exposures to hazardous chemicals and developmental effects or birth defects. Many chemicals can cross the placenta and concentrate in the fetus. Thus, the developing fetus is extremely vulnerable to chemical exposure. Number of recent epidemiologic studies has demonstrated neurobehavioral impairment at low-dose exposure levels of lead that were once thought to be safe. Susceptibility of an individual to the toxic and carcinogenic effects of a chemical mixture is believed to be affected to a significant degree by genetics. The advent of new gene array technology is expected to allow the analysis of global patterns of gene expression in response to xenobiotic/mixture exposure. These new approaches of pharmacogenomics and toxicogenomics are expected to be of paramount importance in unraveling the complex interactions among environmental toxicants and in determining their effects on human health. Conditions such as diabetes, aging, caloric restriction, etc. alter the outcome of hepato- and nephrotoxicity by interfering with the tissue's ability to respond to injury by compensatory tissue repair. There are reports to claim that low-dose exposure to chemical mixtures may play an important role in developmental toxicology because of possible interactions among the components of the mixture, but these reports do not consider the maternal occupational exposure, or lifestyle issues such as use or abuse of medications, drinking, smoking, etc. Elevations in the rates of neural tube defects and major cardiac defects have been found in populations residing in the proximity of toxic waste sites and that consumed contaminated public drinking water. However, there is no conclusive scientific evidence behind it.

Conclusions

The unexpected toxicity due to chemical interaction with or in the biosystem is a paramount public health concern and presents immense challenge for risk assessment. Due to the increased awareness on potential interactive toxicity of chemicals, there is a decline in the amount of chemicals released into the environment reports the US EPA. This is a welcome success of primary prevention, but there seems to be little insight into the potential for joint toxic actions of such chemicals at environmental levels. Although the literature on chemical mixture is growing, our knowledge on underlying biological and pathophysiological processes associated with chemical interaction is meager. In addition, the possibility that subpopulation responds differently to the toxic effects of chemicals than general population further complicates the prediction of biological basis of interaction. Understanding what mixtures of chemicals the public is exposed to as well as the mechanisms involved in the interaction of these mixtures and subsequent health effects is essential. Since it is impossible to conduct studies with all mixtures encountered, toxicity testing of complex environmental mixtures of regulatory importance should be performed. A rational approach to studying mixtures includes prioritizing and identifying chemical mixtures that are based on known human exposures, and applying innovative experimental and computational strategies to dissect the mechanistic basis for interactions of chemicals in a mixture. This would aid in developing qualitative and quantitative health assessment methods for assessments of potential risks for developing multiple health effects.

See also: Common Mechanism of Toxicity; Mixtures, Toxicology and Risk Assessment; Modifying Factors of Toxicity.

Further Reading

US Environmental Protection Agency (EPA) (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. EPA/630/R-00/002.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry (ATSDR) (2002) Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures.

Intercellular Communication See Gap Junctional Intercellular Communication in Epigenetic Toxicity.

Intuitive Toxicology See Toxicology, Intuitive.

Invertebrate Ecotoxicology See Ecotoxicology, Invertebrate.

Investigative New Drug Application

Shayne C Gad

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- AGENCY: US Food and Drug Administration (FDA)
- YEAR PASSED: 1962 Drug Amendment to Food, Drug & Cosmetic Act with subsequent amendments
- GROUPS REGULATED: Drug and biopharmaceutical industries

Synopsis of Law

The 1962 Drug Amendment to the Food, Drug and Cosmetic Act (with subsequent amendments) contains the regulations specifically applicable to human drugs in Subchapter D, Parts 30–399. The definition of a new drug is covered in Part 310 (g): "A new drug substance means any substance that when used in the manufacture, processing, or packaging of a drug causes that drug to be a new drug but does not include intermediates used in the synthesis of such substances."

The regulation then goes on to discuss "newness with regard to new formulations, indications, or in combinations." For toxicologists, the meat of the regulations can be found in Section 312 (investigational new drug application (INDA)) and Section 314 (applications for approval to market a new drug or antibiotic drug or NDA). The major focus for a toxicologist working in the pharmaceutical industry is on preparing the correct toxicology 'packages' to be included to 'support' these two types of applications. The exact nature of these packages will be covered below.

Commencement of clinical trials with a new drug substance requires formal notification to FDA. At least 30 days before the drug's sponsor wishes to begin such trials, the sponsor must submit an IND to the agency (basic IND requirements are set out at title 21 CFR part 312 and Section 117 of the 1997 Food and Drug Administration Modernization Act (FDAMA)). If FDA does not object to the IND within 30 days, it automatically becomes effective and clinical trials may begin. If FDA finds a problem with the application, however, it may impose a 'clinical hold', barring commencement of the investigational studies proposed in the application until the problem is resolved to the agency's satisfaction.

In a nutshell, the law requires solid scientific evidence of safety and efficacy before a new drug will be permitted in clinical trials or (later) on the market. The INDA (covered in 21 CFR 310) is for permission to proceed with clinical trials on human subjects. Once clinical trials have been completed, the manufacturer or 'sponsor' can then proceed to file an NDA (covered in 21 CFR 314) for permission to market the new drug.

As stated in 321.21, "A sponsor shall submit an IND if the sponsor intends to conduct a clinical investigation with a new drug ... [and] shall not begin a clinical investigation until ... an IND ... is in effect." (Similar procedures are in place in other major countries. In the United Kingdom, for example, a clinical trials certificate (CTC) must be filed or a clinical trial exemption (CTX) obtained before clinical trials may proceed.) Clinical trials are divided into three phases, as described in 21 CFR 312.21. Phase I trials are initial introductions into healthy volunteers primarily for the purposes of establishing

tolerance (side effects), bioavailability, and metabolism. Phase II clinical trials are "controlled studies ... to evaluate effectiveness of the drug for a particular indication or disease." The secondary objective is to determine common short-term side effects; hence, the subjects are closely monitored. Phase III studies are expanded clinical trials. It is during this phase that the definitive, large-scale, double-blind studies are performed.

The toxicologist's main responsibilities in the IND process are to design, conduct, and interpret appropriate toxicology studies (or 'packages') to support the initial IND and then design the appropriate studies necessary to support each additional phase of investigation. Exactly what may constitute appropriate studies have varied with time and vary somewhat based on the nature of the drug. The toxicologist's second responsibility is to prepare the toxicology summaries for the (clinical) investigator's brochure (described in 312.23(a)(8)(ii)). This is an integrated summary of the toxicological effects of the drug in animals and *in vitro*. The FDA has prepared numerous guidance documents covering the content and format of INDs. The Guidance for Industry provides an in-depth description of the expected contents of the pharmacology and toxicology sections. The document contains the following self-explanatory passage.

Therefore, if final quality-assured individual study reports are not available at the time of IND submission, an integrated summary report of toxicological findings based on the unaudited draft toxicologic reports of the completed animal studies may be submitted.

If unfinalized reports are used in an initial IND, the finalized report must be submitted within 120 days of the start of the clinical trial. The sponsor must also prepare a document identifying any differences between the preliminary and final reports, and the impact (if any) on interpretation.

Thus, while the submission of fully audited reports is preferable, the agency does allow for the use of incomplete reports.

Once an IND or CTC/X is opened, the toxicologists may have several additional responsibilities. The first is to design, conduct, and report the additional tests necessary to support a new clinical protocol or an amendment to the current clinical protocol (Section 312.20). The second is to bring to the sponsor's attention any finding in an ongoing toxicology study in animals "suggesting a significant risk to human subjects, including any finding of mutagenicity, teratogenicity or carcinogenicity," as described in 21 CFR 312.32. The sponsor has a legal obligation to report such findings within 10 working days. Third, to prepare a "list of the preclinical studies ...

Table 1 Composition of standard INDA^a

- 1. IND cover sheets (form FDA 1571)
- 2. Table of contents
- 3. General (clinical) investigation plan
- 4. (Reserved)
- 5. (Clinical) investigators brochure
- 6. (Proposed) clinical protocol(s)
- 7. Chemistry, manufacturing, and control information
- Pharmacology and toxicology information (includes metabolism and pharmacokinetic assessments done in animals)
- 9. Previous human experience with the investigational drug
- 10. Additional information
- 11. Other relevant information

^aComplete and thorough reports on all pivotal toxicological studies must be provided with the application.

completed or in progress during the past year" and a summary of the major preclinical findings. The sponsor is required (under Section 312.23) to file an annual report (within 60 days of the IND anniversary date) describing the progress of the investigation. INDs are never 'approved' in the strict sense of the word. Once filed, an IND can be opened 30 days after submission, unless the FDA informs the sponsor otherwise. The structure of an IND is outlined in Table 1, though there may be some variation based on the nature of the drug and scope of the proposed trials.

If the clinical trials conducted under an IND are successful in demonstrating safety and effectiveness (often established at a pre-NDA meeting, described in 21 CFR 312.47(b)(2)), the sponsor can then submit an NDA.

Types of INDs

The primary focus of toxicologists is on submissions that are sometimes called 'commercial INDs', which are applications filed principally by companies whose ultimate goal is to obtain marketing approval for new products. There are, however, at least a few types of applications that may be grouped within a second class of filings sometimes referred to as 'noncommercial' INDs. Interestingly, the vast majority of INDs are noncommercial research submissions. These include the following types of INDs.

Investigator IND (also called research IND): The investigator IND is submitted by a physician who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. In most cases, an investigator IND proposes clinical studies on previously studied drugs. A physician might submit a research IND to propose studying an unapproved drug, or an approved product for a new indication or in a new patient population. Generally, however, the physician's motivation is not commercial in nature – in other words, the goal is not to develop data to support marketing approval for an unapproved product or to support new labeling for an approved product. For example, the investigator may simply want to treat patients or obtain data to publish a research paper.

Emergency use IND: The emergency use IND is a vehicle through which the FDA can authorize the immediate shipment of an experimental drug for a desperate medical situation. According to FDA regulations, "need for an investigational drug may arise in an emergency situation that does not allow time for submission of an IND ... In such a case, FDA may authorize shipment of the drug for a specified use in advance of submission of an IND." Emergency use INDs are generally reserved for life-threatening situations in which no standard acceptable treatment is available, and in which there is not sufficient time to obtain institutional review board (IRB) approval.

Treatment IND: Although the treatment IND has a history dating back to the 1960s and 1970s, the FDA took steps to formalize the treatment IND concept in a 1987 regulation. Through the FDA's treatment IND program, experimental drugs showing promise in clinical testing for serious or life-threatening conditions are made widely available while the final clinical work is performed and the FDA review takes place. The FDA Modernization Act of 1997 codified the treatment IND concept as well as other expanded-use programs (e.g., emergency use) into law, and encouraged the FDA to consider changes that might reduce industry reluctance to participate in expanded drug access programs.

Concurrently with an IND filing (or at any later time), a sponsor can request a 'fast-track product' designation for its drug, provided the therapy addresses unmet medical needs related to a serious and life-threatening condition. This fast-track designation, which was created under the FDA Modernization Act of 1997, makes a product eligible for accelerated approval and other benefits.

The Applicability of the IND

The IND is a requirement for all persons and firms seeking to ship unapproved drugs over state lines for use in clinical investigations. However, the FDA offers exemptions from IND submission requirements for certain types of clinical testing and products, including the following:

• Clinical investigations of a drug product that is lawfully marketed in the United States, provided

that all of the following conditions apply: (1) the investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use, or is not intended to be used to support any other significant change in the drug's labeling; (2) the investigation is not intended to support a significant change in the advertising for a prescription drug; (3) the investigation does not involve a change in the route of administration, dosage level, patient population, or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product; (4) the investigation complies with IRB evaluation and informed consent requirements; and (5) the study's sponsor and investigator do not represent in a promotional context that the drug is safe or effective for the purposes for which it is under investigation, or unduly prolong the study after finding that the results are sufficient to support a marketing application. The FDA has stated that this exemption is intended primarily for practicing physicians.

- Drugs intended solely for testing *in vitro* or in laboratory research animals, provided the drug labels and shipments comply with FDA regulations applicable to investigational drugs.
- Clinical investigations involving the use of a placebo, provided that the investigations do not involve the use of a new drug or otherwise trigger IND submission requirements.
- Certain *in vivo* bioavailability and bioequivalence studies in humans. FDA regulations state, however, that INDs are required for *in vivo* bioavailability or bioequivalence studies in humans if the test product is a radioactively labeled drug product, is a cytotoxic drug product, or contains a new chemical entity. Further, INDs are required for the following types of human bioavailability studies that involve a previously approved drug that is not a new chemical entity: (1) a single-dose study in normal subjects or patients when either the maximum single or total daily dose exceeds that specified in the labeling of the approved product; (2) a multiple-dose study in normal subjects or patients when either the single or total daily dose exceeds that specified in the labeling of the approved product; and (3) a multiple-dose study on a controlledrelease product for which no single-dose study has been completed.

In addition to these IND exemptions, FDA regulations provide a mechanism through which individuals and firms can seek an agency waiver from IND requirements. The agency can grant a waiver if certain criteria are met, including that the sponsor's noncompliance will not pose a significant or unreasonable risk to human subjects.

FDA Oversight: Clinical Holds

Through the imposition of a 'clinical hold', FDA may forestall a proposed clinical investigation or suspend an existing one. A clinical hold can be imposed for a number of reasons, including an unreasonable and significant risk to patients, the use of improperly qualified investigators, a deficient or disregarded investigative protocol, or any other serious deficiency in an IND or a particular clinical trial. FDA must communicate the imposition of a clinical hold by telephone or other form of rapid communication, and must provide the drug sponsor, within 30 days, with a written explanation of the basis for the clinical hold. As a general rule, until the agency's consent to lift a clinical hold is obtained, any clinical trial or trials subject to the hold cannot commence or resume. Under FDAMA, a sponsor faced with a clinical hold may submit a written request to FDA that the hold be removed. FDA must respond to such a request in writing within 30 days.

IND Withdrawal

As with the imposition of a clinical hold, FDA can halt further use or distribution of an investigational drug through withdrawal or suspension of an IND. Similar concerns, such as undue patient risk or serious deficiencies in the application or the clinical protocol, trigger both types of agency action, with withdrawal obviously reserved for the more serious cases.

Where the continuation of a clinical study poses, in FDA's judgment, an immediate and substantial danger to human subjects, the agency may order immediate termination of an IND, subject to possible reinstatement. Where no such immediate risk is present, however, if FDA proposes to withdraw an IND, the agency will notify the sponsor in writing and provide 30 days to submit any corrections or explanations. The sponsor's failure to respond within the specified time frame results in the termination of the IND. The sponsor, however, may request a formal hearing if FDA refuses to accept a submitted correction or explanation.

See also: Delaney Clause; Food and Drug Administration, US; Food, Drug, and Cosmetic Act, US; Good Laboratory Practices (GLP); Toxic Torts.

Further Reading

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lodine

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7553-56-2
- SYNONYMS: Diiodine; Iode
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Halogen; Halide
- CHEMICAL FORMULA: I⁺

Uses

Iodine was discovered in 1811 by Courtois, and is classed among the rarer elements. Iodine is used as an antihyperthyroid and a topical anti-infective. It is an ingredient in antiseptics and other medicinal preparations and in germicides. The latter use includes udder washes used on cattle in dairy operations; thus iodine is found in cow's milk. Other uses include disinfectants that may be added to swimming pools or drinking water. It is also used as a chemical reagent and is used in dyes (aniline and phthalein dyes), as an alkylation and condensation catalyst, in iodides, iodates, X-ray contrast media, food and feed additive, stabilizers, photographic film, water treatment, and as an unsaturation indicator. Iodine is found naturally in seaweed and is considered and generally recognized as safe substance by the US Food and Drug Administration (FDA). Iodine is a required element by many species, including humans. It has been recognized as preventative against goiter since 1819, and is used in iodized salt for this purpose. Iodine is also used as a dough oxidizer in commercial bread making.

Exposure Routes and Pathways

Exposure may occur via inhalation, ingestion, or dermal or ocular contact.

Relevant Websites

- http://www.access.gpo.gov CFR Title 21 (Food and Drugs) Chapter 1 Food and Drug Administration, Department of Health and Human Services Part 312 Investigational New Drug Application.
- http://www.kluweronline.com *Investigational New Drugs* (an online journal title by Kluwer).

Toxicokinetics

Iodine is absorbed rapidly and completely as I^- from the gastrointestinal tract. It is also absorbed when applied to the skin. Surgical scrubs containing iodine compounds were found to increase the level of urinary iodine in medical personnel. Iodine compounds are efficiently trapped and concentrated in the thyroid gland. Excretion is primarily via urine, although some iodine is excreted in feces and sweat. There is some salivary recycling. The half-life of iodine in blood is 6–10 h. Prolonged administration of large doses of iodine markedly reduces thyroidal iodine uptake.

Mechanism of Toxicity

Iodine is a powerful oxidizing agent and has a direct action on cells by precipitating proteins. The affected cells may be destroyed. In addition to the primary irritant action of iodine, this compound can act as a potent sensitizer. Iodine is an integral part of thyroid hormones (tetraiodothyronine (thyroxine) and triiodothyronine), and deficiency results in compensatory hyperplasia and hypertrophy of the thyroid gland (endemic goiter). Endemic goiter occurs naturally where soil is deficient in iodine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Iodine is a strong irritant of the mucous membrane, respiratory tract, eyes, and skin application of a 2% solution of iodine in alcohol to rabbit eyes caused reversible damage. Stronger solutions of 7% caused severe damage to rabbit and monkey eyes. The oral LD_{50} for mice is $2 g k g^{-1}$ and the oral LD_{50} for dogs is 200–500 mg kg⁻¹.

Human

Ingestion of large quantities of iodine may cause burning of the mouth, throat, and stomach and abdominal pain, nausea, vomiting, and diarrhea. Sufficient exposure may result in progression of symptoms to fever, shock, delirium, and death. Ingestion of 2–4 g has been fatal. The solid element is intensely irritating to eyes, skin, and mucous membranes. Iodine vapor is more irritating than vapors of chlorine or bromine. Occupational reports indicate that concentrations of 0.1 ppm are tolerable, but concentrations of 0.15 or 0.2 ppm are less tolerable. Concentrations of 1 ppm are highly irritating. Vapor concentrations of 0.57 ppm were tolerated for 5 min without eye irritation; but 1.63 ppm caused irritation within 2 min. Symptoms of inhalation exposure include tightness in the chest, sore throat, and headache. High exposures may result in airway constriction, shortness of breath, difficulty in breathing, pulmonary edema (onset may be delayed several hours), and death. Skin contact may result in corrosive tissue destruction at the site of contact. Individual susceptibility to skin reactions varies widely. Application of tincture of iodine to one-third of the body surface was reported as fatal in one case. Iodine solutions are recognized sensitizing agents.

Chronic Toxicity (or Exposure)

Animal

Dogs exposed by injection in the trachea to vapors of iodine demonstrated inflammation of the lungs, breathing problems, and coughing, which persisted for weeks. The lowest doses causing effects were 7- 12 mg kg^{-1} . Doses of 14–18 mg kg⁻¹ caused pulmonary edema and death within 24 h. In guinea pigs, 0.5 ppm did not cause detectable effects, but 7 ppm caused impaired breathing capacity. Adult female rats fed 500, 1000, 1500, or 2000 ppm iodine (as potassium iodide; KI) from 0 to 35 days prior to giving birth exhibited increased neonatal mortality with increasing dose, and milk secretion was reduced as evidenced by examination of the mammary glands. Reproductive impairment was also noted in studies with rabbits and chickens. Rabbits fed 250 ppm iodine for 2-5 days in late gestation exhibited increased mortality of young, and hens fed 312-5000 ppm KI ceased egg production within 1 week. Clinical signs of excessive dietary iodide in cattle include lacrimation, nasal discharge, conjunctivitis, hair loss, dermatitis, and exopthalmia.

Human

Excessive ingestion in humans can result in iodide goiter resulting from inhibition of the thyroid gland. The resulting lack of thyroid hormone secretion causes compensatory increase in thyrotropin secretion and thyroid enlargement. Patients treated with radioactive iodine (¹³¹I) have been studied for chromosome aberrations (dicentrics) in blood samples taken before and at various times after exposure. The increase in aberrations caused by the exposure to iodine was small but statistically significant. In another study, ¹³¹I induced clastogenic and age-dependent aneugenic effects in the lymphocytes of exposed patients. The X chromosome was not preferentially involved in the aneugenic effect induced by ¹³¹I, and it was concluded that, besides its major clastogenic effect, ¹³¹I can also induce an X chromosome-independent aneugenic activity mainly in patients with spontaneous proneness to chromosome loss. A mild toxic syndrome called iodism results from repeated administration of small amounts of iodine. Iodism is characterized by salivation, coryza, sneezing, conjunctivitis, headache, laryngitis, bronchitis, stomatitis, parotitis, enlargement of the submaxillary glands, and skin rashes.

Clinical Management

Rescue workers should avoid direct contact with the chemical. The source of contamination should be removed or the victim should be moved to fresh air. The worker should immediately wash the skin when it becomes contaminated. Oxygen may be administered by a trained professional. A conscious victim who has ingested excess iodine should rinse his or her mouth with water; however, vomiting should not be induced. The victim should drink 8-10 ounces of water. If vomiting occurs, the victim should lean forward to reduce the risk of aspiration. If contact with the eyes occurs, the eyes should be immediately flushed with lukewarm, gently flowing water for at least 15 min, taking care not to rinse contaminated water into the eye. General supportive measures should be provided.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists ceiling limit is 0.1 ppm, and this is also the (US) Occupational Safety and Health Administration permissible exposure ceiling limit. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (15 min) ceiling value is 0.1 ppm (1 mg m^{-3}) and the NIOSH immediately dangerous to life or health value is 2 ppm. The US recommended daily allowance of iodine is 100–200 mg day⁻¹.

See also: Generally Recognized as Safe (GRAS); Metals; Sensitivity Analysis.

Further Reading

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Ionizing Radiation See Radiation Toxicology, Ionizing and Nonionizing.

Iron

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- REPRESENTATIVE COMPOUNDS: Ferrous sulfate (FeSO₄); Iron oxide (Fe₂O₃)
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7439-89-6
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Metal
- CHEMICAL FORMULA: Fe³⁺

Uses

Iron is one of the major essential elements and one of the most important commercial metals. The total number of products made from iron is greater than that of all other metals combined. Iron is the basis for various steels and is used in pigments, fuel additives, catalysts, magnetic tapes, and animal feeds. As an essential element, iron is the central atom in the hemo of hemoglobin. Medicinally, it is administered to anemic patients and to many premenopausal women.

Exposure Routes and Pathways

The primary exposure pathway for iron is ingestion. Iron is present in practically all foods, in dietary supplements, and in drinking water. In some drinking water, iron concentrations may be especially high as iron pipes have been used extensively in transporting potable water. Iron concentration in surface water varies greatly, from 61 to 2680 ppm. In industrial settings, inhalation is a significant exposure pathway (e.g., arc welders are exposed to a high atmosphere of metal fumes and particles). Analyses of urban air samples show that the iron content averages $1.6 \,\mu g \,m^{-3}$, with the iron and steel industry probably the most likely source of emission. Dermal contact is

not a significant exposure pathway; however, iron is a natural component of soils and its concentration can be influenced by some industries.

Toxicokinetics

The chemical form of iron influences absorption, as do interrelationships with other dietary components. The disposition of iron in the human body is regulated by a complex mechanism to maintain homeostasis. Iron has the capacity to accept and donate electrons readily, and iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage as a result of formation of free radicals. The content of body iron is regulated primarily by absorption since humans have no physiological mechanism by which excess iron is excreted. Iron is absorbed (in a complicated process) through the gastrointestinal tract as the ferrous ion, first into the mucosal cells, where it oxidizes into the ferric state, and then is carried by the plasma. It is bound to the iron protein, transferrin, a globulin which transfers the iron to the various tissues. The enzyme ferroxidase oxidizes the ferrous ion to the ferric state. Most absorbed iron is found bound to hemoglobin (66%), a small amount is found in the protein myoglobin, and a minute amount is found in the iron-dependent enzymes.

Iron is stored in the blood, liver, bone marrow, and spleen. The storage proteins for iron are ferritin and hemosiderin. With 'iron overload', more ferritin is synthesized in the liver to bind this excess iron. Iron is a cofactor for hemoglobin and cytochromes.

The homeostasis mechanism permits up to 15% of ingested iron to be absorbed while the average person only excretes 0.01% of the intake. During periods of increased demand, such as pregnancy or childhood, absorption of iron is greatly increased. Normally, excess iron is excreted and some is contained within shed intestinal cells and in bile and urine. Smaller amounts are excreted in sweat, nails, and hair. Approximately 0.5 mg of total iron is excreted per day.

Mechanism of Toxicity

In some adults, iron overload can be the result of a genetic defect (idiopathic hemochromatosis) that causes malfunction of the normal homeostasis mechanism and, in turn, excessive absorption of iron. Iron overload can also be caused by too many blood transfusions, which results in too much iron in the various iron-containing organs.

Recently, it has been suggested that the presence of increased transferrin concentrations in males is associated with an increased number of heart attacks. This must be corroborated by further research.

Excess iron can lead to diabetes mellitus, faulty liver functions, and endocrine disturbance. Iron is a catalyst for oxidative damage leading to lipid peroxidation. The latest hypotheses link peroxidation to heart disease, cancer, and accelerated aging. Iron is involved in the Fenton Reaction, which catalyzes the formation of free radicals that cause excessive damage to cells and their components.

Acute and Short-Term Toxicity (or Exposure)

Animal

In a few animal experiments, sarcomas have appeared at the site following the injection of a large dose of the dextran salt or the lactate or gluconate.

Human

Most iron toxicity is found in very young children who ingest iron-containing medicines with candylike coatings. Fatalities have occurred from childhood ingestion of iron. After consuming more than 0.5 g of iron, toxic symptoms can be delayed for up to 6 h. The gastrointestinal tract can be ulcerated, which alters the limiting mechanism of iron absorption. Besides nausea, vomiting of blood (due to ulceration of the gastrointestinal tract), and black stools, acidosis and some liver damage follow; this can, in some cases, lead to cirrhosis of the liver, liver failure, or renal failure.

Chronic Toxicity (or Exposure)

Animal

Iron does not appear to be mutagenic or teratogenic. However, these experimental findings of tumor formation are open to question and may be associated with 'solid-state carcinogenesis' (believed to be a result of an irritation-type effect at the site of injection as opposed to a genetic mechanism).

Human

Iron has been identified as a component of asbestos and other mineral and synthetic fibers. Inhalation of iron and iron oxide fumes or dust may result in deposition of iron particles in lungs, producing an X-ray appearance resembling silicosis. The carcinogenicity of iron is still under debate, for example, for colorectal and liver cancer. An increase in the incidence of lung cancer, as well as in that of tuberculosis and interstitial fibrosis, has been noted in hematite miners. Due to inadequate controls, it is possible that the increased incidence of lung diseases noted in the study is due to smoking or exposure to other carcinogens present in the occupational setting. The American Conference of Governmental Industrial Hygienists (ACGIH) assigns an A4 (not classifiable as a human carcinogen) ranking to iron. Excess free circulating iron damages blood vessels, and hypotension can occur.

Clinical Management

Acute iron poisoning is treated by removal of ingested iron from the gastrointestinal tract using emesis (vomiting) or gastric lavage and by providing therapy for the associated systemic effects of shock and acidosis. The chelation agent, deferrioxamine, is also administered to bind iron that was not successfully removed from the gastrointestinal tract and has been absorbed.

Excess iron can be removed by either phlebotomy (letting of blood from a vein) or administration of the chelating agent, deferrioxamine, for cases of chronic excess iron. Ascorbic acid can accelerate iron excretion about twofold.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average, for iron oxide dust and fume is 5 mg m^{-3} . The Environmental Protection Agency's Federal Drinking Water Guideline for iron is $300 \text{ \mug} \text{ l}^{-1}$.

See also: Blood; Metals; Poisoning Emergencies in Humans.

Further Reading

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Irritation Testing See Toxicity Testing, Irritation.

Islip Garbage Barge

Todd Canedy

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On March 22, 1987, an Islip, Long Island garbage barge set sail for Jones County, North Carolina. The Mobro 4000 was loaded with 3168 tons of municipal solid waste (MSW) for an entrepreneurial venture. This barge soon became a martyr of environmentalists, and made a media spectacle of the growing scarcity of landfill space in America. Tipping fees, or fees paid to dispose of waste at landfills are higher where landfill space is more scarce and lower where there is a higher abundance of land suitable for landfills. In a hurried attempt by Lowell Harrelson, a New York businessman, to make a few dollars on the increasing garbage crisis in New York City, the Mobro 4000 was sent south in hopes of cashing in on the lower tipping fees of rural North Carolina. Mr. Harrelson failed to secure a dumping contract prior to setting sail. Rather, he tried to negotiate a contract after the barge was already on its way.

Upon arrival at the North Carolina port, a public outcry began resounding through the media. Concerned citizens spotted the barge offshore, piled high with rotting solid waste. The proper authorities were alerted who soon began an investigation.

In order to save a few dollars, the owners of the barge declined to purchase a tarp to cover the MSW

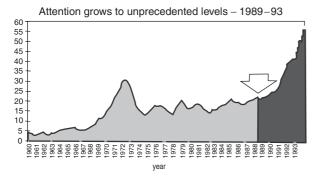


Figure 1 Recycling trends by year.

for its trip south. The said purchase may very well have prevented public dissent. The complaining citizens criticized that the stinking mass of out-of-state waste was floating in their ocean rather than being disposed off in its originating state. Due to the lack of a previous-made dumping contract, concerns were raised about medical, toxic, and other illegal contaminants in the waste.

Local officials called the deal off and sent the barge on its way. The barge eventually spent 164 days at sea, and traveled over 6000 miles before returning to New York City. North Carolina, Florida, Alabama, Mississippi, Louisiana, and Texas all declined port to the barge, as did Mexico, The Bahamas, and Belize.

After the media frenzy that followed the barge's voyage, the boat was forced to incinerate the MSW in a Brooklyn facility and bury the ashes in the same landfill from which the waste began its journey. This public spectacle served as a launch pad for environmentalists everywhere. The Mobro 4000 was an icon of America's excessive consumption and waste, and helped to push hundreds of communities to increase recycling efforts (see Figure 1). This event, coupled with other environmental issues of the time, served as a springboard for public involvement in environmentalism. Interest in waste stream, air pollution, water pollution, and other environmental health issues were never before on public minds to this extent.

See also: Resource Conservation and Recovery Act, US.

Further Reading

Murphy C and Rathje W (2001). *Rubbish!: The Archaeology of Garbage*. TUSCON, AZ: The University of Arizona Press.

Relevant Website

http://www.cfact.org – Committee for a Constructive Tomorrow.

Isocyanates

Robert Kapp

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Isocyanates are a group of low molecular weight aromatic and aliphatic compounds containing the isocyanate group (–NCO). The most widely used industrial isocyanates and their applications are listed below. The most notorious isocyanate is methyl isocyanate, involved in one of the worst industrial tragedies recorded in history. In the early morning hours of December 3, 1984, 200 000 people in Bhopal, India were exposed to methyl isocyanate. The 90 min exposure resulted in at least 2500 deaths and countless cases of severe eye and lung damage. Most of the deaths were related to the pulmonary edema.

HDI – Hexamethylene Diisocyanate

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 822-06-0
- SYNONYMS: 1,6-Diisocyanatohexane; HDI; Hexamethylene-1,6-diisocyanate; 1,6-Hexamethylene diisocyanate; HMDI
- Chemical Formula: $C_8H_{12}N_2O_2$

Uses

Hexamethylene diisocyanate (HDI) is used in the preparation of dental materials, medical adsorbents, and contact lenses, and is used as a polymerizing agent in polyurethane paints and coatings.

Background Information

HDI is a colorless liquid with an irritating odor.

Exposure Routes and Pathways

Inhalation and dermal exposure can occur during the manufacture and use of HDI. Workers and individuals in close proximity to an area where spray applications of polyurethane paints may be exposed.

Acute and Short-Term Toxicity (or Exposure)

Acute inhalation exposure may result in pulmonary edema, coughing, and labored breathing in humans. HDI is extremely irritating to the eyes, nose, and throat. Rodent studies revealed that HDI is extremely toxic by inhalation, and moderately to highly toxic by oral ingestion.

Chronic Toxicity (or Exposure)

Chronic inhalation exposure to HDI is thought to cause chronic lung irritation. In addition, chronic

inhalation exposure has been reported to cause irritation of the nasal tissues and respiratory tract. Dermal exposure has resulted in sensitization in several animal species. The US Environmental Protection Agency (EPA) has set the reference concentration (RfC) for HDI at $0.00001 \,\mathrm{mg \, m^{-3}}$ based upon the degeneration of the olfactory epithelium in rodents. EPA has not established a reference dose (RfD) for HDI.

Reproductive Toxicity

No information is available in the reproductive or developmental effects of HDI in humans. A rat reproductive study found no effects in any reproductive organs.

Carcinogenicity

No information is available on the carcinogenic effects of HDI in humans. Animals exposed to HDI were reported to show no evidence of carcinogenicity in a 2 year inhalation study. EPA has classified HDI as a group D (not classifiable as to human carcinogenicity.)

Clinical Management

Skin or ocular exposure areas should be generously irrigated with saline. All other treatment is symptomatic.

Exposure Standards and Guidelines

The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL), averaged over a 10 h workday is 0.005 ppm $(0.035 \text{ mg m}^{-3})$.

MIC – Methyl Isocyanate

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 624-83-9
- SYNONYMS: Methyl ester of isocyanic acid; MIC
- CHEMICAL FORMULA: C₂H₃NO

Uses

Methyl isocyanate (MIC) is used as a chemical intermediate in the production of carbamate insecticides and some herbicides.

Background Information

MIC is a colorless liquid with a sharp pungent odor.

Exposure Routes and Pathways

Inhalation, ingestion and dermal contact are all possible routes of exposure. Occupational exposure can occur during the use of carbamates produced from MIC. Small amounts of MIC are found in cigarette smoke.

Acute and Short-Term Toxicity (or Exposure)

The acute toxic effects of MIC are essentially similar by either route except for the intensity of the effects. When rats were administered MIC by inhalation or subcutaneous route it produced severe hyperglycemia, clinical lactic acidosis, highly elevated plasma urea, and reduced plasma cholinesterase activity with unaltered erythrocyte acetyl cholinesterase activity. Irrespective of the route of administration, MIC also caused severe hypothermia, which was not ameliorated by prior administration of atropine sulfate. Rats and mice of each sex were exposed by inhalation to MIC at 0, 1, and 3 ppm for $6 h day^{-1}$ for 4 days followed by up to a 91 day recovery period. Only animals exposed to 3 ppm exhibited exposure related changes. Most of the rats exposed at 3 ppm died within 28 days. A prominent decrease in body weight was observed along with severe lung lesions and thymus atrophy. Lesions of the nasal cavity of rats and mice were characterized by regeneration of the olfactory and respiratory epithelium. By day 28, the respiratory epithelium of rats and mice appeared normal, but olfactory regeneration was still present in the surviving rats. Severe lesions of the trachea extending to the bronchi, bronchioles, and alveoli were seen in rats exposed to 3 ppm. Acute inflammation of the airways and hyaline membranes was observed in high dose animals killed on day 7. In the 3 ppm MIC-exposed animals that died during days 8-14, there was lymphatic necrosis of the thymus, atrophy of the splenic white pulp, coagulative necrosis of the liver, and thrombosis of the left cardiac atrium. Gross examination of exposed mice showed minimal differences between exposure groups. Microscopic observations showed treatment-related changes only in the 3 ppm exposure group of mice. The changes involved primarily the bronchial system and were not as severe as those observed in rats. By day 91, bronchial fibrosis was minimal to mild in mice. One group of five male rats was exposed to 3 ppm MIC for a single 6 h exposure. The lesions in the respiratory system were essentially the same as those observed in the 4 day repeated exposure rats that were killed after 7 days.

In another acute toxicity study, rats were exposed only once to 3.52 and 35.32 ppm of MIC for 10 min; in a subacute study, they were exposed to doses of 0.212, 0.265, and 0.349 ppm for 30 min daily for 6 days and were then observed for 90 days for weight gain. At the end of 90 days, damage to the viscera was evaluated. During exposure, the animals had congestion in eyes, lachrymation, nasal secretion and dyspnea, progressively increasing ataxia, immobility, and uncoordinated movements. MIC exposure greatly inhibited weight gain in the animals in a dose-dependent manner. Upon microscopic examination of the viscera, pathological findings were confined to the bronchial tree, lung parenchyma, liver, and kidneys.

Acute inhalation exposure of humans results in pulmonary edema (most of the Bhopal deaths were due to pulmonary edema and secondary respiratory infections from pulmonary edema). Other acute effects include, blindness, nausea, gastritis, sweating, fever, chills, and liver and kidney damage. MIC was studied in in vivo micronucleus test and chromosomal analysis of bone marrow cells. Mice were exposed for 10 min to different concentrations (2.40, 4.80, or 7.20 µl) of MIC at 0 and 24 h. Quantitative analysis failed to exhibit any significant increase in aberration rates in the three treated groups. In another micronucleus assay, mice were exposed to MIC through ip injection for 2 and 5 days in separate experiments, and bone marrow and peripheral blood were sampled 6 and 48 h after the last injection, respectively. MIC did not significantly increase the frequencies of micronucleated erythrocytes in bone marrow and pheripheral blood samples in either twice or multiply treated mice. However, a dosedependent depression in percentage polychromatic erythrocytes observed was significant. This indicates that MIC exposure led to the cytotoxic effect by inhibition of bone marrow cell proliferation.

Chronic Toxicity (or Exposure)

The long-term carcinogenic and pulmonary effects of a single exposure to MIC were examined in rodents. Rats and mice were exposed to MIC by inhalation at 0, 1, 3, or 10 ppm for 2 h. After 2 years, the animals were sacrificed and tissues and organs were examined microscopically. No differences in survival rates or body weight gains were found in the MIC-exposed animals versus controls. Male and female rats exposed to 10 ppm MIC had 42% and 36% incidence, respectively, of intraluminal fibrosis of secondary bronchi; however, no evidence of this lesion was seen in controls or animals exposed to lower concentrations. For male and female mice and female rats, no neoplastic lesions were significantly associated with MIC exposure. Male rats exposed to MIC had marginally increased rates of pheochromocytomas of the adrenal medulla and adenomas of pancreatic acinar cells cells. EPA has not established an RfC or an RfD for MIC.

Reproductive Toxicity

Follow-up of exposed humans after the Bhopal incident found a high level of stillborns, spontaneous abortions and increased infant mortality. There was also a high number of survivors with pelvic inflammatory disease, excessive menstrual bleeding and suppression of lactation. Pregnant mice and rats were exposed to 9 or 20 ppm MIC to determine if the chemical was able to cross the placental barrier and directly affect the fetus. The mice were exposed on day 8 and the rats on day 10 of gestation to 9 ppm MIC for 3 h for evaluation of *in vivo* fetal toxicity. In other experiments the animals were exposed for 2 h to 20 ppm and the embryos removed immediately for culture. MIC exposure reduced maternal progesterone levels in mice that lost but not in mice that retained pregnancy. No relationship was observed between fetal toxicity of MIC and maternal plasma corticosterone levels. Fetal toxicity of MIC was not affected by chronic administration of progesterone or the suppression of pulmonary edema with dexamethasone. A concentration dependent decrease in growth in culture was noted in embryos exposed in utero or in vitro to MIC vapor. No fetal toxicity was noted following exposure to an acute dose, 3 mmol kg^{-1} , of the metabolites. The results indicate that the fetal toxicity of MIC is partly independent of maternal toxicity and may result from the transfer across the placenta and the interaction with fetal tissues.

Monomethylamine, dimethylamine, and trimethylamine are endogenous substances as well as metabolites of MIC. Methylamines exert several toxic effects including inhibition of protein turnover and oocyte RNA synthesis. A study conducted to determine the developmental toxicity of these methylamines using pregnant mice and mouse embryo culture used ip injections (daily from day 1 to day 17 of gestation) of trimethylamine at 2.5 and $5 \,\mu\text{mol}\,\text{kg}^{-1}\text{day}^{-1}$. Trimethylamine significantly decreased fetal body weight but not the placental weight or maternal body weight gain; however, 5 of 11 mice treated with $5 \mu mol kg^{-1}$ trimethylamine died. Similar treatment with dimethylamine or monomethylamine did not exert any obvious maternal or fetal effects. All three methylamines, when added to embryos in culture, caused dose dependent decreases in size, DNA, RNA, and protein content as well as embryo survival; the order of toxicity was trimethylamine > dimethylamine > monomethylamine. The ability of monomethylamines to adversely affect fetal development suggests that these methylamines, especially

trimethylamine, may act as endogenous teratogens under certain conditions.

Carcinogenicity

No information is available on the carcinogenic effects of MIC in humans. Animals exposed via inhalation gave mixed results. EPA has classified MIC as a group D (not classifiable as to human carcinogenicity.)

In Vitro Toxicity Data

MIC was nonmutagenic in the Ames (Salmonella), Drosophila sex-linked recessive lethal assays. MIC induced chromosomal aberrations in cultured Chinese hamster ovary cells.

Environmental Fate

MIC may be released to the environment as a result of its manufacture and use as a chemical intermediate. If MIC is released to soil, it will be expected to rapidly hydrolyze if the soil is moist, based upon the rapid hydrolysis observed in aqueous solution. If released to water, it will be expected to rapidly hydrolyze with half-lives of 20 and 9 min at 15°C and 25°C, respectively, calculated from measured overall hydrolysis rate constants. The products of hydrolysis may include N-carboxymethylamine, methylamine, carbon dioxide, and N,N'-dimethylurea. Since it rapidly hydrolyzes, bioconcentration, volatilization, and adsorption to sediment and suspended solids are not expected to be significant processes. No data were located concerning biodegradation, but MIC will probably abiotically hydrolyze significantly faster than it will biodegrade. If released to the atmosphere, it will be expected to exist almost entirely in the vapor phase based upon its vapor pressure. It will be susceptible to photooxidation via vapor phase reaction with photochemically produced hydroxyl radicals. Hydrolysis of MIC in moist air may be significant based upon its rapid hydrolysis in aqueous solution.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h timeweighted average (TWA) is 0.02 ppm, with a designation that skin exposure is also an important exposure. The (US) Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL), 8 h TWA is 0.02 ppm (with a skin notation). The US NI-OSH REL is 0.02 ppm as a 10 h TWA, and the NI-OSH immediately dangerous to life or health (IDLH) value is 3 ppm. MIC is listed by EPA as a hazardous air pollutant generally known or suspected to cause serious health problems. The Clean Air Act, as amended in 1990, directs EPA to set standards requiring major sources to sharply reduce routine emissions of toxic pollutants.

MDI – 4,4'-Methylenediphenyl Diisocyanate

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 101-68-8
- SYNONYMS: 4,4'-Diphenylmethane diisocyanate; Methylene bis(4-phenyl isocyanate); Methylene di-*p*-phenylene ester of isocyanic acid
- Chemical Formula: C₁₅H₁₀N₂O₂

Uses

4,4'-Methylenediphenyl diisocyanate (MDI) is used to produce polyurethane foams.

Background Information

MDI is a light-yellow fused solid or it may occur in crystalline form.

Exposure Routes and Pathways

Inhalation and dermal exposure can occur during the manufacture and use of MDI. Workers and individuals in close proximity to the plant may inhale emissions from urethane foam production manufacturing facilities.

Acute and Short-Term Toxicity (or Exposure)

Acute inhalation exposure may result in sensitization and asthma in humans. Dermal contact with MDI resulted in dermatitis and eczema in plant workers. Animal studies revealed skin and eye irritation in rabbits, extreme toxicity by inhalation and moderate toxicity by oral ingestion in rodents.

Chronic Toxicity (or Exposure)

Chronic inhalation exposure to MDI is one of the leading causes of asthma in plant workers. In addition, chronic inhalation exposure can cause dyspnea, immune disorders as well as nasal and lung lesions. EPA has set the RfC for MDI at 0.0006 mg m^{-3} based upon irritation of nasal membranes in rodents. EPA has not established an RfD for MDI.

Reproductive Toxicity

No information is available in the reproductive or developmental effects of MDI in humans; however, some effects (decreased placental and fetal weights and increased skeletal variations) were noted in a rat study.

Carcinogenicity

No information is available on the carcinogenic effects of MDI in humans. Animals exposed to polymeric MDI were reported to increase the incidence of pulmonary adenomas. EPA has classified MDI as a group D (not classifiable as to human carcinogenicity).

Exposure Standards and Guidelines

The (US) OSHA PEL 8 h TWA is 0.02 ppm (0.2 mgm^{-3}). The (US) NIOSH REL is 0.005 ppm (0.05 mgm^{-3}) as a 10 h TWA, and the NIOSH IDLH value is 75 mgm $^{-3}$.

TDI – Toluene Diisocyanate

- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 584-84-9
- SYNONYMS: 2,4-TDI; 2,4-Toluene diisocyanate
- Chemical Formula: C₉H₆N₂O₂

Uses

Toluene diisocyanate (TDI) is commonly used as the 2,4 and 2,6 isomers. It is used as a chemical intermediate in the production of polyurethane materials including foams, coatings and elastomers, as a crosslinking agent for nylon-6, and as a hardener in polyurethane adhesives and finishes. Polyurethane elastomers made from TDI are used in coated fabrics and clay-pipe seals. Polyurethane coatings made from TDI are used in floor finishes, wood finishes and sealers, and in coatings for aircraft, tank trucks, truck trailers, and truck fleets.

Background Information

TDI is a colorless, yellow, or brown liquid with a sharp pungent odor. The major metabolites of TDI in animals and humans are toluenediamines and their acetylated products.

Exposure Routes and Pathways

Inhalation and dermal exposure can occur during the manufacture and use of TDI. Workers and individuals in close proximity to the plant may inhale emissions from urethane foam production manufacturing facilities. It can be present as a unreacted impurity in materials, for example, TDI has been found in a urethane foam fabric coating in a concentration of $< 200 \text{ mg kg}^{-1}$.

Toxicokinetics

The toxicokinetics of TDI (2,4- and 2,6-toluenediisocyanates) in chronically exposed workers at two flexible foam polyurethane production plants have been reported. The half-life in urine ranged from 5.8 to 11 days for 2,4- and 2,6-toluenediamines. The differences in exposure were reflected by the plasma toluenediamine concentrations. The mean half-life in plasma was 21 days for 2,4-toluenediamine and 21 days for 2,6-toluenediamine, The study showed that the half-life in plasma of chronically exposed workers for 2,4- and 2,6-toluenediamine was twice as long as for volunteers with short-term exposure. An indication of a two-phase elimination pattern in urine was found. The first phase was related to the more recent exposure and the second, much slower one was probably related to release of toluenediamines in urine from TDI adducts in the body. Two men were exposed to TDI atmospheres in a stainless-steel test chamber. The effective exposure period was 4 h. The isomeric composition of the air in the test chamber was 30% 2,4-TDI and 70% 2,6-TDI. In plasma, 2,4and 2,6-toluenediamine showed a rapid-phase elimination half-life of $\sim 2-5$ h, and that for the slow phase was greater than 6 days. A connection was observed between the concentrations of 2,4- and 2,6-TDI in air and the levels of 2,4- and 2,6-toluenediamine in plasma. The cumulated amount of 2,4-toluenediamine excreted in the urine over 24 h was $\sim 15-19\%$ of the estimated inhaled dose of 2,4-TDI, and that of 2,6toluenediamine was \sim 17–23% of the inhaled dose of 2,6-TDI. In another study, five men were exposed to TDI atmospheres for 7.5 h in a stainless-steel test chamber. The urinary elimination of the toluenediamines showed a possible biphasic pattern, with rapid first phases for 2,4-toluenediamine (mean half-life for the concn in urine, 1.9 h) and for 2,6-toluenediamine (mean half-life for the concn in urine, 1.6 h). The cumulative amount of 2,4-toluenediamine excreted in urine within 28h ranged from 8% to 14% of the estimated dose of 2,4-TDI, and the cumulative amount of 2,6-toluenediamine in urine ranged from 14% to 18% of the 2,6-TDI dose. The average urinary level of 2,4-toluenediamine was $5 \mu g l^{-1}$ in the 6-8h sample, and the corresponding value for 2,6toluenediamine was $8.6 \,\mu g l^{-1}$. Biological monitoring of exposure to 2,4- and 2,6-TDI by analysis of 2,4and 2,6-toluenediamine in urine is feasible.

Acute and Short-Term Toxicity (or Exposure)

In laboratory animals, TDI has caused inflammation and necrosis when applied directly to the skin, conjunctivitis when applied to the eyes, and rhinitis, laryngitis, tracheitis, bronchitis, and pneumonia when inhaled. All workers develop eye, nose, and throat irritation at 0.5 ppm exposure to TDI. Sensitized individuals may manifest symptoms at levels as low as 0.02 ppm. TDI is a potent respiratory irritant and sensitizer, even at low airborne concentrations. Chronic bronchitis, chronic restrictive pulmonary disease, and hypersensitivity pneumonitis have also been described among TDI-exposed people. The mechanism of TDI-induced asthma is still unknown. TDI may produce a true hemorrhagic syndrome affecting the bone marrow and producing primarily thrombocyte series suppression.

Chronic Toxicity (or Exposure)

Chronic inhalation exposure results in severe lung effects that are characterized by asthma-like reactions characterized by dyspnea, wheezing, and bronchial constriction. The US EPA has neither established an RfC (for inhalation exposure) nor an RfD (for oral exposure) for TDI.

Reproductive Toxicity

No information is available in the reproductive or developmental effects of TDI.

Carcinogenicity

Rats and mice were administered commercial grade TDI (80% 2,4- and 20% 2,6-) in corn oil by gavage at doses of 60 or 120 mg kg⁻¹ body weight, 5 days per week for 105 or 106 weeks. Other groups of rats and mice received 120 or 240 mg kg⁻¹ on the same schedule. The results indicated that commercial grade TDI in corn oil was carcinogenic for rats, causing subcutaneous fibromas and fibrosarcomas (combined) in males and females, pancreatic acinar cell adenomas in males, and pancreatic islet cell adenomas, neoplastic nodules of the liver, and mammary gland fibroadenomas in females. TDI was not carcinogenic for male mice, but was carcinogenic for female mice, causing hemangiomas or hemangiosarcomas (combined), as well as hepatocellular adenomas. The International Agency for Research on Cancer (IARC) has judged that TDI is a group B chemical, that is, there is inadequate evidence for the carcinogenicity of TDI in humans, there is sufficient evidence for the carcinogenicity of TDI in experimental animals, and the overall evaluation is that TDI is possibly carcinogenic to humans. The (US) National Toxicology Program (NTP) lists TDI as an anticipated human carcinogen.

In Vitro Toxicity Data

TDI was mutagenic in the Ames Salmonella assay, and induced chromosome aberrations after a 24 h treatment in the absence of metabolic activation in human whole blood lymphocyte cultures. To investigate the role of pharmacological mechanisms in TDI-induced occupational asthma, the effects of TDI on rat trachea ring and lung parenchymal strip were studied *in vitro*. The most prominent effect observed was a stimulation of metacholine-induced contraction of the tracheal ring by $1 \,\mu$ moll⁻¹ TDI. It was concluded that the pharmacological effect of TDI may result from an autonomic imbalance between cholinergic and B-adrenergic neural control.

Environmental Fate

TDI's production and uses may result in its release to the environment through various waste streams. If released to air, TDI will exist solely as a vapor in the ambient atmosphere and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 2.7 days. Atmospheric degradation may also occur through contact with clouds, fog, or rain. If released to water or moist soil, toluenediisocyanate is not expected to leach or adsorb to solids due to its rapid degradation reaction with water. It is not expected to bioconcentrate in aquatic organisms.

Isodrin 645

Exposure Standards and Guidelines

The (US) OSHA PEL, 8 h TWA is 0.02 ppm (0.14 mg m⁻³).

See also: Bhopal; Carbamate Pesticides.

Further Reading

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Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Isocyanates.

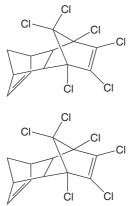
- http://www.tc.gc.ca Isocyanates (from Transport Canada).
- http://www.cdc.gov US National Institute for Occupational Safety and Health (NIOSH). Isocyanates (NIOSH Safety and Health Topic).

Isodrin

K S Rao

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 465-73-6
- SYNONYMS: 1,4;5,8-Dimethanonaphthalene,1,2,3,4,10,10-hexachloro-, 4,4a,5,8,8a hexahydro-endo, Compound 711; Experimental Insecticide 711; SD 3418
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Cyclodiene; Insecticide
- CHEMICAL FORMULA: C₁₂H₈Cl₆
- CHEMICAL STRUCTURE:



Uses

Isodrin, a cyclodiene insecticide has been discontinued and is no longer used in the United States.

Exposure Routes and Pathways

Exposure to isodrin can occur by inhalation, or ingestion; however, the primary exposure is through the dermal route to mixers, loaders, and applicators, during and after normal use.

Toxicokinetics

Isodrin is metabolized by biooxidation to endrin. Isodrin and its metabolite, endrin, have high fat:water partition coefficients and, therefore, tend to accumulate in adipose tissue. At a constant rate of intake, however, the concentration of the insecticide in adipose tissue reaches an equilibrium and remains relatively constant. Following cessation of exposure, it is slowly eliminated from the body. *In vitro* studies have shown that mixed-function oxidase of mouse liver converts isodrin to endrin. Mice excrete 10% of the orally administered dose in urine. Four unidentified metabolites were present in urine, three probably are glucuronide or sulfate conjugates. Feces is the major route of excretion for isodrin in mice. Five metabolites were present in the organic extracts. Acid hydrolysis of the aqueous phase released four metabolites. None were identified.

Mechanism of Toxicity

Isodrin, a cyclodiene insecticide, is a neuropoison, and the locus of primary toxic action is believed to be sensory and motor nerve fibers and the motor cortex. The underlying mechanism of neurotoxic effect is the slowing of the cessation of sodium conductance across the nerve membrane and inhibition of the initiation of the potassium conductance. A prolongation of the afterpotential results in repetitive firing in the presynaptic nerve membrane, which is due primarily to the slowing of the falling phase of the sodium current and partly to the decrease in the steady state potassium current. Isodrin was among 11 cyclodiene insecticidal compounds tested for the ability to induce detoxifying microsomal oxidase. Isodrin showed a maximum of 30% increase in enzyme activity. The induction of enzymes appeared to be a nonspecific phenomenon.

Acute and Short-Term Toxicity (or Exposure)

Animal

From its acute oral toxicity in animals, isodrin would be considered extremely toxic; the oral LD_{50} in rats is 7 mg kg⁻¹. Isodrin is one of the extremely toxic chlorohydrocarbon insecticides. It is a skin irritant. Oral administration of isodrin produces central nervous system (CNS) symptoms, and survivors may develop liver and kidney damage.

Human

The probable oral lethal dose for humans is in the range of $5-50 \,\mathrm{mg \, kg^{-1}}$ (between seven drops to one teaspoon for a 150 lb person). Signs and symptoms of poisoning in humans resulting from high doses of isodrin are due to excitation of the CNS. Acute exposure to isodrin may result in overall discomfort, headache, dizziness, agitation, nervousness, disturbed behavior, tremors, seizures, and/or coma. Convulsive episodes may alternate with periods of severe CNS depression. Seizures may be the first symptom of acute exposure, occurring within minutes to hours of a sufficient exposure to isodrin. Nausea, vomiting, and diarrhea are common side effects. Hypertension (high blood pressure), tachycardia (rapid heart rate), and cardiac arrhythmias (abnormal heart beating) may be noted. Respiratory

depression may lead to respiratory arrest. Contact of isodrin with the skin, eyes, and mucous membranes may result in redness and irritation. Victims often have an elevated temperature.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of isodrin to rats results in electroencephalogram abnormalities and seizures.

Human

Epileptiform convulsions and abnormal electroencephalographic patterns have been found in studies of insecticide manufacturing workers suffering from intoxication by isodrin. Fourteen patients with convulsions caused by the insecticide all showed specific anomalies in the electroencephalogram, consisting of bilateral synchronous theta wave activity and occasional bilateral synchronous spike and wave complexes believed to be associated with brain stem injury.

Clinical Management

Acute exposure to isodrin may require decontamination and life support for the victims. Emergency personnel should wear protective clothing appropriate to the type and degree of contamination. Airpurifying or supplied-air respiratory equipment should also be worn, as necessary. Rescue vehicles should carry supplies such as plastic sheeting and disposable plastic bags to assist in preventing the spread of contamination. After acute exposure, vital signs should be evaluated, including pulse and respiratory rate, and any trauma should be noted. If no pulse is detected, cardiopulmonary resuscitation (CPR) should be provided. If the victim is not breathing, artificial respiration should be provided. If breathing is labored, oxygen or other respiratory support should be administered. In case of inhalation exposure, the victims should be moved to fresh air. In case of dermal exposure, the contaminated clothing should be removed as soon as possible. The exposed skin areas should be washed three times. Initially, washing should be done with soap and water, followed with an alcohol wash, and then again with soap and water. If eye exposure has occurred, the eyes must be flushed with lukewarm water for at least 15 min.

Periodic electroencephalographic examination is valuable for the detection of early subclinical intoxication. Persons with a history of convulsive disorders would be expected to be at increased risk from isodrin exposure. The concentration of isodrin in the blood is helpful in determining the extent of absorption.

Treatment in acute exposure is symptomatic and supportive. Oils should not be used as either cathartics or dermal cleansing agents, as they increase absorption. Gastric lavage and use of activated charcoal and sodium sulfate are indicated for ingestion. Management of seizures is with valium or phenobarbital.

In cases of chronic ingestion exposure, isodrin can accumulate in the adipose tissue, due to its lipophilicity. It is encouraging that a means of hastening the excretion of stored isodrin has been developed. This involves the use of anion exchange resin, cholestyramine, which, when given orally to patients, enhances fecal excretion of isodrin. The rationale for the use of cholestyramine relates to the biliary-enterohepatic circulation, which cycles isodrin; hence, cholestyramine by binding the insecticide, interrupts the reabsorption phase and shifts the equilibrium from reabsorption and storage to fecal excretion.

Environmental Fate

If released into the soil, isodrin may undergo microbial oxidation to endrin. The behavior of isodrin in soil may range from moderately mobile to immobile and isodrin is not expected to hydrolyze since it contains no hydrolysable functional groups. The soil half-life of isodrin has been estimated to be 0.5–1 year. It is absorbed by the roots of plants and is likely to be translocated to above ground parts of plants. If released into water, isodrin may bioconcentrate in aquatic organisms, adsorb onto suspended soils and sediments and undergo very slow microbial transformation. The bioconcentration factor of isodrin was found to be 4500 in molluscs.

See also: Cyclodienes; Organochlorine Insecticides.

Further Reading

Gosselin RE, Smith RP, and Hodge HC (1984) *Clinical Toxicology of Commercial Products*, 5th edn. Baltimore, MD: Williams and Wilkins.

Isoniazid

Lisa Vivero

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 54-85-3
- SYNONYMS: Isonicotinic acid hydrazide; INH
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Isonicotinic acid derivative; Antitubercular agent
- CHEMICAL STRUCTURE:



Uses

Isoniazid is an antibiotic used for the treatment and prevention of tuberculosis infection.

Exposure Routes and Pathways

Isoniazid is usually ingested orally as a tablet, capsule, or liquid. An injectable formulation is also available.

Toxicokinetics

Isoniazid is rapidly absorbed from the gastrointestinal tract reaching peak plasma concentrations within 1-2h of ingestion. The extent of absorption may be reduced by oral administration with food or aluminum-containing antacids. Isoniazid binds negligibly to plasma proteins and distributes into all body fluids with a volume of distribution of 0.61 kg^{-1} . The metabolism of isoniazid follows Michaelis-Menten kinetics and occurs mainly in the liver by acetylation. The rate of acetylation is genetically determined and is expressed phenotypically as 'fast' (90% of Asians and Inuits) and 'slow' (50% of White and African-Americans) acetylators. Fast acetylators metabolize isoniazid up to 6 times faster, producing plasma concentrations 30-50% lower than in slow acetylators. The mean elimination half-life of isoniazid is about 1 h for fast acetylators and up to 5 h for slow acetylators. Fast acetylators eliminate 11% of isoniazid

unchanged, and slow acetylators excrete 27% unchanged. Approximately 50–70% of an isoniazid dose is renally eliminated within 24 h of ingestion, mainly as metabolites. Metabolites include acetylisoniazid, isonicotinic acid, acetylhydrazine, diacetylhydrazine, and hydrazine.

Mechanism of Toxicity

Isoniazid may cause toxicity directly through toxic intermediates, and immunologic responses, or indirectly through the depletion of pyridoxine (vitamin B_6), and interference with several enzymes and cofactors including those needed to produce γ -aminobutyric acid (GABA), nicotinamide adenine dinucleotide (NAD), and niacin (vitamin B_3).

The exact mechanism of isoniazid-induced hepatotoxicity is unknown. However, the metabolite acetylhydrazine is believed to be responsible for hepatic injury when it is converted to toxic intermediates via cytochrome P-450 (CYP)2E1. Persons with the CYP2E1c1/c1 genotype may be more susceptible to hepatotoxicity. The role acetylator status plays in hepatotoxicity continues to be debated, but it is currently thought that slow acetylators are at greater risk. Other risk factors include increasing age, chronic isoniazid overdosage, comorbid conditions such as malnutrition, pregnancy, diabetes, HIV, renal dysfunction, hepatic dysfunction, alcoholism, and concomitant use of enzyme inducing drugs.

Isoniazid-induced seizures are caused by the depletion of GABA, a primary inhibitory neurotransmitter that requires the cofactor pyridoxal-5'-phosphate for its synthesis from glutamate. Isoniazid-induced GABA deficiency is brought on by at least three processes: (1) metabolites form complexes with pyridoxine increasing its urinary excretion; (2) metabolites block pyridoxine-5'-phosphokinase, the enzyme that activates pyridoxine to pyridoxal-5'-phosphate; and (3) metabolites inactivate pyridoxal-5'-phosphate. Prolonged seizures commonly result in plasma lactic acid accumulation that can lead to a metabolic acidosis. Isoniazid may worsen the severity of acidosis by inhibiting the production of NAD, a cofactor necessary for the conversion of lactate to pyruvate. Longterm exposure to isoniazid therapy commonly causes peripheral neuropathy due to pyridoxine deficiency, and may induce pellagra, a niacin deficiency disorder. Niacin requires the cofactor pyridoxal-5'-phosphate for its production from tryptophan.

Other enzymes inhibited by isoniazid include the cytochrome P450 mixed function oxidases, monoamine oxidase, glutamate decarboxylase, and histaminase. The consequences of these extensive enzymatic disturbances are mood elevation, decreased central nervous system GABA levels, depressed catecholamine synthesis, defects in glucose and fatty acid oxidation, and impaired metabolism of other drugs. Important drug interactions include those with phenytoin, carbamazepine, warfarin, and rifampin.

Acute and Short-Term Toxicity (or Exposure)

Animal

When taken or administered in overdose to dogs, isoniazid produces seizures, metabolic acidosis, and, if untreated, death.

Human

Isoniazid intoxication is characteristically presented by generalized seizures, metabolic acidosis, and coma. Acute ingestions of more than 1.5 g may produce mild symptoms of malaise, nausea, vomiting, dizziness, slurred speech, and tachycardia. Ingestions of more than 2–5 g produce moderate toxicity, and ingestions of >6 g are typically fatal unless there is aggressive intervention. Seizures generally occur within 1 h, but may be delayed up to 5 h postingestion. Status epilepticus may occur with seizures lasting for hours followed by severe anion-gap metabolic acidosis. Coma may ensue after or between seizures.

Chronic Toxicity (or Exposure)

Animal

Rats dosed at 35 mg kg^{-1} isoniazid per day in drinking water for 48 weeks had slightly increased rates of liver and lung tumors compared to controls. Although studies in rats and rabbits have shown that Isoniazid is embryocidal, it has not been shown to be teratogenic in rats, mice, or rabbits.

Human

Chronic therapeutic ingestion of isoniazid is associated with several common adverse effects including rash, fever, and elevated liver function tests (in up to 20% of patients). Isoniazid-induced hepatitis occurs less frequently (0.1–2%) and generally occurs within the first 2 months of therapy. Complete hepatic failure may result if isoniazid therapy is continued. Neurologic symptoms of stocking-glove peripheral neuropathy, optic neuritis, hallucinations, pellagra, and seizures may occur in the absence of an overdose, especially in predisposed persons. Autoantibody production with resulting hemolytic anemia, thrombocytopenia, arthritis, or vasculitis may also develop.

In Vitro Toxicity Data

Mutagenicity studies of isoniazid have yielded mixed results. Some models of sister-chromatid exchange were positive. *Salmonella* assays have been positive and negative. *In vivo* nonhuman primate carcinogenicity studies have been positive.

Clinical Management

In the patient who presents with seizures, airway protection and seizure control are primary goals. Disturbances in cardiac rhythm or function also require immediate attention. Ipecac-induced emesis is contraindicated due to the risk of seizures and the resulting potential for aspiration. Gastrointestinal decontamination via administration of activated charcoal should be considered for substantial recent ingestions. Pyridoxine is administered intravenously to all symptomatic and potentially serious asymptomatic overdoses as it provides rapid relief or prevention of severe toxicity, including seizures. The pyridoxine dosage is equal to the estimated isoniazid dose. If the quantity of isoniazid taken is unknown, 5 g of pyridoxine is empirically given. Repeat doses may be necessary should signs of toxicity persist. Pyridoxine may be supplemented with a benzodiazepine or a barbiturate anticonvulsant. Seizure control usually resolves metabolic acidosis; however, intravenous sodium bicarbonate may be necessary to correct severe acidosis. Hemodialysis is considered for those unresponsive to all other therapy.

See Also: Niacin; Pyridoxine.

Further Reading

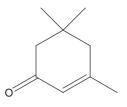
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- Wason S, Lacouture PG, and Lovejoy FH (1981) Single highdose pyridoxine treatment for isoniazid overdose. *Journal* of the American Medical Association 246: 1102–1104.

Isophorone

Leonard I Sweet

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 78-59-1
- SYNONYMS: Isoacetophenone; 1,1,3-Trimethyl-3-cyclohexene-5-one; 1,5,5-Trimethyl-1-cyclohexen-3-one; 3,5,5-Trimethyl-2-cyclohexen-1one; 3,5,5-Trimethyl-2-cyclohexenone; 3,5,5-Trimethylcyclohex-2-enone; 3,5,5-Trimethylcyclohexenone; Alpha-isophorone; Isoacetophorone; Isoforon
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Ketones
- CHEMICAL FORMULA: C₉H₁₄O
- CHEMICAL STRUCTURE:



Uses

Isophorone is used as an intermediate in the production of certain chemicals, and as a solvent for resins, polymers, and pesticide formulations. It is used as a solvent for concentrated vinyl chloride/acetate based coating systems, as an adhesive for plastics, and is found in metal paints, nitrocellulose finishes, printing inks for plastics, and some herbicide and pesticide formulations. Isophorone may occur naturally in cranberries.

Exposure Routes and Pathways

Exposure to isophorone can occur via inhalation, ingestion, skin or eye contact, and there is potential for skin absorption. Inhalation and dermal contact is expected to be the primary route of occupational exposure. The general population may be exposed to isophorone via ingestion of contaminated drinking water.

Toxicokinetics

Rapid absorption and elimination is expected after oral or inhalation exposure, though the rate, extent, and relative tissue distribution is not well characterized. Pharmacokinetic studies in rats indicated that the majority of orally administered isophorone was eliminated in the urine (predominantly), expired air, and feces, within 24 h. Studies in experimental animals suggest that isophorone is metabolized to dihydroisophorone, isophorol, diisophorone glucuronide, and other products after oral exposure; though different metabolic pathways may operate following other routes of exposure.

Mechanism of Toxicity

The toxicological mechanisms of isophorone are not well characterized. Critical effects include irritation, narcosis, malaise, fatigue, and central nervous system (CNS) depression. Isophorone may induce its neurological effects by interference with neuronal impulse transmissions via physical interaction with nerve membrane components. In animal models, isophorone may also act by inducing neuropathy, involving binding to globulin proteins, although this mechanism may not be relevant to humans. Lesions of the liver have been observed after overexposure in mouse models, although it is not clear whether isophorone elicited the lesions directly or by enhancing an age-related process. DNA-binding studies in mice have shown no significant covalent binding of isophorone or its metabolites to DNA from liver or kidney cells, supporting a potential nongenotoxic mechanism of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Isophorone causes irritation of the eyes, skin, mucous membranes, and respiratory tract, and CNS depression. Systemic effects of isophorone toxicity in animals include pulmonary congestion and hemorrhaging, hyperkeratosis of the forestomach, and liver and kidney damage. Short-term overexposure of animals to high levels of isophorone resulted in inactivity and coma. The acute toxicity if isophorone is low, with oral LD_{50} values >1500 mg kg⁻¹ in the rat, > 2200 mg kg⁻¹ in the mouse, and > 2000 mg kg⁻¹ in the rabbit. Dermal LD_{50} values were 1700 mg kg^{-1} in the rat and $> 1200 \text{ mg kg}^{-1}$ in the rabbit. Acute effects from dermal exposure in experimental animals ranged from mild erythema to scabs. Conjunctiva and corneal damage have been reported after direct application to the eye. Skin sensitization potential has been shown to be low.

In acute and short-term studies on rats given high doses of isophorone $(>1000 \text{ mg kg}^{-1})$ degenerative effects in the liver as well as CNS depression were observed, and there were some deaths. In 90 day oral studies with laboratory animals, no-observed-effect levels ranged from 150 to 500 mg kg⁻¹ body weight per day. Acute inhalation exposure of test animals

to isophorone resulted in irritation, decreased body weights, hematological effects, and pulmonary congestion.

Human

Adverse effects of isophorone reported by people who have been exposed include irritation of the skin, eyes, nose, and throat, as well as dizziness and fatigue. Irritant effects have been reported at air concentrations above 1 mg m^{-3} , whereas nausea, headache, and dizziness have been reported at above 1142 mg m^{-3} . The sharp odor of isophorone may induce olfactory fatigue. Dermal exposures have caused irritation including burning. The lowest published toxic concentrations for humans via inhalation are 140 mg m^{-3} for eye, nose, and pulmonary system effects. The estimated immediately dangerous to life or health air concentration is 200 ppm.

Chronic Toxicity (or Exposure)

Animal

Isophorone has been investigated for potential genotoxicity, and has generally been shown to lack significant activity in the mouse lymphoma, unscheduled DNA synthesis, and micronucleus assays. Animal models are generally negative with regard to potential reproductive and developmental toxicity of isophorone at high doses.

Isophorone has been investigated for potential carcinogenicity, and in male rats, caused an increase in tumors of the kidney, liver, lymph, and reproductive glands when exposed by ingestion. There was no increase in tumors in female rats or mice.

Human

The US Environmental Protection Agency has classified isophorone as a possible human carcinogen. The National Toxicology Program tested isophorone for evidence of carcinogenicity and found the following: male rat – some evidence; female rat – no evidence; male mice – equivocal evidence; female mice – no evidence. European Risk Phrases suggest there is possible risk of irreversible effects upon repeated overexposure.

In Vitro Toxicity Data

Isophorone induced sister chromatid exchanges but not chromosome aberrations in Chinese hamster ovary cells. Isophorone was positive in L5178Y tk +/tk mouse lymphoma cell forward mutation assay without metabolic activation. Isophorone was negative in tests with *Salmonella typhimurium* bacterial strains TA98, TA100, TA1535, TA1537, with or without metabolic activation.

Clinical Management

Management of individuals overexposed to isophorone begins with removing those individuals from the source of exposure, flushing eyes and skin with copious amounts of water, and removing contaminated clothing. If ingested, oral administration of charcoal as a slurry may prove therapeutic in limiting the absorption of isophorone from the intestine. In cases of respiratory overexposure, the victim should be moved to fresh air immediately, breathing should be monitored and oxygen supplied if difficult, and treatment given according to severity of irritation. Medical tests are not well characterized to determine the extent of overexposure to isophorone, although tests for kidney and liver function, as well as examination of the eyes and nose for chronic inflammation may be useful.

Environmental Fate

If released to the environment, isophorone is expected to preferentially partition to the soil and water. Bioconcentration and bioaccumulation potential is expected to be low, based on the estimated bioconcentration factor and experimental octanol-water partition coefficient. Biodegradation is not expected to occur rapidly. Volatilization is expected to be an important fate and transport process based on the Henry's law constant and vapor pressure. When released into the air, isophorone is expected to have a short half-life of much less than 1 day.

Ecotoxicology

The available data suggest that the aquatic toxicity of isophorone is low, with short-term toxicity values for freshwater algae, invertebrates, and fish ranging from 100 to 300 mg l^{-1} .

Other Hazards

Flammable and explosive when exposed to heat or flame; can react with oxidizing materials.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for isophorone include the following:

- American Conference of Governmental Industrial Hygienists (5 ppm Ceiling);
- Australia (5 ppm Peak);
- Belgium (5 ppm short-term exposure limit (STEL));
- Canada (5 ppm Ceiling);
- China $(30 \text{ mg m}^{-3} \text{ Ceiling});$
- Denmark (5 ppm Ceiling);
- Finland (1 ppm time-weighted average (TWA));
- Germany (4 ppm Peak; 2 ppm TWA);
- Mexico (5 ppm TWA);
- Portugal (5 ppm Ceiling);
- United Kingdom (5 ppm STEL); and
- US Occupational Safety and Health Administration permissible exposure limit (25 ppm TWA).

Miscellaneous

Isophorone is colorless to light yellow liquid with a peppermint or camphor-like odor. Odor is generally detected at concentrations ranging from 0.2 to 2 ppm. It is soluble in water and with most organic solvents.

See also: Neurotoxicity.

Relevant Websites

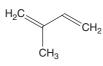
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- http://www.ecetoc.org ECETOC Joint Assessment, vol. 10 (1989). Isophorone: CAS 78-59-1. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.
- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Isophorone.

Isoprene

Kathryn A Wurzel

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 78-79-5
- SYNONYMS: 2-Methyl-1,3-butadiene; 2-Methylbutadiene; β-Methylbivinyl; Isopentadiene
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Reactive branched diene
- CHEMICAL FORMULA: C₅H₈
- CHEMICAL STRUCTURE:



Uses

The isoprene unit is the most important building block for lipids, steroids, terpenoids, and a wide variety of natural products. The only chemical reaction of commercial importance (other than polymerization) is its conversion to terpenes. Isoprene is used in the manufacture of 'synthetic' natural rubber, butyl rubber, and as a copolymer in the production of synthetic elastomers.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal exposure are exposure pathways for isoprene.

Toxicokinetics

Hemoglobin adduct formation is linearly related to administered doses of isoprene up to ~55 µmol kg⁻¹; the concentration of hemoglobin adducts may therefore be used as an indicator of previous exposure. Mice exhaled approximately twice as much butadiene as isoprene following exposure to isoprene. The percentage of inhaled isoprene metabolized decreased with increasing exposure concentrations and vapor concentration. Approximately 75% of the total metabolites are excreted in the urine, independent of the inhaled concentration. A higher percentage of metabolites are excreted in the feces following highconcentration exposures.

Saturation kinetics is observed in rats and mice. The half-life in rats and mice are 6.8 and 4.4 min, respectively, following inhalation exposure. The presence of isoprene products in the respiratory epithelium

even after short exposure durations suggests that, significant metabolism occurs in this tissue. Human studies have demonstrated 20% isoprene absorption in the upper respiratory tract with 70–99% being retained in the lungs.

Isoprene is metabolized to epoxides and diepoxides. Body fat appears to be a reservoir for isoprene and its metabolites.

Mechanism of Toxicity

A mutagenic metabolite, isoprene dioxide, was tentatively identified in all examined tissues following exposure to isoprene. It is believed that the formation of reactive epoxides following exposure to isoprene results in tumor induction.

Acute and Short-Term Toxicity (or Exposure)

Animal

A 2% isoprene air concentration did not cause central nervous system (CNS) depression in mice but did produce bronchial irritation.

Human

Acute contact with isoprene may irritate skin, eyes, and mucous membranes. Upper respiratory tract irritation is associated with exposure via inhalation. CNS depression is possible with exposure to high concentrations.

Chronic Toxicity (or Exposure)

Animal

Isoprene is nonmutagenic in bacterial test systems. However, isoprene forms adducts of blood hemoglobin in mice and rats. Increases in frequency of sister chromatid exchanges in bone marrow cells and in levels of micronucleated polychromatic erythrocytes were detected. Based on these results, isoprene is expected to induce tumors at multiple sites in exposed mice. Developmental toxicity has been indicated in mice, including decreased fetal body weight and ossification; these impacts were not noted in rats.

Mice exposed to 7000 ppm isoprene via inhalation showed decreased weight gain, testicular atrophy in males, and microscopic lesions. In addition, there is sufficient evidence of the carcinogenicity of isoprene in animals.

Human

There are no epidemiological data relevant to the carcinogenicity of isoprene in humans. It is considered a possible human carcinogen based on sufficient evidence of carcinogenicity in animals.

Clinical Management

There is no information available specifically for isoprene. Exposed skin and eyes should be flushed with copious quantities of water following exposure. Humidified oxygen should be administered after excess inhalation exposure. Induced emesis should be avoided if ingestion has occurred. Measures to decrease gastrointestinal absorption should be instituted (gastric lavage, activated charcoal, or dilution by administration of liquids).

Environmental Fate

Isoprene will exist as a vapor in the atmosphere where it will be degraded by reaction with photochemically produced hydroxy radicals, ozone molecules, and nitrate radicals. Isoprene is moderately mobile in soil and will volatilize from moist soil surfaces. In water, both volatilization and hydrolysis are expected to be important fate processes.

Miscellaneous

Isoprene occurs widely in nature as it is produced by plants during photosynthesis. It is also produced endogenously in humans.

See also: Carcinogenesis.

Further Reading

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Isopropanol

Michael D Reed

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- SYNONYMS: Numerous derivatives and brand names available. Isopropyl alcohol; 2-Propanol; 'Rubbing' alcohol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alcohol, disinfectant
- CHEMICAL FORMULA: C₃H₈O
- CHEMICAL STRUCTURE: CH₃-CHOH-CH₃

Uses

Isopropanol is used as a solvent in numerous industrial and commercial products including synthetic resins, coatings, lacquers, and paint removers. It is also used in drug and cosmetic formulations, including many toiletries, perfumes, and colognes. It is also found in consumer products such as windshield cleaning fluids and glass cleaners. Because of its widespread availability and low cost, isopropanol has been abused by alcoholics.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to isopropanol. Isopropanol may also enter the systemic circulation via the inhalation, cutaneous, and rectal routes.

Toxicokinetics

Isopropanol is rapidly (within 30 min) and well absorbed (~70% bioavailability) after oral administration. The V_d of isopropanol is 0.6–0.71kg⁻¹ with minimal to no protein binding. Isopropanol is rapidly metabolized by alcohol dehydrogenase in a first-order, concentration-dependent manner to acetone. This apparent first-order metabolism of isopropanol is probably a result of extensive pulmonary clearance of the acetone. Approximately 80% of systemic isopropanol is metabolized to acetone with the remainder excreted unchanged via the kidneys. A very small amount of isopropanol may be eliminated via the lungs. The presence of ethanol will competitively

antagonize isopropanol metabolism via alcohol dehydrogenase prolonging the isopropanol $t_{1/2}$

Mechanism of Toxicity

Isopropanol is a potent central nervous system (CNS) depressant; it is believed to exert this effect via a similar mechanism as ethanol by modulating ion transport at the cell membrane in excitatory and inhibitory neurons. Ethanol enhances inhibitory or antagonizes excitatory neurotransmission. The metabolite, acetone, may potentiate and lengthen the duration of CNS symptoms observed upon isopropanol exposure. Although early animal studies suggested that the CNS depressant effects of isopropanol is approximately twice that of ethanol, this increased CNS depressant activity is probably a result of the combined effects of isopropanol and acetone.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals develop similar effects as those seen in humans (gastrointestinal effects, CNS depression, confusion, respiratory depression, and death may occur).

Human

Given its widespread availability, use, and cheap cost, isopropanol has resulted in relatively few reports of serious, acute adverse effects in humans. In cases of poisoning or intentional ingestion, the major signs of isopropanol toxicity are those of alcoholic intoxication, including nausea, vomiting, abdominal pain, gastritis, lethargy, weakness, hypotension, ataxia, and hypothermia. In extreme cases, isopropanol depression of the CNS can produce unconsciousness, leading to coma and death due to respiratory depression. Skin absorption of isopropanol in toxic amounts has not been routinely reported, but one case involving a child intoxicated after being sponged with isopropanol suggests that dermal absorption should not be underestimated, particularly in children.

The presence of isopropanol in the blood will result in an increase in the serum osmolality without a concurrent metabolic acidosis. The measured serum osmolality represents the summation effect of both isopropanol and acetone.

Exposure to $\sim 400 \text{ ppm}$ isopropanol vapors for 3 min can cause mild irritation of the eyes, nose, and throat.

Chronic Toxicity (or Exposure)

Animal

Several chronic exposure studies in various animal models have been performed to check for increased tumor incidence. Most models (rats, mice) have not shown increased tumor formation. Rats exposed to 0.5-1% isopropyl alcohol in drinking water for 27 weeks developed only decreased weight gain.

Human

Chronic exposure (abuse) to isopropanol will result in more serious gastritis (hemorrhagic gastritis) than is routinely observed with chronic ethanol exposure. Similarly, all of the negative social and physical consequences of ethanol exposure would be observed, probably to a greater extent, with chronic isopropanol exposure.

In Vitro Toxicity Data

Cell multiplication tests in various models have demonstrated inhibition only when concentrations were greater than 1000 mgl^{-1} (e.g., *Pseudomonas putida* 1086 mgl^{-1} , *Microcystis aeruginosa* (algae) 1000 mgl^{-1} , *Scenedesmus guadricauda* (green algae) 1800 mgl^{-1} , *Entosiphon sulcatum* 4930 mgl⁻¹, *Uronema parduczi* 3425 mgl⁻¹).

Clinical Management

Individuals overexposed to isopropanol should be removed from exposure, affected areas of the skin should be washed with soap and water, and the eyes should be irrigated with water. Isopropanol is rapidly absorbed from the gastrointestinal tract. Efforts to decrease absorption are unlikely to be beneficial. Severe isopropanol overdoses have been managed successfully with either peritoneal dialysis or hemodialysis. Since the vast majority of patients respond completely with only supportive therapy, dialysis (hemodialysis much more effective than peritoneal) should be instituted in those patients with a history and physical exam consistent with a very large ingestion (blood isopropyl alcohol >400 mg dl⁻¹), those patients with hemodynamic instability (hypotension) and coma.

Environmental Fate

Because of its broad range of uses and large production, isopropyl alcohol can be released into various waste streams. At ambient atmosphere, release of liquid isopropanol will rapidly change to vapor state. Vapor will react with oxygen radicals in the atmosphere with a half-life of ~ 3.2 days. Isopropanol has also been identified as metabolic product from aerobic and anaerobic microbes, fungi, and yeast.

See also: Acetone; Ethanol; Fragrances and Perfumes.

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Itai-Itai

Rika Shuto

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Introduction

Itai-Itai disease is a well-known chronic cadmium poisoning, occurring among inhabitants of the Jinzu River basin in Toyama Prefecture, Japan, during and after World War II. In 1955, Dr. Hagino first reported the disease that is characterized by severe pain resulting from osteomalacia and named the disease, 'Itai-Itai disease', meaning 'ouch, ouch' or 'painful' in English. According to the various studies, undertaken by many researchers and Toyama Prefecture, the Ministry of Health and Welfare of Japan declared in May 1968 that itai-itai disease was caused by chronic cadmium poisoning. Between 1967 and 2003, 187 total cases of itai-itai disease (184 women and 3 men) had been officially recognized by the Japanese government, and only four among them were still alive. Most of the victims were middle-aged women who were deficient in calcium due to lactation, multiple pregnancies, and postmenopausal loss of calcium and were living in this community for more than 30 years.

Exposure Routes and Pathways

The Jinzu River basin had been polluted by heavy metals, mainly zinc, cadmium, and lead from the Kamioka mine, located ~ 20 miles from Toyama Plain. Waste water from upstream mine had been discharged into the river for 50 years between the 1910s and the 1950s. The polluted river water had been used for irrigation in this area; consequently the soil, rice, vegetables, and fish were highly polluted. According to a study by Fukushima, cadmium concentrations in rice paddy soils in this area were higher than those in unpolluted areas. As a result of

pollution of the river, the inhabitants living in this cadmium-polluted area for a long time had accumulated high concentrations of cadmium in their bodies through the diet. The intake of cadmium among *itaiitai* patients was $1000 \,\mu g \, day^{-1}$, which was about 200 times higher than the normal intake in unexposed populations. High concentrations of cadmium were detected in urine, blood, bone, kidney, and other organs of patients living in or near this area.

Symptoms

In the early stage of the disease, patients suffer from pains in the lumbar areas, shoulders, and eventually the entire body due to renal tubular dysfunction and decrease in the bone mass. In the later, more serious stage, patients may experience difficulty in mobility due to osteomalacia with severe pains, and may further experience spontaneous bone fracture caused by the slightest external pressure, such as coughing. Finally, patients waste away and eventually die due to significant weight loss.

Mechanism of Toxicity

Itai-itai disease is mainly characterized by renal tubular dysfunction, severe osteomalacia, pesudofractures, and anemia. Chronic cadmium exposure induces renal tubular dysfunction resulting in decreased reabsorption of many substances, including calcium, phosphorus, and vitamin D. Chronic cadmium exposure also can lead to excessive urinary excretion of calcium, phosphorus, glucose, amino acids, and low molecular-weight proteins (such as β 2-microglobulin, lysozyme, retinol binding protein, and vitamin D binding protein). Hypophosphatemia resulting from excessive excretion of phosphate can lead to decreased serum calcium levels and cause osteomalacia, accompanying pseudofractures and severe bone pains.

Clinical Treatments

Renal tubular dysfunction with *itai-itai* disease is irreversible and progressive even if the cadmium exposure is reduced. There is no specific treatment for renal tubular dysfunction by chronic cadmium poisoning. Long-term administration of vitamin D could be useful in the treatment of osteomalacia; however, its effectiveness is limited, and the reappearance of osteomalacia could be observed due to the latent renal tubular dysfunction.

Resolutions

After the official declaration for the disease by Japanese government in 1968, Agricultural Land Soil Pollution Prevention Law was enacted in December 1970. Maximum concentration of cadmium is regulated as less than 1 mg kg^{-1} in rice for agricultural land. Corresponding to this law, Toyama Prefecture had conducted surveys on the cadmium in the polluted area along the Jinzu River and found that cadmium concentration was 1 ppm or more in brown rice and soils in this area. Based on these surveys, Toyama Prefecture declared that the upper soil layer of total 1500.6 ha of paddy fields along the Jinzu should be restored to bring the soil back to normal. Since the restoration work started in 1980, the average cadmium concentrations in soils has reduced to 0.16 ppm from 1.12 ppm (before restoration), and 0.09 ppm from 0.99 ppm (before restoration) in brown rice until 1997. The restoration work is expected to be completed in 2004.

See also: Cadmium; Kidney; Metals; Minamata; Pollution, Water.

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Ivermectins *See* Avermectins.

Jequirity Bean

Brenda Swanson-Biearman

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- SYNONYMS: *Abrus precatorius*; Deadly crab's eye; Indian bean; Love bean; Lucky bean; Mienie mienie; Prayer bean; Rosary bean; Rosary pea; Seminole bead
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Toxalbumins

Uses

There are no known therapeutic uses for the jequirity bean, but they are used decoratively.

Exposure Routes and Pathways

Ingestion of the bean is the most common route of exposure.

Toxicokinetics

The mature bean is innocuous if the hard outer coat is intact. Any interruption in the integrity of the seed coat (e.g., chewing) or ingestion of the soft-coated immature bean may cause toxicity. The inner core contains the amino acid *n*-methyltryptophan, abric acid, glycyrrhizin, and abrin. Abrin is stable in the gastrointestinal tract where it is slowly, but erratically absorbed. In rats, distribution sites occur primarily in the liver (12%) and spleen. Biotransformation and elimination of toxalbumins are poorly defined.

Mechanism of Toxicity

Abrin exerts its necrotizing toxic action by attaching itself to the cell membranes and direct inhibition of protein synthesis on the parenchymal cells (e.g., liver and kidney cells) and red blood cells. It is responsible for the toxic effects of the bean by causing inhibition of protein synthesis. It has been determined that abrin does not inhibit mitochondrial respiration *in vitro*, but it does interfere with amino acid incorporation in the liver of rats.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute manifestations of jequirity bean toxicity in animals are similar to those found in humans.

Human

Clinical effects include an initial aggregation of red blood cells within 1 h, severe gastroenteritis accompanied by serosal hemorrhage, swelling, inflammation of Peyer's patches, and retroperitoneal lymph nodes. Hepatic and renal necroses have been reported. Retinal hemorrhages may appear. Abrin combines with the cell stroma and agglutinates red blood cells leading to thrombus and embolus formation. Profound endothelial damage and profound capillary hemorrhage may occur in severe cases. Adrenal insufficiency and adrenal failure may also be noted. Symptoms may begin after a delay of up to several days and may persist for as long as 10 or 11 days.

Clinical Management

Supportive measures, including administration of blood products, parenteral fluid, and electrolytes, are recommended. Removal of the seeds from the gastrointestinal tract can be done with charcoal. Whole bowel irrigation may also be considered after a dose of charcoal for patients with voluminous ingestions. Alkalinization of the urine with sodium bicarbonate has been recommended for the prevention of hemoglobin precipitates in the renal tubules.

See also: Ricin and other Toxalbumins.

Further Reading

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Jet Fuels

Udayan M Apte and Harihara M Mehendale

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- SYNONYMS: Jet propellant; JP-4; JP-7; JP-8
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Mixture of aliphatic and aromatic hydrocarbons

Uses

Jet propellants are the most widely used aviation fuels used by United States and other North Atlantic Treaty Organization (NATO) militaries.

Background Information

Jet fuels are aviation fuels used mainly by the United States and other North Atlantic Treaty Organization (NATO) nations for military establishments. Other fuels called Jet A and Jet A-1 are closely related fuels used by commercial airlines. JP are a complex mixture of primarily aliphatic (but also aromatic) hydrocarbons, derived from crude oil and/or kerosene by refining and adding various other additives such as fuel icing inhibitors, antioxidants, corrosion inhibitors, metal deactivators, and static dissipaters. Gas chromatographic analysis of JP-8, the most recent JP, indicates that it is made up of complex mixture of 9 to 17 different hydrocarbons, including thousands of isomers and three to six performance additives. They are generally colorless liquids and smell like kerosene.

Exposure Routes and Pathways

Primary exposure routes to JP are inhalation and dermal exposure to the aircraft maintenance personnel working directly with the JP. Additionally, populations residing near air force bases may be exposed to the JP by inhalations route in the form of vapors and aerosol.

Toxicokinetics

No definitive quantitative data are available on the absorption, distribution, metabolism, and excretion of JP in humans or in experimental animals in inhalation, dermal, or oral route of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure of male Sprague–Dawley rats to as high as 5000 mg m^{-3} via inhalation for 4 h did not produce

apparent signs of toxicity or mortality in 2 weeks postexposure observation period. Similarly, 5000 and 8000 mg kg^{-1} of acute oral administration of JP-4 did not produce any conclusive data on mortality. Dermal exposure to JP-4 and JP-7 resulted in skin irritation, necrosis, and erythema.

Human

Although no studies have been reported with acute exposure to JP in humans, a case was reported where an air force pilot was exposed to high levels of JP fumes and suffered from immediate intoxication but no long-term adverse effects.

Chronic Toxicity (or Exposure)

Animal

Extensive information has been gathered on subchronic and chronic exposures of JP in laboratory rodents, mainly via inhalation and dermal routes of exposure. No mortality has been observed related to chronic exposure to JP in animals but significant systemic effects have been recorded. F344 rats exposed to $500-1000 \text{ mg m}^{-3}$ of 24 h day⁻¹ of JP-4 for periods of 90 days to 6 months demonstrated increases in liver and kidney weight along with fatty degeneration in the liver. In the same study, renal tubular hyperplasia, hyaline degenerations, and α -2 μ -globulin nephropathy were reported. Subchronic exposure to JP induced decrease in white blood cell counts in rats. A 1 year exposure to JP in mice resulted in nesolacrimal hyperplasia and testicular atrophy at the end of 12 months exposure. Although a 8 months inhalation exposure to JP-4 in F344 rats did induce intestinal tumors no other cancers were reported in any of the species (rats and mice) tested. Therefore, the risk of cancer occurrence by inhalation exposure to JP is considered minimal.

Dermal exposure to JP resulted in irritation, necrosis of skin, and visible separation and sloughing of the skin. A 105 weeks dermal exposure to mice resulted in increased incidence of squamous cell carcinoma and fibrosarcoma.

Human

No data are available on the human exposure to JP via the dermal route.

Ecotoxicology

No data are available on the effects of environmental exposure of JP on any animal species or plants but

considerable evidence exists that JP is biodegradable, mainly by bacteria, in the environment.

See also: Occupational Exposure Limits; Occupational Toxicology.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Jet Fuels.

Jimsonweed

Brenda Swanson-Biearman

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 8063-18-1
- SYNONYMS: Datura species; Datura arborea; Datura cornigera; Datura folium; Datura suaveolens; Datura stramonium; Angel's trumpet; Downy thorn apple; Horn-of-plenty; Stinkweed; Thornapple; Black henbane
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Anticholinergic

Uses

Datura species are abused for psychedelic properties. Historically, *D. Stramonium* had been used by the American Indians as a folk medicine and in religious activities. *Stramonium* has been used in homeopathic asthma preparations. *Datura* does not have a therapeutic use.

Exposure Routes and Pathways

Exposure occurs via ingestion of the seeds, tea made from seeds, or smoking leaves.

Toxicokinetics

Studies of the pharmacokinetics of Jimsonweed are incomplete. Decreased gastrointestinal motility may delay or prolong absorption.

Mechanism of Toxicity

The toxins in Jimsonweed are tropane belladonna alkaloids possessing strong anticholinergic properties. They include: hyoscyamine (leaves, roots, seeds); hyoscine (roots); atropine (D,L-hyoscyamine), and scopolamine (L-hyoscine). They act as competitive antagonists to acetylcholine at peripheral and central muscarinic receptors at a common binding site. The peripheral receptors are on exocrine glands, affecting perspiration, salivation, smooth and cardiac muscle. As tertiary amines, there is central nervous system (CNS) absorption, inhibition of CNS receptors, and resultant central anticholinergic syndrome of acute psychosis or delirium.

Acute and Short-Term Toxicity (or Exposure)

Animal

In farm animals, muscle tremors, ataxia, drowsiness, tachypnea, and sudden death have been reported.

Human

Common symptoms of exposure include mydriasis, sinus tachycardia, hypertension or hypotension, anxiety, hallucinations, psychoses, choreoathetosis, delirium, seizures, dry mouth, flushed skin, decreased gastrointestinal motility, ileus, urinary retention, and hyperpyrexia. Anticholinergic agents may be detected in the urine, but this does not direct clinical management. Due to multiple plant variations, the alkaloid content differs greatly.

Clinical Management

Gastric decontamination with activated charcoal up to 36 h after ingestion may be useful. Supportive care is the cornerstone of therapy. Sedation with benzodiazepines may control tachycardia associated with agitation and hallucinations. Esmolol can be considered for the treatment of hemodynamically compromising tachyarrhythmias. Physostigmine may be used in the presence of severe incapacitating or life-threatening anticholinergic effects that are unresponsive to conventional therapies.

See also: Acetylcholine; Anticholinergics; Atropine.

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Joint FAO/WHO Expert Meetings (JECFA and JMPR)

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History

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) are international scientific expert committees that are administered jointly by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations. Their purpose is to perform the toxicological evaluation of chemicals in food.

JECFA has been meeting since 1956, initially to evaluate the safety of food additives. Its mandate has been expanded to contaminants, natural toxins, and residues of veterinary drugs in food. The Committee has also developed principles for the safety assessment of chemicals in food that are consistent with the current thinking on risk assessment, and take account of recent developments in toxicology and other relevant sciences. These principles were originally published in 1987, as Environmental Health Criteria 70: Principles for the safety assessment of food additives and contaminants in food.

JMPR has been meeting regularly since 1963 to evaluate pesticide residues in food. The Meeting has also developed principles originally published in 1990, as Environmental Health Criteria 104: Principles for the toxicological assessment of pesticide residues in food.

The principles for evaluations have been continuously reviewed and updated to take account of new scientific knowledge. FAO and WHO have recently initiated a project to update and consolidate principles for the assessment of food additives, contaminants, residues of veterinary drugs in food, and pesticide residues in food.

Mission and Purpose

JECFA and JMPR provide independent, international scientific advice on chemicals in food, including their toxicological evaluation.

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Klein-Schwartz W and Oderda GM (1984) Jimsonweed intoxications in adolescents and young adults. *American Journal of Diseases of Children* 138: 737–739.

All countries need to have access to reliable risk assessments of chemicals in food, but relatively few have the expertise and funds available to carry out separate risk assessments on large numbers of chemicals. In this context, JECFA and JMPR serve as scientific advisory bodies to FAO, WHO, to FAO and WHO member governments, and to the Codex Alimentarius Commission.¹ The risk assessments provided by JECFA and JMPR form the basis for food standards, on national, regional, or international level.

Procedures and Membership of the Scientific Committees

The selection of members is made only after a careful consideration of the scientific credentials of the various candidates. Individuals participate as independent scientific experts, and do not represent any country or organization. A balance of scientific expertise and other experience is considered essential. FAO and WHO meet the costs of experts' attendance at JECFA and JMPR meetings.

JECFA

JECFA normally meets twice a year with individual agendas covering either (1) food additives including flavors, contaminants, and naturally occurring toxicants in food, or (2) residues of veterinary drugs in food. The membership of the meetings varies accordingly, with different sets of experts being called upon depending on the subject matter of the meeting. FAO and WHO have complementary functions in selecting members for JECFA. FAO is responsible for selecting members to deal with (1) the development of specifications for the purity of food additives and (2) the assessment of residue levels of veterinary drugs in

¹The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines, and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. The main purposes of this Programme are protecting health of the consumers, ensuring fair trade practices in the food trade, and promoting coordination of all food standards work undertaken by international governmental and nongovernmental organizations.

food. WHO is responsible for selecting members to perform the toxicological evaluations of the substances under consideration. Both FAO and WHO invite members who are responsible for assessing intakes.

For food additives including flavours, contaminants, and naturally occurring toxicants, the Committee:

- Elaborates principles for evaluating their safety.
- Conducts toxicological evaluations and establishes acceptable daily intakes (ADIs) or tolerable intakes.
- Prepares specifications of purity for food additives.
- Assesses intake.

For residues of veterinary drugs in food, the Committee:

- Elaborates principles for evaluating their safety.
- Establishes ADIs and recommends maximum residue limits (MRLs).
- Determines criteria for the appropriate methods of analysis for detecting and/or quantifying residues in food.

JMPR

JMPR usually meets once every year. FAO and WHO call on experts with complementary responsibilities. The FAO experts are responsible for reviewing the residue and analytical aspects of the pesticides under consideration, including data on their metabolism, fate in the environment, and use patterns, and for estimating the maximum residue levels that might occur as a result of the use of the pesticides according to good agricultural practices. The WHO experts are responsible for reviewing toxicological and related data in order to estimate, where possible and considered necessary, ADIs for chronic intake or acute reference dose (ARfD) for short term (24 h) acute intake of the pesticides under consideration.

In summary, for pesticide residues in food JMPR experts:

- Elaborate principles for evaluating their safety.
- Establish ADIs, ARfDs, and recommend MRLs.
- Determine criteria for the appropriate methods of analysis for detecting and/or quantifying residues in food.

Evaluations

JECFA

To date, JECFA has evaluated more than 1500 food additives, ~ 40 contaminants and naturally

occurring toxicants, and residues of ~ 90 veterinary drug residues in food.

For food additives, JECFA normally establishes ADIs on the basis of available toxicological and other relevant information. Specifications of purity are also developed for food additives, which help to ensure that the product in commerce is of appropriate quality, can be manufactured consistently, and is equivalent to the material that was subjected to toxicological testing.

For contaminants and naturally occurring toxicants, levels corresponding to 'tolerable' intakes such as the provisional maximum tolerable daily intake (PMTDI) or provisional tolerable weekly intake (PTWI) are established when there is an identifiable no-observed-effect level, that is, a threshold of effect can be assumed based on available data. When a noobserved-effect level cannot be identified, the Committee provides other advice, such as identification of the food(s) that contributes most to intake. This allows for targeted management actions in order to decrease exposure.

With veterinary drugs, data on good practice in the use of veterinary drugs are evaluated and corresponding MRLs in animal tissues, milk, and/or eggs are recommended. Such MRLs are intended to provide assurance that when the drug has been used properly, the intake of residues of the drug present in food is unlikely to exceed the ADI.

JMPR

To date, JMPR has evaluated ~240 pesticides, many of them repeatedly. JMPR establishes ADIs (based on chronic toxicity) and acute reference doses (based on acute toxicity) on the basis of the toxicological data and related information available on the substances that are being evaluated. In addition, JMPR reviews pesticide use patterns, data on the chemistry and composition of pesticides, and methods of analysis of pesticide residues. It recommends MRLs for pesticides that occur in food commodities following their use according to Good Agricultural Practice. The potential intake of pesticide residues is compared with the ADI and acute reference dose to estimate the potential dietary risks associated with the adoption of the MRLs.

In recent years, the scope of the toxicological evaluations has been expanded to include assessment of other routes of exposure that are relevant for public and occupational health. In addition, some environmental hazard assessments have been performed.

Reports and Publications

Summary Reports

A summary report of the meetings is published electronically on the FAO and WHO websites within 2 weeks of the meetings. It provides basic details relating to acceptable or tolerable intakes (ADIs/TDIs) and other toxicological conclusions, and specifications or MRLs.

Reports

The conclusions of JECFA meetings are summarized in reports published in the WHO Technical Report Series. Reports reflect the agreed view of the Committee as a whole, and describe the basis for the conclusions. In the very rare event in which some members cannot accept all of the conclusions, a minority report may be included as an annex. These reports are usually published 6–8 months after the meeting. The conclusions of JMPR meetings are summarized in reports published in the FAO Plant Production and Protection Paper series. Reports reflect the agreed view of the Committee as a whole, and describe the basis for the conclusions. JMPR reports also include the dietary risk assessments.

Monographs

JECFA Toxicological and intake monographs are published after JECFA meetings in the WHO Food Additive Series. These summarize all the data used in the Committee's risk assessments and provide full references to the relevant literature. Specifications for the identity and purity of food additives, which are accessible electronically (see Relevant Websites section), and monographs on veterinary drug residues summarizing the data used for recommending MRLs, are published in the FAO Food and Nutrition Paper (FNP) series. Specifications for food additives are published in a Compendium and addenda to FNP 52, and residues monographs on the assessment of veterinary drugs are published in addenda to FNP 41 (see Relevant Websites section). JMPR Toxicological evaluations on pesticides residues in food are published by the WHO International Programme on Chemical Safety (IPCS). These summarize all the data used in JMPR's risk assessments and provide full references to the relevant literature.

Residues monographs, which contain information on pesticide use patterns, data on the chemistry and composition of pesticides, methods of analysis for pesticide residues, and information on MRLs, are published in the FAO Plant Production and Protection Paper series.

Most of the JECFA and JMPR toxicological monographs are available online through IPCS INC-HEM (see Relevant Websites section).

Further information, including future agenda, call for data, and call for experts, is available at the websites listed in the Relevant Websites section.

See also: Acceptable Daily Intake (ADI); Food Additives; Food and Agriculture Organization of the United Nations; Food and Drug Administration, US; Pesticides.

Relevant Websites

- http://www.who.int The Chemicals in Food Programme focuses on principles and methods for the assessment of chemicals in food.
- http://www.fao.org JECFA at FAO is within the Food and Nutrition division which publishes specifications for food additives in a Compendium and addenda to FNP 52, and residues monographs on the assessment of veterinary drugs are published in addenda to FNP 41. JMPR is within the Plant Production and Protection Division.
- http://www.inchem.org Most of the JECFA and JMPR toxicological monographs are available online through IPCS INCHEM.

K

Kava

Molly Broderick and Teresa Dodd-Butera

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• SYNONYMS: Kava-Kava; Kawa; Ava; Awa; Intoxicating pepper; *Piper methysticum*

Uses

Kava is used in the South Pacific islands as a beverage to induce relaxation prior to ceremonies. It may also be used as a sedative and to treat anxiety. The herb has been postulated to have anticonvulsant, antifungal, aphrodisiac, and antiseptic properties.

Background Information

Kava is a dried black pepper root from the *Piper methysticum* species found in Polynesia and Micronesia but widely available in Europe and the United States as a herbal medicine.

Exposure Routes and Pathways

Kava is available as a powder, capsule, tincture, extract, or root. Concentrations of active ingredients vary. The root extract has a higher available distribution than single compounds.

Mechanism of Toxicity

The active constituents are resinous compounds called kava lactones. The exact mechanism of action is unclear though it is thought to act on GABA receptors resulting in the sedative effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animal studies, kava lactones decreased muscle action potentials and disrupted voltage-gated Na⁺ channels. *In vitro*, kava was found to block

norepinephrine intake. In addition, inhibition of certain types of cDNA P450 isoforms was demonstrated.

Human

Toxic effects can include sedation, shortness of breath, alteration in blood pressure, conjunctival redness, visual disturbances, ataxia, and oral paresthesias. Isolated cases of psychotic episodes and dystonic reactions have occurred.

Chronic Toxicity (or Exposure)

Animal

Due to a lack of sufficient scientific evidence regarding safety, further tests are needed to evaluate genotoxicity, reproductive toxicity, neurotoxicity, chronic and carcinogenicity.

Human

Scientific reports address a risk of liver and renal dysfunction. Hepatic failure, hepatitis, and cirrhosis have been reported with chronic use. The most common side effect from chronic, large doses of kava is a scaly skin rash called kava dermopathy.

Clinical Management

Liver and renal function tests should be monitored in symptomatic patients or patients on chronic usage of the herb. Individuals using kava regularly should be cautioned about the potential for adverse interactions with other pharmacologically active substances, including ethanol, barbiturates, and some benzodiazepines. A toxic dose has not yet been established.

Further Reading

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Piper methysticum (kava kava). Alternative Medicine Review 1998; 3(6): 458–460.

Relevant Websites

http://www.cfsan.fda.gov – Consumer advisory: Kava-containing dietary supplements may be associated with severe liver injury, 3/25/02. http://www.cdc.gov – Centers for Disease Control.

Kerosene

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 8008-20-6
- SYNONYMS: Straight-run kerosene (petroleum); Range oil; Fuel oil no. 1; Deobase
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Petroleum hydrocarbon
- CHEMICAL FORMULA: Kerosene is a mixture of petroleum hydrocarbons, chiefly of the methane series having from 10 to 16 carbon atoms per molecule. It constitutes the fifth fraction in the distillation of petroleum. C_xH_{2x+2}

Uses

Kerosene, originally used for lighting and heating, is also used as a diesel fuel, as a component in blending aviation fuels, as a solvent and carrier for a wide range of products (including cleaning compositions and pesticides), and as a mold-release agent in the ceramic and pottery industry.

Exposure Routes and Pathways

Kerosene may enter the water or soil environment as a result of regular use (e.g., evaporation of pesticide solvent), from spills during use or transportation, or from leaking storage facilities. The relatively low vapor pressure of kerosene makes inhalation exposure unlikely under ordinary occupational conditions unless conditions of poor ventilation exist. The combustion product of burned kerosene, carbon monoxide, is of real concern when kerosene heaters are not vented. Exposure to kerosene mist can occur as kerosene is often applied in the form of a spray. Eye and skin contact with kerosene and kerosene mists and vapors can occur. The exposure pathway usually of most concern is ingestion because this is the most common means of acute poisoning, especially in children.

Toxicokinetics

No or little quantitative data are available concerning the absorption, distribution, metabolism, and excretion of kerosene. Indirect evidence suggests that kerosene may be absorbed through the respiratory tract, the gastrointestinal tract, and percutaneously.

Mechanism of Toxicity

The specific mechanism of toxicity of kerosene has not been completely determined. The primary risk from ingestion of kerosene is aspiration during emesis, which may cause pneumonitis. The biochemical mechanism of lung response to large concentrations of aerosolized kerosene (resulting in bronchoconstriction and asthma-like symptoms) may involve the parasympathetic nervous system via a direct effect on the vagus nerve or by inhibition of acety1cholinesterase. The mechanism(s) of central nervous system (CNS) depression from kerosene exposure has not been elucidated, but undoubtedly includes disruption of the membranes of nerve cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicities (LD_{50}/LC_{50}) for kerosene are $>5 \text{ g kg}^{-1}$ (oral; rats), $>2 \text{ g kg}^{-1}$ (dermal; rabbit), and $>5 \text{ mg l}^{-1}$ per 4 h (inhalation; rats). Skin exposure for more than 4 h resulted in mild to severe irritation in rabbits and other experimental animals. Prolonged eye exposure caused mild irritation in rabbits. Skin sensitization did not occur in guinea pigs when treated with kerosene.

Human

Kerosene is of low-order toxicity following oral, dermal, or inhalation exposure. Symptoms from exposure to high levels of kerosene may include hypoactivity, ataxia, and prostration, consistent with CNS depression, including coma and death. Pneumonia (chemical pneumonitis) is the major lethal complication after ingestion of kerosene due to aspiration into the lungs after vomiting or emesis. Tachycardia, nausea, abdominal cramps, and diarrhea have also been associated with the ingestion of kerosene. Skin irritation, which can be severe, can occur especially after prolonged or repeated exposure. Respiratory tract irritation may occur after inhalation of mists or aerosols. Slight eye irritation may occur if exposure is prolonged. Kerosene was not identified as a skin sensitizer in experimental animals.

Chronic Toxicity (or Exposure)

Animal

Chronic dermal applications of kerosene caused skin carcinoma in mice. Kerosene has been reported to have weak cancer-promoting activity but no cancerinitiating activity. Repeated applications of kerosene to the skin of laboratory animals caused moderate to severe skin irritation with an increase in skin tumors after long latency periods. The increase in skin tumors was considered to be the result of the severe skin damage. This explanation is consistent with the general lack of activity of kerosene in genotoxicity assays.

Human

Kerosene can cause chemical pneumonia. Prolonged or repeated contact of the skin with kerosene may result in drying of the skin and dermatitis, which may lead to severe skin damage with degenerative changes. Repeated inhalation of kerosene vapors may cause symptoms consistent with CNS depression such as headache and vertigo. Other symptoms reported in experimental animals and/or humans after repeated exposure to kerosene include neuralgia, loss of memory, blood changes, kidney effects, and respiratory disturbances.

Clinical Management

Asymptomatic individuals should be observed for 48 h after exposure for the development of symptoms. Respiratory and cardiovascular functions should be supported in symptomatic individuals. If there is suspicion of aspiration, breathing should be observed and the patient should be treated symptomatically.

If kerosene has been ingested, nothing should be administered by mouth and vomiting should not be induced. Gastric decontamination is not usually indicated after ingestion due to the possibility of aspiration. If necessary, gastric lavage must only be performed after cuffed endotracheal intubation. Treatment should be symptomatic. Antibiotic and corticosteroid therapy may be considered for treatment of possible chemical pneumonitis resulting from aspiration. If aspiration is expected to have occurred (i.e., a coughing symptomatic individual), arterial blood should be monitored to ensure adequate ventilation. The development of pulmonary edema may be delayed in onset up to 24–72 h after exposure.

For skin contact, affected areas should be washed thoroughly with soap and water. For eye contact, the eyes should be gently flushed with copious amounts of water for at least 10 min.

Environmental Fate

Kerosene is biodegradable in soil although some components of the mixture adhere strongly to the soil. Kerosene is also biodegradable in surface water. However, some components of the mixture may bioconcentrate in fish and other aquatic organisms.

Exposure Standards and Guidelines

The recommended exposure limit, 10 h time-weighted average, is 100 mg m^{-3} .

See also: Petroleum Hydrocarbons; Pollution, Water.

Further Reading

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Kidney

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Introduction

The kidney is an organ that performs several important functions essential to sustain life. These functions include the regulation of volume and electrolyte homeostasis, control of acid–base balance, and the excretion of waste products. The kidney also has endocrine functions including renin secretion, stimulation of erythropoietin formation, and activation of vitamin D. Numerous disease states (e.g., infections, shock, diabetes, gout) can affect the ability of the kidney to perform its normal functions, and if these diseases are not properly treated, serious illness or death can result.

The kidney is also a major target organ for the toxic effects induced by numerous chemical and physical agents. Renal toxicity or nephrotoxicity can be the result of a direct toxic effect of an agent on renal tissue (e.g., mercuric salts, cyanide ion) or a secondary event following toxicity or tissue damage at a site other than the kidney (e.g., carbon monoxide poisoning, crush injury). The kidney may also be damaged by toxic metabolites that originate at renal or extrarenal sites. In addition, the nephron, the functional unit of the kidney, is composed of several distinct anatomical segments. Some compounds or their metabolites induce toxicity to only one segment of the nephron and are considered to be site-selective nephrotoxicants, while other agents may induce widespread renal damage and are considered to be nonsite-selective toxicants.

An understanding of how and why chemicals induce nephrotoxicity requires some familiarity with the anatomy and physiology of the kidney. In addition, interpretation of renal toxicology studies will require a working knowledge of the various techniques used for evaluating renal function. It is also important to be aware of which nephrotoxicants require biotransformation before they induce nephrotoxicity, nephrotoxic mechanisms when known, and the site(s) of renal damage for the various nephrotoxicants.

Renal Anatomy and Physiology

In mammalian species, the two kidneys carry out the normal physiological and endocrine functions

described above. The kidney receives blood via the renal artery, and blood leaves the kidney by way of the renal vein. In adult humans, the rate of blood flow through both kidneys is $\sim 1.21 \text{ min}^{-1}$ or $\sim 20\%$ of the cardiac output for a 70 kg individual. Urine formed within the kidneys is transported from kidneys through the ureters to the bladder, a reservoir for the urine until it is excreted.

Each kidney is subdivided anatomically into three zones: (1) an outer zone called the cortex, (2) an intermediate zone called the medulla, and (3) an innermost zone called the pelvis. The cortex and medulla have important characteristics that help facilitate the formation of urine and control the composition of waste products, electrolytes, and water.

Each kidney also contains over one million nephrons, which are the functional units of the kidney. Nephrons originate in the cortex where an afferent arteriole forms a specialized capillary bed known as the glomerulus (**Figure 1**). Some nephrons originate near the surface of the kidney and are called superficial nephrons, while other nephrons originate near the cortical-medullary region and are called juxtamedullary nephrons.

The glomerulus, which forms within Bowman's capsule, is a special capillary bed with large pores, and substances that are not bound to plasma proteins and have molecular weights less than albumin (~69 000) can be filtered at the glomerulus and enter Bowman's capsule. This glomerular filtrate is essentially protein-free plasma, although a small amount of low molecular weight protein is also filtered by the glomerulus. The rate at which the glomerular filtrate is formed, the glomerular filtration rate (GFR), is ~ 125 ml min⁻¹ in humans and serves as an important measure of renal function.

The glomerular filtrate flows from Bowman's capsule into the proximal tubule. The proximal tubule can be subdivided into three segments (S_1 , S_2 , and S_3). The S_1 segment is closest to the glomerulus and is localized in the cortex, while the S_3 segment is furthest from the glomerulus and is found in both the cortex and the outer portion of the medulla. The S_1 and S_2 segments comprise the pars convoluta, while the latter portion of the S_2 segment and the S_3 segment comprise the pars recta or straight portion.

The proximal tubule cells contain numerous finger-like projections on the luminal surface, which markedly increase the luminal surface area of the cells and help promote reabsorption of substances filtered at the glomerulus. Under normal conditions,

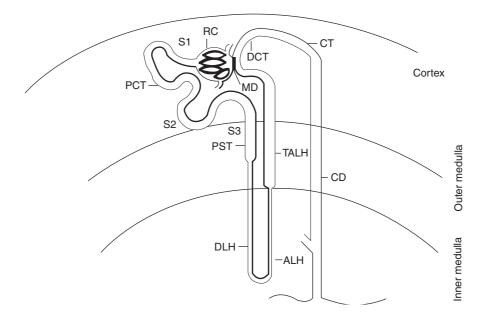


Figure 1 Anatomy of the nephron. RC, renal corpuscle (includes glomerulus and Bowman's capsule); PCT, proximal convoluted tubule; PST, proximal straight tubule; DLH, descending limb of the loop of Henle; ALH, ascending limb of the loop of Henle; TALH, thick ascending limb of the loop of Henle; MD, macula densa; DCT, distal convoluted tubule; CT, connecting tubule; CD, collecting duct.

~65–70% of filtered sodium, chloride, calcium, and water; 80–90% of filtered bicarbonate, phosphate, and urate; and essentially all of the filtered amino acids, glucose, and low molecular weight proteins are reabsorbed from the glomerular filtrate as the filtrate passes the length of the proximal tubule. Reabsorption occurs without a major change in the osmolality of the filtrate such that the fluid leaving the S₃ segment of the proximal tubule has essentially the same osmolality as the fluid entering the S₁ segment. However, the quantity of the glomerular filtrate is markedly reduced during its transit through this nephron component.

Proximal tubule cells also contain active transport systems on the basolateral or antiluminal side of the cells, which are capable of transporting weakly acidic or weakly basic compounds from the interstitial space into the proximal tubular cells. These transport systems are distinct systems and transport either organic anions (weakly acidic compounds) or organic cations (weakly basic and guaternary ammonium compounds). As a result of transport into proximal tubule cells, weak acids and bases can achieve intracellular concentrations that are hundred times higher than the corresponding plasma concentration for the compound. The prototypic organic anion for studying the organic anion transporter is *p*-aminohippurate (PAH), and the prototypic organic cations for examining organic cation transport are N-methylnicotinamide (NMN) and tetraethylammonium (TEA). Accumulated material can then enter luminal fluid via either passive diffusion or facilitated transport.

The location of the organic anion and cation transport systems is not homogeneous along the entire proximal tubule. In the rabbit, PAH transport is greatest in the S₂ segment and lower in the S₁ and S₃ segments. In contrast, organic cation transport is highest in the S_1 segment and lowest in the S_3 segment. In superficial nephrons, the transport of organic cations in the S₂ segment is intermediate between the S_1 and S_3 segments, but is about equal to the capacity of S₁ segments in juxtamedullary nephrons. Although the organic anion and cation transporters are clearly localized in the proximal tubules for all mammalian species studied to date, the segmental localization of these transporters has not been studied in great detail in species other than the rabbit. Nevertheless, the ability of many nephrotoxicants to induce nephrotoxicity to proximal tubular segments is dependent on the compound or its metabolites being accumulated in proximal tubule cells via one of these organic transport systems. The function of these systems also serves as a sensitive measure of renal function and is routinely used to monitor renal function in animal models.

Luminal fluid leaving the proximal tubule enters the descending limb of the loop of Henle. This nephron segment passes deeper into the medulla than the proximal tubule, and the high medullary interstitial osmolality causes water to move from the luminal fluid and into the medullary interstitial space. As a result, the luminal fluid becomes more concentrated as it passes through the descending limb. Organic compounds (e.g., sulfonamides, methotrexate), which have poor water solubility, can precipitate in this nephron segment once their water solubility limit is reached, and they block luminal flow and glomerular filtration.

When the luminal fluid reaches the thick ascending limb of the loop of Henle, water no longer can freely move from the luminal fluid into the medullary interstitial space. Instead, this portion of the nephron is impermeable to water reabsorption and actively reabsorbs sodium, chloride, and potassium ions. Approximately 20–25% of filtered sodium and calcium ions are reabsorbed at this location. In addition, most, if not all, of the potassium ions reaching the thick limb of the loop of Henle are reabsorbed as well. Thus, as the luminal fluid passes through the ascending limb, the luminal fluid becomes more dilute.

Although the ascending limb of the loop of Henle begins in the medulla, it ends in the cortex where it joins the distal tubule. Sodium chloride is also reabsorbed in the early segment of the distal tubule, and the combination of this segment with the cortical portion of the thick ascending limb of the loop of Henle is referred to as the cortical diluting segment of the nephron. In the late distal tubule, sodium ions are reabsorbed in exchange for potassium or hydrogen ions. The secretion of hydrogen ions in exchange for sodium ions results in the acidification of the urine. The process of sodium exchange is partially under the control of mineralocorticoids (e.g., aldosterone) and accounts for the reabsorption of 2–3% of filtered sodium ions.

The kidney also has the ability to respond to changes in the GFR through the action of specialized distal epithelial cells called the macula densa. These cells are in close contact with the glomerular apparatus of the same nephron and can detect even small changes in the flow of luminal fluid. Increases in the flow rate activate the macula densa cells to communicate with the granular cells and vascular components of the juxtaglomerular apparatus and stimulate the release of renin. Renin release results in the formation of the vasoactive peptide angiotensin II and subsequent vasoconstriction that leads to a decrease in the GFR and the luminal flow rate. Thus, the distal tubule is not only important for urine formation, but also plays a role in regulating the GFR.

Another important urinary regulatory mechanism involves the effects of vasopressin (AVP) also known as antidiuretic hormone (ADH). A decrease in blood pressure or an elevation in plasma osmolality will result in the release of ADH from the posterior pituitary. ADH is carried by the blood to the nephron where ADH increases the reabsorption of water from the collecting tubule. Thus, in the presence of ADH, water reabsorption is increased and urine becomes more concentrated, while decreased ADH secretion will result in diuresis and a more dilute urine.

Mechanisms of Nephrotoxicity

The kidney is a target organ for many chemicals, in large part because of the physiology of the kidney described above. The substantial blood flow through the kidneys results in the kidneys being exposed to significant amounts of chemicals and their metabolites. The ability of the proximal tubular cells to transport organic anions and cations can lead to the intracellular accumulation of weakly acidic or basic chemicals as well as amino acid conjugates and quaternary compounds (e.g., paraquat) in this renal nephron segment. Such accumulations can eventually lead to toxic levels of the chemical in proximal tubular cells. In addition, the large demand for energy to support the reabsorption and secretion processes makes the kidney particularly susceptible to compounds (e.g., cyanide ion) which inhibit the production of cellular energy and/or oxygen utilization.

There are several mechanisms by which renal toxicity can be induced (**Table 1**). Direct tubular toxicity is one of the most common mechanisms by which chemicals or their toxic metabolites induce nephropathy. Proximal tubule cells are especially susceptible to toxicity via this mechanism because (1) the proximal tubule is one of the first segments of the nephron to be exposed to toxicants and (2) these cells can accumulate toxicants by actively transporting the

Table 1 Mechanisms of nephrotoxicity

Direct tubular toxicity Examples: amphotericin B, aminoglycosides, cephaloridine, cisplatin, heavy metals, cysteine conjugates, 4-aminophenol, many others
Altered renal hemodynamics
Examples: angiotensin converting enzyme (ACE) inhibitors, cyclosporine, nonsteroidal antiinflammatory drugs (NSAIDs)
Tubular obstruction
Examples: sulfonamides, methotrexate, oxalic acid, acyclovir
Tubulointerstitial nephritis
Examples: analgesics, penicillins, cephalosporins, heavy metals, cisplatin, nitrosoureas, NSAIDs, cimetadine, many others
Glomerular injury
Examples: heavy metals, D-penicillamine, captopril, methimazole, heroin, puromycin aminonucleoside
Fluid/electrolyte imbalance
Examples: chlorpropamide, lithium, captopril, NSAIDs, fluoride, diuretics, ACE inhibitors

toxicants into this tubular segment and achieving high intracellular concentrations of toxic chemical species or their precursors. The exact mechanism of direct tubular toxicity or the critical cellular target is not known with certainty for most toxicants. However, nephrotoxicants can induce direct toxicity by several mechanisms including (1) alkylation of cell macromolecules (e.g., phosgene formed from chloroform), (2) complexation with cellular sulfhydryl groups and other ligands (e.g., heavy metals), (3) generation of free radials and/or initiation of lipid peroxidation (e.g., cephaloridine, paraquat), or (4) disruption of mitochondrial function and energy production (e.g., cysteine conjugates, cyanide).

Nephrotoxicity can also occur following an alteration in renal hemodynamics. Since renal blood flow (RBF) is important for maintaining a steady supply of oxygen and nutrients to renal cells, a reduction in blood flow to the kidney can result in decreased oxygen delivery (ischemia), decreased energy production, and decreased renal function. The vasodilatory prostaglandins PGE₂ and PGI₂ can be important in maintaining proper renal perfusion. Inhibition of renal cyclooxygenase by administration of nonsterodial antiinflammatory drugs (NSAIDs), such as indomethacin, can result in a decrease in production of the vasodilatory prostaglandins, overriding renal vasoconstriction, and ultimately renal failure. In disease states, such as congestive heart failure and cirrhosis with ascites, where renal perfusion may be augmented by the synthesis of PGE₂, NSAIDs can cause reversible acute renal failure.

The obstruction of renal tubules can occur following the precipitation of compounds with low water solubility. The obstruction of tubules prevents the filtration of blood at the glomerulus and can lead to oliguric acute renal failure characterized by a decrease in urine volume and a rise in the blood urea nitrogen (BUN) concentration. Precipitation of the toxicant occurs as the luminal fluid passes through the descending limb of the loop of Henle and the fluid becomes more concentrated. When the solubility limit of the toxicant is exceeded, the toxicant begins to precipitate and obstruct flow. Tubular obstruction was occasionally seen following the use of some of the early sulfonamide antimicrobial agents, but is less of a problem with the currently used drugs. High-dose methotrexate therapy or rapidly infused acyclovir can also result in tubular obstruction. The use of cancer chemotherapeutic agents can lead to rapid cell killing and the delivery of large amounts of uric acid to the kidney. Uric acid is particularly prone to deposit in acidic urine so that therapy to prevent uric acid deposition can include alkaline diuresis, hydration of the patient with intravenous fluid

administration, and allopurinol, an inhibitor of xanthine oxidase, which decreases uric acid formation. Oxalic acid, which is formed from the biotransformation of ethylene glycol and other compounds, can also deposit in renal tubules and may contribute to the nephrotoxicity induced by these agents.

Tubulointerstitial nephritis can be either acute or chronic in nature. Acute interstitial nephritis is characterized by an acute renal interstitial inflammatory response with urinary eosinophils and nonoliguric acute renal failure. The more common drugs that induce acute interstitial nephritis include penicillins, rifampicin, sulfonamides, and cimetadine. Chronic tubulointerstitial nephritis is most commonly associated with the long term use of large amounts of analgesics and antiinflammatory agents (e.g., NSAIDs).

Glomerular toxicity is frequently seen as a 'leaky sieve' effect. Normally, the glomerulus serves as a barrier to high molecular weight (>50 000–60 000) proteins, however, when the glomerulus is damaged, proteinuria can be observed. Chemically induced glomerular disease is frequently immune mediated with the observation of immunoglobulin and complement deposits in renal biopsies noted in some cases. Heavy metals such as mercuric salts may induce their glomerular effects via an immune mediated pathway, but the exact mechanism of this effect is unclear.

Many drugs and other chemicals can adversely affect renal function by directly or indirectly affecting the reabsorption of electrolytes and water in the kidney. Chlorpropamide can enhance the secretion of ADH and promote the water conservation actions of the hormone, while lithium use can lead to a nephrogenic diabetes insipidus. NSAIDs block the formation of renal prostaglandins, which can result in hyperkalemia. Hyperkalemia may also result from the use of beta blockers, potassium-sparing diuretics, and cyclosporine.

Methods for Evaluating Nephrotoxicity

There are numerous methods to determine the nephrotoxic potential of a chemical or to study the mechanism(s) by which a chemical induces nephrotoxicity. In humans, the concern is most often related to either drug-induced or occupationally associated nephrotoxicity. Evaluation of nephrotoxicity in humans is limited primarily to the measurement of urinary changes (e.g., volume, enzymes, protein, etc.), BUN or serum creatinine concentrations, creatinine clearance, or renal biopsy. The measurement of an increase in urinary N-acetyl- β -D-glucosaminidase (NAG) or alanine aminopeptidase (AAP) levels, enzymes localized primarily on proximal tubule cells, has been used as an early marker for chemotherapyinduced nephrotoxicity. However, in laboratory animals, many techniques and models are available for monitoring renal function both *in vivo* and *in vitro*.

In Vivo Techniques

There are two general models for evaluating the nephrotoxic potential of chemicals that utilize whole animals. In one model, conscious animals are administered the test compound and renal functional parameters (Table 2) evaluated over a period of hours or days. Some of the urinary parameters routinely monitored using *in vivo* nephrotoxicity studies include volume, osmolality, and contents. Urine volume can increase (polyuria), decrease (oliguria), or approach a zero value (anuria). Urinary osmolality usually decreases from control levels, while in oliguric states urine tends to be more concentrated and urinary osmolality values rise above the control level.

The urinary contents also can provide important information concerning the presence of nephrotoxicity. The presence or elevated levels of enzymes, protein, amino acids, glucose, blood, or casts in the urine can signal renal injury. Enzymuria results primarily from the loss of the brush border (microvilli)

 Table 2
 Common parameters of renal function monitored in in vivo studies

Clearance of organic compounds; creatinine, urea, inulin, PAH, phenol red, TEA
Comment: allows for the determination of GFR or RBF (PAH)
Urinary volume
Comment: reflects absorption capability of nephron or altered GFR
Urinary free water/osmolality
Comment: represents the ability of the kidney to concentrate urine
Enzymuria/proteinuria
Comment: changes reflect cellular toxicity and/or altered glomerular function
Glucosuria/amino aciduria
Comment: increasing amounts of either in urine suggest proximal tubular damage, extrarenal effects also possible (e.g., diabetes)
Electrolyte excretion; pH
Comment: may be influenced by many factors
Kidney weight
Comment: can increase (edema and hypertrophy) or decrease (atrophy)
Morphological changes
O

Comment: provides information on the sight and nature of the lesion

of proximal tubular cells or from the rupture of necrotic tubular cells with the release of cytosolic enzymes and organelles. The appearance of significant enzymuria has been used as an early indicator of nephrotoxicity and for predicting the injured nephron site. Proteinuria is also an index of chemically induced nephrotoxicity. The amount of protein present in the urine can be used as a measure of the relative degree of renal damage, while the nature of the protein can provide information on the site of the lesion. Low molecular weight proteins such as β_2 microglobulin ($\sim 12\,000\,\text{Da}$) are freely filtered at the glomerulus and almost completely reabsorbed by the proximal tubule, while high molecular weight proteins (e.g., albumin, $\sim 69\,000\,\text{Da}$) are not normally filtered at the glomerulus. Thus, the appearance of increased amounts of albumin can indicate glomerular damage, and an increased excretion of β_2 -microglobulin or a decreased ratio of albumin to β_2 -microglobulin can be indicative of proximal tubule damage.

The appearance of amino acids and/or glucose in urine can also indicate proximal tubule toxicity. Both nutrients are almost entirely reabsorbed in the proximal tubule and proximal tubular damage can lead to the increased excretion of both compounds. However, reabsorption of these materials is dependent on membrane transport systems which can become saturated and lead to the increased excretion of the amino acid or glucose (e.g., uncontrolled diabetes). Recent studies have suggested that examination of urine using nuclear magnetic resonance technology might be useful in characterizing the degree and type of nephrotoxicity present (i.e., tubular versus glomerular) following exposure to a renal toxicant.

Creatinine, an endogenous end product of muscle metabolism, is often measured in plasma and urine to determine creatinine clearance. Since, creatinine is freely filtered at the glomerulus and is not reabsorbed or secreted by the proximal tubule of most species, creatinine clearance provides a good measure of the GFR. Another endogenous compound, urea, is also cleared mainly by renal filtration and excretion. Increases in the blood or serum concentration of urea are indicative of decreased GFR. However, increases in BUN concentration occur only after substantial renal damage has been established. Thus, BUN concentration is not a sensitive indicator of nephrotoxicity and changes usually occur later than changes in other parameters (e.g., enzymuria).

At the end of a screening protocol in animal models, the kidneys can be removed and examined for morphological changes (light or electron microscopy) or used in an *in vitro* model as described below to further evaluate renal function following *in vivo*

PAH, *p*-aminohippurate; TEA, tetraethylammonium; GFR, glomerular filtration rate; RBF, renal blood flow.

exposure to the test agent. Kidney weight can also be easily measured at the end of a screening period as one index of nephrotoxicity. Some nephrotoxicants induce an increase in kidney weight, while exposure to other nephrotoxicants results in decreased kidney weight.

A second in vivo model involves the use of anesthetized animals. The animals used in these studies are either untreated or have received the test agent or procedure before anesthesia. Changes in GFR are frequently measured by determining the urinary clearance of inulin, an exogenously administered polysaccharide that is freely filtered at the glomerulus but is not secreted or reabsorbed by the nephron. Changes in RBF, a measure of renal hemodynamics, can be monitored by measuring the renal clearance of PAH or other organic compounds that are essentially 100% extracted from the peritubular fluid but poorly reabsorbed from the luminal fluid or by the use of electromagnetic flow probes placed on one or both renal arteries. In addition, to determining changes in GFR and RBF, the excretion patterns of electrolytes, protein, enzymes, glucose, water, and other urinary components can be determined both before and after exposure to a nephrotoxicant to provide information on the relative renal toxicity of test compound, the temporal aspects of toxicity and the nephron segment where toxicity occurs. Kidneys may be perfused with fixative solutions at the end of the experiment to allow for histological examination of tissue.

In Vitro Techniques

There are a large number of *in vitro* techniques available for evaluating the nephrotoxic potential of an agent or examining potential mechanisms by which an agent induces nephrotoxicity (Table 3). These techniques employ various levels of tissue organization including whole kidneys, tubules or tubule segments, cortical slices, cells, and isolated cellular components. *In vitro* techniques offer the advantage of allowing the investigator to study the direct effects of a compound on renal function without the contribution of extrarenal or indirect mechanisms (e.g., extrarenal biotransformation or altered renal hemodynamics). However, many *in vitro*

Table 3 Common in vitro models for examining nephrotoxicity

Isolated perfused kidney/tubule Renal cortical slices Nephron segments Isolated renal epithelial cells Renal cell cultures Isolated renal organelles (e.g., mitochondria) Membrane vesicles (basolateral or luminal) systems remove the integral nature of the kidney, and in other *in vitro* models the *in vivo* cellular characteristics change over time (e.g., cell culture techniques). The relative importance of these factors for the induction of nephrotoxicity by a test compound will determine the toxic potential of that agent in the various *in vitro* systems. Therefore, the use of *in vitro* systems may not always provide the same results as will be obtained in whole animals. Nonetheless, valuable information can be obtained once the toxicant species has been identified and an appropriate *in vitro* model is selected for use. In the remainder of this section, a general description of the more common *in vitro* systems for examining nephrotoxicity is provided.

The isolated perfused kidney allows the investigator the ability to monitor the effects of chemicals on the intact kidney without the effects of extrarenal systems. Transport of chemicals occurs via normal mechanisms as vasculature and tubular lumen remain open in the perfused state, and renal handling and biotransformation of chemicals can be evaluated using this technique. However, to utilize an isolated perfused kidney preparation, a special apparatus must be used.

Renal cortical slices offer a convenient and sensitive model for determining the induction of nephrotoxicity following either in vivo or in vitro exposure to a nephrotoxicant. Slices can be prepared freehand or by the use of a mechanical tissue slicer. When isolated from different depths of the cortex, renal cortical slices can provide one method for examining cell- or site-specific damage. Superficial slices contain S_1 and S_2 segments of the proximal tubule, while deeper slices can be prepared which contain mainly the S₃ segment. Thus, effects of nephrotoxicants on S_1, S_2 segments versus S_3 segments can be performed. Renal function parameters monitored in renal slices can include organic ion (PAH, TEA) accumulation, gluconeogenesis, LDH release, oxygen consumption, electrolyte levels, ATP production, and morphology. In addition, the order of nephrotoxic potential of several agents (mercuric chloride > potassium dichromate > hexachloro-1,3-butadiene > cephaloridine > gentamicin) is the same *in vivo* and in renal cortical slices from Fischer 344 rats.

Renal tubules or tubule segments can be isolated from the kidneys of a variety of species and either perfused with or suspended in an appropriate medium. The rabbit is commonly used as the model for tubule perfusion studies, although renal tubules from many species, including humans, have been used. Tubule segments allow the renal cells to remain in contact, but tubule collapse may occur in nonperfused segments, which could hinder absorption of toxicants from the lumen. If the normal route of entry into renal tissue for a toxicant is via absorption at the luminal surface, then a reduction in the nephrotoxic potential of the toxicant might be observed using nonperfused tubule segments. However, the perfused tubule allows ready access to the luminal surface when the toxicant is added to the perfusate. Also, addition of a toxicant to the bathing media provides exposure of the basolateral membrane to the toxical parameters as well following exposure of this cell surface to the test compound. Thus, the perfused tubule segment can provide an *in vitro* model with many of the characteristics of the *in vivo* model.

Isolated renal cortical epithelial cells and cultured cell lines have become standard in vitro model systems for examining the nephrotoxic potential of toxicants, toxicant bioactivation and direct mechanisms of toxicity. Enriched populations of freshly isolated proximal and distal tubular cells can be obtained to examine the effects of toxicants on these distinct cell populations. Growing freshly isolated cells for several days can provide primary cultures of renal cells that can be used to study transport and toxicity of compounds. Such primary cultured cells can be obtained from proximal tubule or distal tubule/collecting duct cells. There are also several cell lines (e.g., LLC-PK₁, MDKC, HK-2) to study the effects of chemicals on renal tissue. In addition, cell lines have been developed from specific segments of the proximal tubule. Overall, these cell systems allow for the rapid screening of chemicals, exposure to apical and/ or basolateral surfaces, and more detailed studies of specific cell populations. However, cells in culture can exhibit reduced functional and metabolic characteristics with time. These changes might impact on the potential effects of some chemicals in cell culture systems. Therefore, toxicity in the specific cell line to be used should be validated prior to mechanistic studies.

Isolated renal cell components (cytosol, organelles, membranes) are also commonly used *in vitro* systems. Renal microsomes and cytosol are useful in examining the renal biotransformation and bioactivation of nephrotoxicants. Since mitochondria are frequently targets for nephrotoxicants, isolated renal mitochondria are also an important model system for determining the toxic mechanism(s) of some compounds. Also, the direct effects of toxicants on renal cell membranes can be studied in vesicles prepared from either the luminal (brush border) or basolateral (antiluminal, peritubular) membrane of renal cortical cells. The use of isolated cell components is helpful in answering specific questions about mechanisms of nephrotoxicity and bioactivation of nephrotoxicants.

Nephrotoxicants

Nephrotoxicants can be classified in many different ways including chemical class (e.g., heavy metals, halogenated hydrocarbons), intended use (e.g., drugs, agricultural agents), site of toxicity (e.g., glomerular toxicants, proximal tubule toxicants), and mechanism of toxicity (e.g., crystalluria, interstitial nephritis, direct tubular toxicity). Nephrotoxicants can also be considered either primary or secondary nephrotoxicants. Primary nephrotoxicants (e.g., heavy metals) are capable of inducing nephrotoxicity as the parent compound, while secondary nephrotoxicants (e.g., trichloroethylene) require bioactivation to the ultimate nephrotoxicant species. In the following sections on specific nephrotoxicants, nephrotoxic agents will be divided into therapeutic and environmental nephrotoxicants. Sites of toxicity, mechanisms of toxicity, and the ultimate nephrotoxicant species, if known, will be discussed.

Therapeutic Nephrotoxicants

Many drugs have the potential to alter renal function. In the case of the diuretic drugs, such as furosemide and hydrochlorothiazide, the increased urine flow rate and sodium excretion which these drugs induce is a desirable, therapeutic response. However, for most therapeutics, drug-induced effects on renal function are not desirable and constitute a toxic response. Examples of some drug classes whose members induce nephrotoxicity include the antimicrobials, nonsteroidal antiinflammatory agents, analgesics, cancer chemotherapeutic agents, immunosuppressives, and radiocontrast media. In addition, there are several miscellaneous drugs within other pharmacological classes which affect renal function. The nature and magnitude of the nephrotoxic response varies greatly among these drugs, and a single drug may induce more than one type of nephrotoxic response depending on dose, duration of therapy, age of the patient, and other patient variables.

Antimicrobials Several different classes of antimicrobial agents contain members that induce nephrotoxicity. The primary groups of drugs which induce nephrotoxicity include the β -lactams (penicillins, cephalosporins, carbapenems), aminoglycosides, the antifungal agent amphotericin B, vancomycin, and the tetracyclines. The spectrum of nephrotoxic effects and mechanism(s) of nephrotoxicity vary among these agents, and the potential for a nephrotoxic

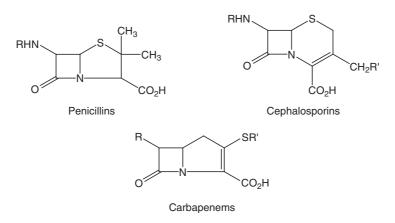


Figure 2 Structure of β -lactam antimicrobials.

response can be increased by combining two antimicrobial drugs that can induce renal toxicity or by combining a nephrotoxicant antimicrobial with other nephrotoxicant drugs.

The β -Lactam Antimicrobials The β -lactam antimicrobial drugs (penicillins, cephalosporins, carbapenems; Figure 2) are related chemically by the presence of a four-membered ring containing a nitrogen atom adjacent to a carbonyl group (a β -lactam ring). This β -lactam ring also confers antimicrobial activity to these drugs, since hydrolysis of the ring results in inactive drugs. Because these drugs are weakly acidic drugs, they are actively secreted by the organic anion transport system of the proximal tubule and can concentrate in this nephron segment during the secretory process. The nephrotoxicity observed following administration of a β -lactam antibiotic can occur by different mechanisms depending on the antibiotic used and patient variables.

Penicillin-induced renal toxicity is most commonly seen as allergic acute interstitial nephritis (AIN). Methicillin is the most common penicillin to induce AIN, but the use of penicillin G, ampicillin, amoxacillin, oxacillin, and carbenicillin also can lead to the development of AIN. Typically, acute renal failure follows 1 or 2 weeks of treatment with fever or rashes sometimes occurring before overt renal dysfunction. Removal of the penicillin generally allows renal function to return to normal within a few days or weeks. AIN can also be induced by certain cephalosporins (e.g., cephalothin, cephalexin, cephradine, cefoxitin, cefotaxime) and non- β -lactam antimicrobials (e.g., sulfonamides, rifampicin, tetracyclines, erythromycin).

In addition to inducing AIN, several of the cephalosporins (e.g., cephaloridine, cephaloglycine, cefaclor, and cephalothin) are directly toxic to the proximal tubule. Accumulation in proximal tubular cells via the organic anion transporter is an important step in cephalosporin nephrotoxicity, since inhibition of cephalosporin transport by probenecid also attenuates cephalosporin nephrotoxicity. In addition, the site of the cephalosporin-induced renal lesion correlates with the proximal tubular segment having the greatest capacity for organic anion transport for a particular species.

The cellular mechanism of direct cephalosporininduced nephrotoxicity may include several possible actions of the cephalosporins. Nephrotoxic cephalosporins are known to induce lipid peroxidation and cellular membrane damage, acylate cellular proteins, and/or interfere with mitochondrial respiration. Mitochondrial respiration appears to be inhibited due to acylation of mitochondrial transporters for metabolic substrates, thereby depriving mitochondria of the necessary intermediates to utilize oxygen. Ultimately, the formation of adenosine triphosphate (ATP), needed to supply cellular energy, also declines to inhibit energy-dependent cellular functions.

Imipenem, a carbapenem antimicrobial, also possesses nephrotoxic potential. In animal models, nephrotoxicity is dose dependent and characterized by tubular necrosis. Interestingly, imipenem nephrotoxicity is markedly attenuated by co-administration of cilastatin, an inhibitor of the cytosolic and brush border enzyme dehydropeptidase I (DHP). Although DHP is responsible for hydrolyzing imipenem to inactive metabolites, the major protective effect of cilastatin appears to be due to inhibition of renal imipenem accumulation rather than DHP inhibition.

Aminoglycosides The aminoglycosides are important antimicrobial drugs used alone or in combination with β -lactam antibiotics for the treatment of certain serious gram-negative infections. Chemically, the aminoglycosides consist of various sugars containing amino groups and linked by glycosidic bonds. The amino groups are ionized at physiological pH and give the aminoglycosides polycationic character and a highly polar nature.

Aminoglycosides concentrate in the S_1 and S_2 segments of the proximal tubule via a high-capacity, adsorptive endocytotic mechanism following binding to cellular membrane acidic (anionic) phospholipids. This endocytotic process occurs primarily at the brush border membrane. Following adsorption, aminoglycoside-containing vesicles bind to secondary lysosomes where the drug becomes sequestered. Lysosomes become early targets for aminoglycoside-induced effects with inhibition of phospholipid degradation and subsequent myeloid body formation being characteristic of aminoglycoside nephrotoxicity. Some lysosomes may rupture to release lysosomal enzymes into the cytosol of renal cells. Changes in brush border microvilli, alterations in rough endoplasmic reticulum, and increased numbers of cytoplasmic vacuoles also occur. Mitochondrial swelling, decreased mitochondrial respiration, and inhibition of basolateral Na⁺,K⁺-ATPase activity precede tubular necrosis. Ultimately, nonoliguric renal failure results, which may not completely reverse following cessation of aminoglycoside administration.

Although all aminoglycosides possess the ability to induce nephrotoxicity, differences exist in the nephrotoxic potential of the various drugs. Neomycin and gentamicin are the most potent nephrotoxicant aminoglycosides, while amikacin and netilmicin are the least nephrotoxic aminoglycosides. Other aminoglycosides are intermediate in nephrotoxic potential.

The exact mechanism of aminoglycoside nephrotoxicity is unclear. As discussed above, release of lysosomal enzymes can contribute to altered cellular function. Hydroxy radicals may also play a role in aminoglycoside mitochondrial effects, since catalase inhibits *in vitro* alterations of mitochondrial function by gentamicin and the use of hydroxy radical scavengers or iron chelators reduces gentamicin nephrotoxicity *in vivo*. Additionally, aminoglycoside-induced decreases in cellular pyridoxal 5'-phosphate may contribute to nephrotoxicity by removing an important cellular cofactor.

Amphotericin B The drug of choice for treating many serious systemic fungal infections is the broad spectrum antifungal agent amphotericin B. Unfortunately, nephrotoxicity is a common side effect of amphotericin B administration with up to 50–80% of amphotericin B-treated patients experiencing adverse renal effects. Amphotericin B nephrotoxicity is characterized by a distal renal tubular acidosis, potassium wasting and a defect in urinary concentration. In some cases, nephrotoxicity may progress to renal failure with azotemia.

Amphotericin-induced renal effects are due to the ability of the drug (1) to induce renal vasoconstriction which leads to a decrease in RBF and GFR, and (2) to cause an increase in tubular permeability, particularly in the distal segments of the nephron. Tubular permeability changes are the result of the association of amphotericin B with the sterols in the cell membrane to form a pore or channel. Chemically, amphotericin B is a cyclic molecule composed of lipophilic (multiple conjugated double bonds) and hydrophilic regions (Figure 3) such that insertion of amphotericin B into the cell membrane facilitates passive movement of water, potassium ions, hydrogen ions, and other small molecules through the newly created pore and across the membrane. These fluxes appear to account for the decrease in urinary concentrating ability, potassium wasting and renal tubular acidosis. Although it is not clear why the distal segments of the nephron are the major targets for amphotericin B, one explanation may be that the greater level of sterols found in distal membranes might facilitate the binding of amphotericin B to these sites.

Vancomycin Vancomycin is an antibiotic produced by *Streptococcus orientalis*. It is bactericidal, but vancomycin-induced toxicity has primarily limited its use to the treatment of serious infections (e.g.,

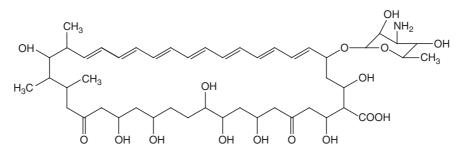


Figure 3 Structure of the antifungal drug amphotericin B.

methicillin-resistant staphalococci). Nephrotoxicity is manifest as proximal tubular toxicity. Elimination of impurities from early preparations and careful monitoring of vancomycin blood levels have reduced the number of cases of nephrotoxicity. However, vancomycin use may potentiate aminoglycoside nephrotoxicity when the two agents are used concurrently.

Tetracyclines Tetracyclines are broad spectrum antibiotics obtained from Streptomyces strains or prepared semisynthetically. Use of tetracyclines has resulted in three types of renal effects. First, the use of outdated tetracyclines results in direct proximal tubular toxicity characterized by the increased excretion of amino acids, glucose, and phosphate (Fanconi syndrome). The mechanism of this response is unclear, but appears to be due to the formation of the degradation product anhydro-4-epi-tetracycline. Second, administration of some tetracyclines, particularly demeclocycline, can result in a dose-dependent, reversible nephrogenic diabetes insipidus, which appears to result from an inhibition of ADH effects on water reabsorption. Lastly, in patients with preexisting compromised renal function, tetracyclines can induce increased sodium excretion and azotemia. The mechanism of the naturesis may be due to an effect of tetracyclines on luminal membrane sodium conductance, while the azotemia appears to result from the antianabolic effects of the tetracyclines.

Nonsteroidal Antiinflammatory Drugs The NSAIDs as a class possess the ability to induce renal failure characterized by a rapid decrease in urine volume and significant sodium and water retention which is also rapidly reversed when the drug is removed. Conditions that decrease renal perfusion (e.g., congestive heart failure, decreased blood volume, chronic renal disease, etc.) also predispose individuals to the renal effects of NSAIDs. Under these conditions, RBF and GFR are maintained by a balance of the vasoconstrictor actions of angiotensin II and the vasodilatory effects of prostaglandin E₂ (PGE2). NSAID-induced renal failure results as a consequence of NSAID inhibition of renal cyclooxygenase with a subsequent inhibition of PGE2 synthesis. Renal vasoconstriction predominates under these conditions resulting in acute oliguric renal failure. While all NSAIDs have the potential to induce acute renal failure, sulindac appears to have less of an effect on renal PGE2 synthesis and may be the drug of choice in patients with preexisting conditions which would predispose them to NSAID-induced renal effects.

Nonnarcotic Analgesics Nonnarcotic analgesic drugs are widely used for the relief of minor pain,

to reduce fever and/or as antiinflammatory agents. Acetaminophen (paracetamol) is perhaps the most commonly used agent in this class of drugs which also includes aspirin and phenacetin. Analgesic use can result in acute or chronic nephrotoxicity depending on the amount of drug ingested and patient variables. Normally, acute nephrotoxicity results from acute overdose, while chronic use of single or combination products can result in renal papillary necrosis and chronic interstitial nephritis.

Acute overdose with acetaminophen (> 300 mg kg⁻¹) results in hepatotoxicity and/or nephrotoxicity. Although hepatotoxicity is frequently the predominant toxicity, acetaminophen nephrotoxicity can occur in the absence of marked hepatic toxicity. In these cases, liver function returns to normal or near normal levels before the onset of nephrotoxicity. Acute acetaminophen nephrotoxicity is generally characterized as oliguric acute renal failure with acute tubular necrosis. Acetaminophen can also induce acute nephrotoxicity in therapeutic doses, but chronic alcohol intake usually accompanies renal toxicity in these patients.

The mechanism of acute acetaminophen nephrotoxicity is related to the bioactivation of acetaminophen and/or its metabolites to highly reactive species which are capable of arylating renal macromolecules or generating reactive oxygen species. Acetaminophen hepatotoxicity is the result of conversion of acetaminophen to the reactive intermediate N-acetyl-p-benzoquinoneimine (NAPQI), which can covalently bind to hepatic macromolecules. It is less clear what role formation of NAPQI in the kidney plays in acetaminophen nephrotoxicity. In some species (e.g., the Fischer 344 rat) deacetylation appears to be an important biotransformation step in acetaminophen nephrotoxicity, while in other species (e.g., the CD-1 mouse), bioactivation does not appear to require deacetylation of acetaminophen before the ultimate nephrotoxicant species is produced. Therefore, the role of NAPQI in acute acetaminophen nephrotoxicity might be species dependent.

Biotransformation of acetaminophen by deacetylase enzymes in liver or kidney produces the metabolite 4-aminophenol (Figure 4). Evidence suggests that acetaminophen nephrotoxicity may result from 4-aminophenol formation. In animal studies, 4-aminophenol is a more potent nephrotoxicant than acetaminophen and inhibition of deacetylase enzymes also attenuates acetaminophen nephrotoxicity. Deacetylase enzymes are also present in higher levels in renal cortex, the target for acetaminophen nephrotoxicity, than in liver or renal medulla and there is a positive correlation between renal cortex

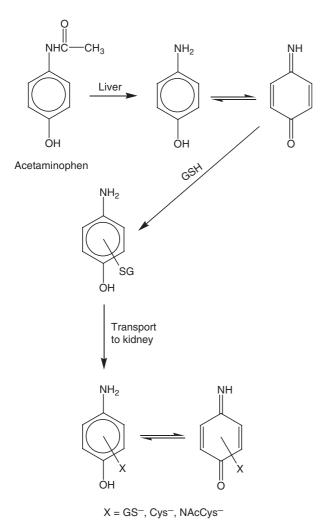


Figure 4 Bioactivation of acetaminophen.

deacetylase activity and susceptibility to acetaminophen nephrotoxicity in various animal models.

The mechanism of 4-aminophenol nephrotoxicity remains to be determined with certainty. The current hypothesis suggests that 4-aminophenol is oxidized by cytochrome P450 isozymes or peroxidases to *p*-benzoquinoneimine which can arylate renal macromolecules and/or redox cycle between 4-aminophenol and *p*-benzoquinoneimine to form reactive oxygen species. Recent studies have suggested that 4-aminophenol might be converted to a glutathione conjugate in the liver prior to transport to the kidney (Figure 4), and that the glutathione conjugate or one of its metabolites is the form that accumulates in kidney from extrarenal sources. Thus, acetaminophen nephrotoxicity could result from production of more than one reactive intermediate.

Chronic analgesic nephrotoxicity is characterized by renal papillary necrosis and interstitial nephritis rather than the proximal tubular necrosis observed in acute nephrotoxicity. In most cases, chronic

 Table 4
 Cancer chemotherapeutic drugs capable of inducing nephrotoxicity

Alkylating agents	
Cisplatin	
Nitrosoureas	
Cyclophosphamide	
Antibiotics	
Mitomycin C	
Mithramycin	
Doxorubicin	
Antimetabolites	
Methotrexate	
5-Fluorouracil	
6-Thioguanine	
Cytosine arabinoside	
5-Azacytidine	
Miscellaneous	
Interferon	
Celiptinium	

nephropathy results from abuse of combination analgesic preparations (phenacetin, acetaminophen and/or a salicylate) over a long period of time. In these situations, the primary nephrotoxicant appears to be acetaminophen, which concentrates more in the renal medulla than in renal cortex or blood. Within the medulla, acetaminophen can be converted to the reactive intermediate NAPQI by the prostaglandin hydroperoxidase component of the prostaglandin H synthase complex. NAPQI interactions within medullary tissue result in a depletion of the cytoprotective tripeptide glutathione. As glutathione becomes depleted, arylation of medullary macromolecules by NAPQI occurs. In addition, acetaminophen in therapeutic doses can increase prostaglandin hydroperoxidase activity and therefore, stimulate formation of its own reactive metabolite NAPOI.

Aspirin also has the potential to increase acetaminophen nephropathy. Aspirin inhibits the cyclooxygenase component of prostaglandin H synthase without effect on the prostaglandin hydroperoxidase component, while salicylic acid (the deacetylated metabolite of aspirin) decreases renal glutathione concentrations. Thus, coadministration of aspirin with acetaminophen (or phenacetin) results in a synergistic nephrotoxicity.

Cancer Chemotherapeutic Drugs Cancer chemotherapeutic drugs can be life saving in the treatment of certain cancers. Unfortunately, host systems are also a target for these agents with several antineoplastic drugs possessing the potential to induce nephrotoxicity (Table 4). In some cases, nephrotoxicity normally occurs only at high doses (e.g., methotrexate, 6-thioguanine) or is a low risk (e.g., 5-fluorouracil, interferon). However, nephrotoxicity can be a

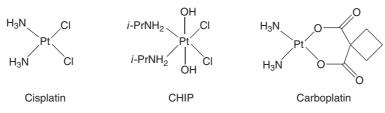


Figure 5 Platinum cancer chemotherapeutic drugs.

significant toxicity following administration of certain antineoplastic drugs (e.g., cisplatin) which can require additional efforts to minimize the development of renal failure.

Alkylating Agents Perhaps the cancer chemotherapeutic drug which is most commonly associated with the induction of nephrotoxicity is cisplatin (**Figure 5**). Cisplatin is a member of the platinum complex class of antineoplastic drugs which also includes carboplatin and iproplatin (CHIP). However, the nephrotoxic potential of cisplatin appears to be greater than the nephrotoxic potential of the other clinically useful platinum agents.

In rat models of cisplatin nephrotoxicity, cisplatin nephrotoxicity is observed as acute proximal tubular necrosis with the primary target being the S₃ segment. However, in humans, cisplatin nephrotoxicity is characterized by tubular necrosis with the distal tubules and collecting ducts affected along with proximal tubules. Early changes in renal function include enzymuria (e.g., *N*-acetyl- β -D-glucosaminidase) and β_2 -microglobinuria, which suggests that the proximal tubule is an initial target for cisplatin. Diuresis, increased BUN concentration, decreased creatinine clearance and magnesium wasting soon develop, indicating the presence of renal failure.

The mechanism of cisplatin nephrotoxicity is unclear, although numerous hypotheses have been proposed. Cisplatin is primarily excreted via the kidneys by both filtration and secretion using the organic cation transporter, which indicates that the kidney will be exposed to a large percentage of the administered dose. It is believed that the chloride groups are replaced by water molecules *in vivo*, ultimately forming a hydrated or hydroxylated platinum species which might interact with renal macromolecules (e.g., DNA) to lead to nephrotoxicity. Other postulated targets for cisplatin are renal ATPase enzymes and renal mitochondria.

The nitrosoureas include streptozotocin, an agent useful in treating pancreatic (islet cell) tumors, and the carmustine, lomustine and semustine group, useful in treating brain and gastrointestinal tumors. Streptozotocin induces a reversible, mild nephropathy characterized by proteinuria in 50–70% of patients and decreased creatinine clearance in 20–30% of patients. Renal phosphate wasting and proteinuria are early signs of nephrotoxicity. The primary target in the kidney for streptozotocin is the proximal tubule with glomerular abnormalities also noted. Removal of the drug results in return to normal renal function within weeks.

Semustine is the most common agent to induce nephrotoxicity among the second group of nitrosoureas. Semustine nephrotoxicity results from bioactivation of the nitrosourea to an alkylating metabolite which mainly attacks proximal tubular cells. Carbamoylating metabolites of semustine are also formed *in vivo* but do not appear to contribute to renal toxicity. Renal failure occurs most often following high dose (>1200 mg m⁻², total dose) administration. Onset of nephrotoxicity (glomerulosclerosis, renal interstitial nephritis, proximal tubular degeneration) can be delayed for over one year following therapy and may progress to irreversible renal failure.

Cyclophosphamide, a nitrogen mustard alkylating agent, is a widely used cancer chemotherapeutic drug to treat lymphomas, leukemias, multiple myeloma and a numerous solid tumors. Cyclophosphamide can induce nephrotoxicity characterized as decreased water excretion and an inappropriate concentration of urine. These effects are due to a direct effect of one or more alkylating cyclophosphamide metabolites at distal tubules and collecting ducts. Special caution is warranted to avoid water-induced diuresis or diuretic therapy in these patients as hyponatremia can become a problem.

Antimetabolites Nephrotoxicity is generally not a major toxicity of antimetabolite therapy, except when these drugs are administered in high doses or in susceptible patients. Acute renal failure is the most common type of nephropathy induced by the antimetabolites with methotrexate treatment possessing the greatest risk. Acute renal failure has also been reported as a potential toxicity for 5-fluorouracil, 6-thioguanine, cytosine arabinoside, and 5-azacytidine.

Methotrexate is an antimetabolite of folic acid useful in combination therapy for a wide range of cancerous conditions. When methotrexate is administered in high doses (> $50-250 \text{ mg kg}^{-1}$ to $1-7 \text{ g m}^{-2}$), solubility limits may be exceeded with a resultant precipitation of methotrexate and its 7-hydroxy metabolite within the renal lumina. Tubular obstruction can reduce GFR by as much as 50%. Methotrexate might also have a direct effect on proximal tubular function, since proximal tubular necrosis may be seen in the absence of precipitated material within the renal lumina. In addition, a direct effect of methotrexate on glomerular hemodynamics has been postulated. Renal toxicity induced by methotrexate also can enhance other methotrexate toxicities (e.g., myleosuppression) by decreasing the excretion of the drug from the body.

Antibiotics The clinical use of three antibiotic cancer chemotherapeutic drugs (mitomycin C, mithramycin, and doxorubicin) has been associated with the development of nephrotoxicity. Each of these drugs is commonly used in combination chemotherapy which in some cases might result in additive or enhanced nephrotoxicity.

Mitomycin C is isolated from *Streptomyces caespitosis* and is used in the treatment of solid tumors. Renal failure (elevated BUN and serum creatinine concentration, proteinuria) induced by mitomycin C is dose dependent and cumulative. When administered alone, the incidence of nephrotoxicity is less than 1%, but in combination with 5-fluorouracil nephrotoxicity occurs more frequently and can be marked. The chemical species responsible for mitomycin C nephrotoxicity appears to result from the formation of alkylating metabolites.

Mithramycin is an antibiotic antineoplastic drug isolated from *Streptomyces tanashiensis*. In early studies, treatment with mithramycin (25 or $50 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 1 week or three times per week each month) resulted in decreased GFR in up to 40% of patients and proteinuria in 78%. Morphological changes included proximal and distal tubular necrosis, atrophy, or swelling. Single dose administration of mithramycin to treat the hypercalcemia sometimes associated with cancer generally does not induce renal toxicity. However, a few isolated case reports suggest that in patients with compromised renal function, nephrotoxicity may occur following single dose therapy.

Nephrotoxicity associated with doxorubicin use is also dose dependent and occurs at the same time as doxorubicin-induced cardiotoxicity. Studies in animal models reveal glomerular effects, renal interstitial fibrosis, and vacuolization of tubules. However, clinical evidence of nephrotoxicity in the absence of cardiotoxicity is limited suggesting that dose reduction efforts to minimize cardiotoxicity also reduce the risk of nephrotoxicity.

Miscellaneous Cancer Chemotherapeutic Agents The lack of curative treatments for most malignancies has stimulated the search for newer, more efficacious cancer chemotherapeutic agents. Interferon- α has recently been obtained using molecular biology techniques in sufficient quantities to begin clinical testing against various cancerous conditions including hairy cell leukemia, non-Hodgkin's lymphoma and Karposi's sarcoma. A few reports suggest that reversible acute renal insufficiency associated with proteinuria may occur following continued interferon- α administration. Morphological changes are consistent with the presence of acute interstitial nephritis.

Celiptinium is useful in the treatment of metastatic breast cancer and is useful in combination therapy because of minimal hematotoxicity. Acute and chronic renal failures have been detected in patients treated with celiptinium. Acute renal failure is dose dependent, while chronic effects appear to be cumulative in nature. The primary manifestation of celiptinium nephrotoxicity is tubular necrosis with celiptinium-induced lipid peroxidation in proximal tubular cells proposed as the mechanism of toxicity.

Immunosuppressive Drugs The modern development of drugs to suppress the immune system has made organ and bone marrow transplants possible and saved countless lives. Two important drugs in this class of agents are cyclosporine (cyclosporin A) and tacrolimus (FK506), fungal products with immunosuppressive properties. Cyclosporine acts primarily by inhibiting helper T-cell activation following the binding of cyclosporine to a cytoplasmic receptor protein, cyclophilin. Other effects on the immune system are also observed, but appear to be less important than T-cell effects. Tacrolimus also inhibits T-cell activation but via interaction with a different cytoplasmic receptor than cyclosporine. A newer drug OKT₃ is a monoclonal antibody that can destroy effector T-cells to act as an immunosuppressive drug.

Nephrotoxicity is a common toxicity and significant problem associated with the use of cyclosporine in humans. Three types of nephrotoxicity have been observed in patients receiving cyclosporine: (1) an acute, reversible renal failure; (2) acute vasculopathy or thrombotic microangiopathy; and (3) chronic renal failure with interstitial fibrosis that may not be reversible.

Cyclosporine-induced acute renal failure is characterized by decreased GFR and urine volume, and elevated BUN and serum creatinine concentrations. These effects are dose dependent and rapidly reverse when cyclosporine therapy is discontinued. The mechanism of the acute renal failure appears to be related to cyclosporine-induced renal vasoconstriction to reduce glomerular filtration. The precise mechanism responsible for the resultant vasoconstriction remains to be determined with certainty. However, stimulation of the renin–angiotensin system, alteration of renal prostaglandin status (increased vasoconstrictor and/or decreased vasodilatory prostaglandin levels), and stimulation of adrenergic nerves have been proposed as possible mechanisms.

A second form of cyclosporine-induced nephrotoxicity is acute thrombotic microangiopathy. The mechanism for induction of this toxicity is unclear but may be due to a direct toxic effect of cyclosporine on renal arterioles and glomerular capillaries. Histologically, arterioles exhibit protein deposits while glomeruli show thrombosis and endothelial cell damage. These effects are similar in nature to transplant rejection thrombotic microangiopathy, but arcuate and interlobular arteries rather than arterioles are primarily affected with transplant rejection.

Chronic cyclosporine nephrotoxicity can develop in patients receiving the drug for 1 year or longer. In these patients, there is a gradual decline in renal function. While GFR may not be markedly reduced, significant morphological changes including vascular changes, glomerular sclerosis, interstitial fibrosis, and tubular atrophy have been reported. Chronic effects may not be reversible upon discontinuation of cyclosporine, and the renal effects may progress to end-stage renal failure. The mechanism underlying cyclosporine-induced chronic renal failure is unclear. However, it has been proposed that the renal vasoconstriction induced by cyclosporine results in both acute and, ultimately, chronic renal failure.

Tacrolimus nephrotoxicity can occur as proximal tubular vacuolization, proximal tubular necrosis, or glomerular capillary/arteriolar thrombi. Although there are reports that tacrolimus may be less potent as a nephrotoxicant than cyclosporine, tacrolimus potentiates cyclosporine nephrotoxicity in humans.

 OKT_3 , an immunosuppressive monoclonal antibody, can induce systemic vascular changes (leaky syndrome) and prerenal azotemia, presumably by stimulating the release of cytokines (e.g., tumor necrosis factor). These effects are seen more often in poorly hydrated patients. There is also evidence that OKT_3 may induce a direct tubular toxicity, since significant numbers of patients developing renal insufficiency also exhibit enzymuria. Radiocontrast Media The use of radiocontrast media to visualize organs in the body has been a common practice for over 50 years. However, the use of these agents is now recognized as a significant cause of hospital-acquired acute renal failure with up to 10% of all cases due to the administration of a radiocontrast agent. Numerous factors may increase the risk of acute renal failure developing following a diagnostic procedure with a radiocontrast agent including existing renal insufficiency, diabetes mellitus, dehydration, anemia, cardiovascular disease, age, and many others.

Typically, radiocontrast-induced acute renal failure is diagnosed when oliguria or a >50% rise above baseline in serum creatinine develops within 24–48 h following the radiocontrast procedure. The most predominant morphological change seen in the kidney is extensive vacuolization of proximal tubular cells.

The agents currently used as radiocontrast media are triiodinated benzoic acid derivatives and may be ionic (e.g., sodium diatrizoate) or nonionic (e.g., iotrol, iopamidol). The mechanism of nephrotoxicity induced by radiocontrast media involves both renal hemodynamic changes and direct tubular injury, and these effects are related to the high osmolarity of the drugs (up to 1965 mOsm l^{-1}).

Radiocontrast agents can enter the nephron by filtration and/or secretion, depending on the agent administered. The changes in renal hemodynamics induced by radiocontrast media result primarily from the large osmotic load delivered to the distal segment of the nephron. At the level of the macula densa, this osmotic load is detected and the tubuloglomerular feedback system is activated to stimulate vasoconstriction and decrease GFR. While evidence exists for direct tubular toxicity (enzymuria, naturesis, diuresis greater than an osmotic effect), the mechanism is unclear. However, tubular injury may be exacerbated by the production of reactive oxygen species generated from mesangial cells and polymorphonuclear leukocytes and macrophages which migrate into glomerular and tubular sites following radiocontrast administration.

Environmental Nephrotoxicants

Exposure to nephrotoxicants not only occurs in the clinical setting, but also occurs from environmental sources. Environmental nephrotoxicants are defined as nephrotoxicants found in the natural, home, and/ or work environment which have no therapeutic utility. Unlike therapeutic nephrotoxicants, which are administered to obtain a beneficial health effect, exposure to environmental nephrotoxicants usually

Aluminum	Iron
Antimony	Lead
Arsenic	Lithium
Beryllium	Manganese
Bismuth	Mercury
Cadmium	Molybdenum
Chromium	Nickel
Cobalt	Platinum
Copper	Silver
Gallium	Thallium
Germanium	Uranium
Gold	Vanadium
Indium	

Table 5	Nephrotoxicant metals
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occurs accidentally or in suicide/intentional poisoning cases.

Although environmental nephrotoxicants differ markedly in their chemical nature, there are several distinct classes of environmental nephrotoxicants. These classes include the metals, halogenated solvents, agricultural agents, and natural products. A diverse array of miscellaneous nephrotoxicants also exists and will be discussed briefly at the end of this section.

Metals A wide range of metals induce nephrotoxicity in humans and/or in animal models (Table 5). Some of these metals (e.g., iron, cobalt, copper) are essential elements required for normal body function, while others can be useful in treating diseases. For example, gold salts are useful in treating rheumatoid arthritis; lithium salts are indicated for the treatment of manic-depressive illness; and aluminum and bismuth salts are available to treat indigestion and stomach aches. However, exposure to these and other metals can occur from environmental sources and in excessive concentrations, can lead to nephropathy.

Many of the metals are potent nephrotoxicants, inducing marked renal effects at concentrations far lower than many other classes of nephrotoxicants. The proximal tubule is a major target for metal toxicity and, in some cases, renal hemodynamic changes are also important for initiating and/or maintaining the renal damage. Although metal-induced nephrotoxicity has been studied for many years, the precise cellular mechanisms that underlie the potent nature of this class of toxicants are not fully elucidated. However, there are several reasons why the kidney is susceptible to metal-induced effects.

The large reabsorptive nature of the kidney insures that filtered proteins, electrolytes, and water will be conserved and homeostasis maintained in the body. Metals make use of these reabsorptive processes to gain entry into renal cells. For example, chromate can enter proximal tubular cells via the sulfate transporter, while cadmium binds to metallothionein, a low molecular weight metal-binding protein, and enters proximal tubular cells along with the protein. Once inside kidney cells, the metals may substitute for endogenous molecules. Arsenate can substitute for phosphate in oxidative phosphorylation within mitochondria to cause a decrease in ATP synthesis. Also, lead can substitute for calcium to alter a large number of calcium-mediated events within cells. Metals also have a high affinity for sulfhydryl and amino ligands and form complexes or chelates with these organic functional groups. Formation of these chelates with essential functional groups of cellular macromolecules can markedly alter cell function to eventually lead to cell death. In addition to direct interaction of metals with cellular targets, radioactive metals that accumulate in renal tissue can release radiation to initiate the formation of cellular free radicals to disrupt cell function and membrane integrity.

The chemical form of a metal that accumulates in the kidney may vary among the metals. However, the ionic form of a metal is normally much more potent as a nephrotoxicant than the elemental form. Once in the body, metal ions interact with numerous molecules (albumin, metal-binding proteins, glutathione, amino acids, etc.) and move around the body primarily as reversible complexes. Unfortunately, little information is available on the chemical form of most metals that actually enters proximal tubular cells and additional research is needed in this area.

Mercury The effects of various forms of mercury on renal function have been known for centuries. Therapeutically, the first class of highly efficacious diuretic drugs was the organomercurials (e.g., mersalyl), but these agents have been now replaced by the loop or high-ceiling diuretics. Toxicological interest has centered on inorganic mercury salts, primarily in the form of mercuric chloride (HgCl₂), and organic mercury, mainly methylmercury salts (CH_3Hg^+) . In the natural environment, elemental mercury can be converted by microorganisms to both inorganic mercury salts and organic mercurials which can find their way into the food chain. Mercurials are also used agriculturally as insecticides and fungicides so that exposure to these agents can occur occupationally, from industrial wastes or agricultural runoff.

Acute exposure to mercuric salts targets the S_3 segment of the proximal tubule to induce severe necrosis. Acute renal failure develops rapidly characterized by decreased RBF and GFR, oliguria, glucosuria, proteinuria, and elevated BUN concentration. Renal vasoconstriction contributes to the developing nephrotoxicity and may be due to activation of the renin–angiotensin system. At higher doses or later time points, S_1 and S_2 segments of the proximal tubule are also damaged.

The mechanism of proximal tubule toxicity following administration of mercuric chloride has been extensively studied. However, the key sequence of events leading to cell death remains to be determined with certainty. Mercuric ions induce mitochondrial toxicity, alter cell membrane function, disrupt cell calcium homeostasis and cause changes in membrane phospholipid composition. Binding of mercuric ions to enzymatic sulfhydryl groups and ischemia-induced mitochondrial toxicity have been proposed as the basic mechanisms leading to these cellular effects and ultimately to cell death.

Another aspect of mercuric ion-induced tubule toxicity relates to the nature of the chemical species responsible for proximal tubule damage. Recent studies have suggested that, *in vivo*, mercuric ion forms diconjugates with thiol-containing molecules such as glutathione or homocysteine. When the kidney is exposed to these diconjugates (e.g., glutathionyl–Hg–glutathionyl or cysteinyl–Hg–cysteinyl), the conjugates are accumulated via amino acid transporters or processing enzymes to increase the proximal tubule cell concentration of mercury. The accumulated mercury then interacts with critical intracellular targets to induce toxicity. Thus, the conjugates provide a mechanism for facilitating mercuric ion entry into renal cells which leads to toxicity.

In addition to tubule effects, glomerular changes are also noted. These effects are due to the formation of an autoantibody which localizes along the glomerular basement membrane. Glomerular damage then occurs from complement-mediated events or circulating lymphocytes. Chronic exposure to low levels of mercuric salts or mercury vapor can also induce immune-mediated glomerular toxicity which is more common clinically than necrosis.

Organic mercurials are capable of inducing nephrotoxicity in S_2 and S_3 segments of the proximal tubule. Part of the S_3 damage results from the biotransformation of the organic mercurial to release mercuric ions. Methylmercury (CH₃Hg⁺) readily concentrates in renal proximal tubular cells and alters mitochondrial function and lysosomes. At least part of methylmercury-induced nephrotoxicity may be due to homolytic scission of methylmercury to release methyl radicals and to lipid peroxidative toxicity.

Cadmium Cadmium has a variety of uses including electroplating, galvanizing, as a color pigment and in

the manufacture of batteries. Industrial exposure is a major source of cadmium in humans, but cadmium is also found in food. Concentrations of cadmium in food varies widely, with shellfish (e.g., oysters, mussels, scallops) being a major dietary source of cadmium $(100-1000 \,\mu g \, kg^{-1})$. Cigarettes also contain cadmium and it has been estimated that smoking one or more packs of cigarettes per day may double the body burden of cadmium.

Cadmium nephrotoxicity occurs when renal accumulation of cadmium exceeds 200 µg Cd²⁺ per gram tissue. Nephrotoxicity is characterized by low molecular weight proteinuria (e.g., β_2 -microglobulinuria in humans), glucosuria, calciuria, aminoaciduria, phosphaturia, and interstitial inflammatory reactions and fibrosis. These effects are due primarily to damage of the S₁ and S₂ segments of the proximal tubule. Glomerular damage may also occur, since albuminuria is also occasionally observed.

Exposure to cadmium results in hepatic stimulation of the metal-binding protein metallothionein, a small protein with a molecular weight of ~ 6500 -6800 Da. Metallothionein is composed of 20 cysteine residues whose sulfhydryl groups readily complex with seven metal ions such as cadmium. The metallothionein-cadmium complex is released from damaged hepatocytes and is carried via the blood to the kidney. The low molecular weight of the metallothionein-cadmium complex allows it to be readily filtered at the glomerulus and enter the tubular lumen. The complex is absorbed from luminal fluid via a pinocytotic mechanism in the S_1 and S_2 segments and degraded by lysosomal proteases to release free cadmium. The free cadmium can then bind to renal metallothionein or attack targets such as calmodulin within proximal tubular cells.

Lead Lead is the most ubiquitous of the nephrotoxicant metals in the environment. Like mercury, the health effects of lead have been recognized for centuries with the nervous system as well as the kidney being a target for certain forms of these metals. Sources of exposure to lead include food ($\sim 100 \,\mu g$ or less per day for adults), lead-based paints, industrial emissions, lead dusts and leadglazed pottery.

Clinically, lead nephropathy is seen as either reversible tubular dysfunction or as an irreversible interstitial nephropathy. Tubular toxicity occurs most commonly in children following acute exposure and is characterized by glucosuria, phosphaturia, aminoaciduria, and occasionally proteinuria. One unique morphological feature of lead exposure is the formation of nuclear inclusion bodies within renal tubular cells. These bodies are complexes between lead and an acidic protein aggregate. Recently, it has been suggested that proteins in humans similar to rat α_{2u} globulin bind lead in the liver (or other tissues) and transport lead to the kidney where the lead:leadbinding protein complex is absorbed by an endocytotic mechanism. Within the renal cells, most of the lead is associated with the nuclear inclusion bodies. However, the lead-protein complex can be reversed with ethylenediaminetetraacetic acid (EDTA) and lead excretion promoted.

The cellular mechanism of lead nephrotoxicity appears to be due to an alteration of calcium homeostasis. Lead (Pb^{2+}) competes with calcium (Ca^{2+}) for transport, binding to calmodulin and at other cell calcium regulatory sites. Lead can accumulate in mitochondria using the calcium transporter and disrupt respiration. Interactions of lead with calmodulin can result in a disruption of the calcium messenger system to adversely affect normal cell function. The nuclear inclusion bodies may also alter the cellular function of DNA, although this interaction has not been fully elucidated.

Chronic exposure to low levels of lead results in lead accumulation within the body. Workers who have been chronically exposed to lead develop interstitial fibrosis, vascular and glomerular sclerosis, and tubular atrophy and/or hypertrophy. Although acute lead nephropathy is reversible with chelator therapy and/or removal from exposure, chronic effects may be irreversible. In addition, chronic exposure to lead may result in a gouty nephropathy as lead reduces uric acid excretion and elevates blood uric acid levels.

Halogenated Hydrocarbons Halogenated hydrocarbons encompass a large group of chemicals with a wide range of applications. Many of these compounds are organic solvents (e.g., chloroform, trichloroethylene) or chemical intermediates (e.g., bromobenzene, chloroanilines) used in laboratory, industrial, or commercial applications. Halogenated hydrocarbons are also used in agriculture as pesticides (e.g., 1,2-dibromoethane). The majority of nephrotoxicant halogenated hydrocarbons contain chloro and/or bromo groups with only a few nephrotoxicants substituted with fluoro or iodo groups.

The site and severity of the nephrotoxic lesion also varies widely among the halogenated hydrocarbons depending on chemical class, number and position of halogen groups, age, sex, species, dose, and preexisting conditions. Unlike the metals, halogenated hydrocarbons are usually not direct nephrotoxicants and require bioactivation before the ultimate nephrotoxicant species is produced. Several mechanisms of bioactivation have been identified for the halogenated hydrocarbons including oxidation, free radical formation, intramolecular cyclization, and conjugation with glutathione (Table 6). In the following sections, specific examples of halogenated hydrocarbons and their mechanisms of bioactivation will be discussed.

Chloroform Chloroform (trichloromethane, Figure 6) has been used in the past as an anesthetic and as an additive in pharmaceutical preparations. Today, chloroform is used primarily as an organic solvent. Chloroform-induced nephrotoxicity is primarily seen as proximal tubular toxicity with minimal changes in distal tubular function and no evidence of glomerular effects. Nephrotoxicity is characterized by proteinuria, glucosuria, elevated BUN concentration, and kidney weight, decreased accumulation of organic ions by renal cortical slices, and a fatty degeneration of proximal tubular cells. Species and sex differences exist in the susceptibility to the nephrotoxic effects induced by chloroform with certain strains of male mice (e.g., DBA/2J) being particularly sensitive.

 Table 6
 Mechanisms of bioactivation of halogenated hydrocarbons

Oxidation
Example: Chloroform
Free radical formation
Example: Carbon tetrachloride
Intramolecular cyclization
Example: 2-Bromoethylamine
Glutathione conjugation
Intramolecular cyclization
Example: 1,2-Dibromoethane
Cysteine conjugate β -lyase activation
Example: Trichloroethylene
Facilitated transport
Example: Bromobenzene

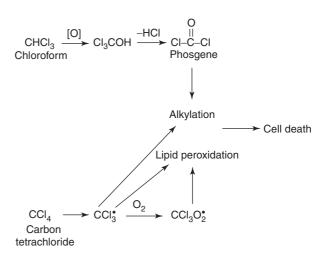


Figure 6 Bioactivation of chloroform and carbon tetrachloride.

The mechanism of chloroform nephrotoxicity involves the oxidation of chloroform to trichloromethanol by renal cytochrome P-450 isozymes (Figure 6). The trichloromethanol readily eliminates HCl to form the highly reactive toxicant phosgene (COCl₂). The phosgene can (1) be detoxified by conjugation with two molecules of glutathione, (2) react with water to form two molecules of HCl and one molecule of CO₂, or (3) covalently bind to renal macromolecules to disrupt cellular function and induce nephrotoxicity.

There are several lines of evidence that support the formation of phosgene as the ultimate nephrotoxicant species following exposure to chloroform in mice and rabbits. First, susceptible strains of male mice oxidize chloroform faster than resistant strains. Secondly, deuterium labeling of chloroform, to form CDCl₃, results in the formation of a chloroform derivative which is oxidized much slower than chloroform and is less potent as a nephrotoxicant. In addition, trapping experiments with cysteine have documented the formation of phosgene as a product of chloroform biotransformation. Although these results support phosgene as the toxicant species in mice and rabbits, it is not known with certainty if the same mechanism for nephrotoxicity is operating in humans. In humans, chloroform nephrotoxicity has been documented in both males and females, while only male mice exhibit nephrotoxicity following chloroform administration. Thus, additional or alternate mechanisms may be contributing to chloroform nephrotoxicity in humans.

Carbon Tetrachloride Carbon tetrachloride (CCl₄) was widely used as a dry cleaning solvent until its potential as a hepatotoxicant, nephrotoxicant, and carcinogen was recognized. Currently, carbon tetrachloride is used as an organic solvent. Nephrotoxicity associated with dermal or inhalation exposure to carbon tetrachloride is seen as acute tubular necrosis which is delayed in onset. Death occurs from acute renal failure, usually within three weeks of intoxication. Interestingly, humans appear to be more sensitive to acute carbon tetrachloride-induced nephrotoxic to carbon tetrachloride tetrachloride-induced nephrotoxic to acute carbon tetrachloride-induced nephrotoxic to acute the most animal models.

The mechanism of carbon tetrachloride nephrotoxicity involves the initial homolytic cleavage of carbon tetrachloride by cytochrome P450 to form the trichloromethyl and chlorine free radicals (Figure 6). The trichloromethyl free radical can then alkylate renal macromolecules or interact with membrane unsaturated fatty acids to initiate lipid peroxidation. The trichloromethyl free radical may also combine with molecular oxygen to form a peroxy free radical that is more reactive than the trichloromethyl free radical. The peroxy radical could also interact with unsaturated fatty acids in membranes to induce lipid peroxidative damage. Recently, it has been proposed that under anaerobic conditions, carbon tetrachloride is converted to a carbene metabolite (Cl_3C_2), which can covalently bind to cell macromolecules in the liver. However, the role of the carbene metabolite in carbon tetrachloride nephrotoxicity is less clear.

N-(3,5-Dichlorophenyl)succinimide During the 1970s, a large number of N-(halophenyl)succinimides were synthesized and evaluated as agricultural fungicides. The most promising agent in this class of compounds was N-(3,5-dichlorophenyl)succinimide (NDPS), which had a broad spectrum of antifungal activity. NDPS, marketed as Ohric, also proved to be a nephrotoxicant and to promote the carcinogenic activity of several nephrocarcinogens. As a result of potential health hazards NDPS production was halted for many years. However, recently NDPS manufacture and sales have begun again in China for use as an agricultural antimicrobial agent.

Acute NDPS nephrotoxicity is characterized by diuresis, proteinuria, glucosuria, hematuria, and elevated BUN concentration and kidney weight, decreased organic ion accumulation by renal cortical slices and marked proximal tubular necrosis. Sex differences exist for NDPS nephrotoxicity with female Fischer 344 rats being twice as sensitive as males. Interestingly, the primary site of the renal lesion in males is the S₁ and S₂ segments of the proximal tubule, while the S₃ segment is the most affected segment in females. Chronic NDPS nephrotoxicity is seen as marked renal interstitial nephritis.

The ultimate nephrotoxicant species responsible for acute or chronic NDPS nephrotoxicity is related to the formation of sulfate and possibly glucuronide conjugates of NDPS metabolites. Oxidation of the succinimide ring in the liver via phenobarbital-inducible cytochrome-P450 isozymes forms N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS, Figure 7), an essential bioactivation step in acute nephrotoxicity. Both NDHS and its hydrolysis product, N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (NDHSA), are four times more potent as nephrotoxicants than NDPS, while the decarboxylation metabolite of NDHSA is a nonnephrotoxicant. However, neither NDHS nor NDHSA appears to be directly toxic to renal cortical slices, proximal tubule suspensions or renal mitochondria. Studies also suggest that NDHSA can cyclyze in vivo to form NDHS which further clouds the identity of which NDPS metabolite ultimately gives rise to the toxicant species.

Recent reports suggest that the sulfate conjugate of NDHS (NSC) may be the ultimate or penultimate

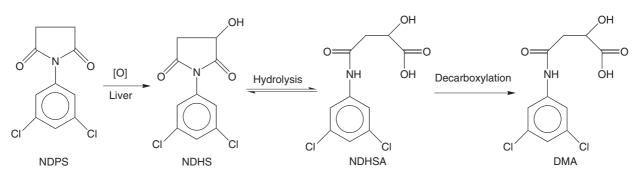


Figure 7 Oxidative biotransformation of N-(3,5-dichlorophenyl) succinimide (NDPS).

toxic metabolite. NSC is formed in the liver and is directly toxic to proximal tubule cells *in vitro*. Whether NSC directly interacts with intracellular targets or breaks down to release *N*-(3,5-dichlorophenyl)maleimide (NDPM), a highly reactive chemical species, as the ultimate toxic species remains to be determined. However, NSC and NDPM exhibit similar nephrotoxic potential *in vitro* with freshly isolated renal cortical cells.

2-Haloethylamines The 2-haloethylamines are model nephrotoxicants that target different segments of the nephron depending on the halogen atom. The bromo derivative, 2-bromoethylamine (BEA), concentrates in the renal medulla and induces renal papillary necrosis. The renal effects of BEA are dependent on the urinary concentrating ability of antidiuretic hormone (ADH). In the absence of ADH, urine is not concentrated in collecting ducts and BEA nephrotoxicity is diminished. The chemical species responsible for BEA nephrotoxicity is believed to be ethyleneimine formed by the intramolecular cylization of BEA which then alkylates renal macromolecules (Figure 8).

The chloro derivative, 2-chloroethylamine (CEA), is less potent as a papillitoxin than BEA, presumably due to the fact that the bromo group is a better leaving group than the chloro group. Thus, the reactive intermediate, ethyleneimine, would form faster from BEA than CEA. The fluoro derivative, 2-fluoroethylamine (FEA), is more lethal than BEA, but at nonlethal doses is toxic to the S₃ segment of the proximal tubule rather than the renal papilla. Since the fluoro group is a poorer leaving group than chloro or bromo groups, it is not clear if FEA nephrotoxicity is due to FEA, ethyleneimine or a FEA metabolite.

Glutathione Conjugates Glutathione (GSH) is a tripeptide (gamma-Glu-Cys-Gly), which forms bonds between the sulfhydryl group of cysteine and electrophilic sites in xenobiotics or their metabolites. The

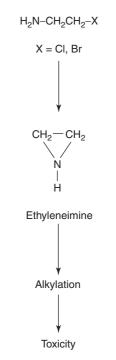


Figure 8 Intramolecular cyclization of haloethylamines.

formation of these glutathione conjugates is catalyzed by one or more of the glutathione *S*-transferase enzymes. The α forms of glutathione *S*-transferase can also catalyze detoxification of organic peroxides to protect cells against free radical toxicity as well as electrophilic attack. Thus, interactions with glutathione serve as an important mechanism for detoxifying reactive chemical species within cells.

Although interactions between glutathione and electrophilic molecules normally leads to detoxification, there are also several examples of bioactivation of chemicals to nephrotoxicants following glutathione conjugation. Glutathione conjugates can be formed in most segments of the kidney with glutathione S-transferase activity being highest in proximal tubular cells. However, glutathione conjugates of many electrophiles are formed primarily in the liver and transported to the kidney either as the glutathione conjugate or as a glutathione conjugate metabolite.

Glutathione conjugates formed in the liver can leave hepatocytes and enter the blood or the bile. Biliary excretion of the glutathione conjugate can result in degradation of the glutathione conjugate to the cysteine conjugate within the biliary tract and small intestine. This degradation is catalyzed by gamma-glutamyl transpeptidase (removes glutamate) and peptidases (remove glycine). The N-acetylcysteine conjugate, also known as a mercapturate, can be formed in the intestinal tract or liver following absorption of the cysteine conjugate from the small intestine by action of an N-acetylase enzyme. The relative amounts of the three conjugates that reach the kidney from extrarenal sites depend primarily on the chemical nature of the halogenated hydrocarbon and the animal species studied.

The kidney also can process and transport glutathione, cysteine, and N-acetylcysteine conjugates. Glutathione conjugates can be converted to the cysteinylglycine conjugate by gamma-glutamyl transpeptidase, which is located in kidney primarily at the brush border membrane. The action of brush border dipeptidase enzymes (e.g., aminopeptidase M) then removes the glycine residue from the dipeptide conjugate to release the cysteine conjugate, which is usually accumulated within proximal tubular cells. Glutathione conjugates may also be directly transported into renal cells at the basolateral membrane via a sodium dependent uptake mechanism. Once inside the cell, the glutathione conjugate may be a substrate for gamma-glutamyl cyclotransferase and peptidases to release the cysteine conjugate. Cysteine conjugates can also be substrates for amino acid uptake systems and accumulate in proximal tubular cells via the amino acid transporters, while mercapturates accumulate in proximal tubular cells via the organic anion transporter at the basolateral membrane. Accumulated mercapturates may then be secreted into the urine or deacetylated to release the corresponding cysteine conjugate. Thus, the kidney can be exposed to metabolites formed from glutathione conjugation originating at renal or extrarenal sites.

Several mechanisms have been identified for the generation of nephrotoxicants following conjugation of halogenated hydrocarbons with glutathione (**Table 6**). These mechanisms include intramolecular cyclization, activation by cysteine conjugate β -lyase, and facilitated renal accumulation of the toxicant. Examples of each of these bioactivation mechanisms will be discussed in the following sections.

Intramolecular Cyclization The 1,2-dihaloethanes (XCH₂CH₂X) are used as pesticides, lead scavengers,

industrial solvents and/or grain fumigants. Both 1,2-dichloroethane and 1,2-dibromoethane are hepatotoxicants, nephrotoxicants, and potential carcinogens. Acute renal effects induced by these alkyl halides are characterized as proximal tubular necrosis, primarily in the juxtaglomerular regions. The mechanism of the nephrotoxicity induced by the 1,2dihaloethanes is believed to initially involve conjugation of the halogenated hydrocarbon with glutathione (**Figure 9**). Both 1,2-dichloroethane and 1,2-dibromoethane form glutathione conjugates in liver, but only 1,2-dibromoethane forms a glutathione conjugate in the kidney.

The glutathione conjugate formed from 1,2dibromoethane is relatively unstable and quickly undergoes intramolecular cyclization via displacement of the second bromo group by the glutathionyl sulfur to form an episulfonium ion (Figure 9). The episulfonium ion then readily forms adducts with renal cell macromolecules, including DNA, which leads to altered cell function and toxicity. Because of the highly reactive nature of the glutathione conjugate formed from 1,2-dibromoethane, it is likely that only the renally formed glutathione conjugate contributes to nephrotoxicity.

The glutathione conjugate formed in the liver from 1,2-dichloroethane is more stable than the conjugate

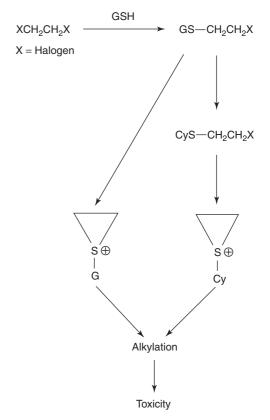


Figure 9 Glutathione conjugation with 1,2-dihaloethanes.

formed from 1,2-dibromoethane, and may be transported to the kidney as the glutathione or related conjugate. Within the kidney, both glutathione and cysteine conjugates appear to contribute to 1,2-dichloroethane nephrotoxicity by forming episulfonium ions which can interact with cellular macromolecules (Figure 9). Reactivity of the glutathione and cysteine conjugates toward DNA is greatest for the cysteine conjugates to the carcinogenic mechanism for 1,2-dichloroethane remains to be determined.

Two additional compounds containing 1,2-dihaloethyl groups are also nephrotoxicants. The nematocide and soil fumigant 1,2-dibromo-3-chloropropane and the flame retardant tris(2,3-dibromopropyl)phosphate both induce acute tubular necrosis. Although conjugation with glutathione may play a role in the toxicity induced by these agents, the mechanism responsible for the nephrotoxicity induced by these compounds is not known.

Cysteine Conjugate β -Lyase Activation There are numerous glutathione and/or cysteine conjugates of unsaturated halogenated hydrocarbons that are nephrotoxicants and/or nephrocarcinogens (Table 7). These conjugates induce nephrotoxicity following cleavage of the cysteine conjugate by the enzyme cysteine conjugate β -lyase to form pyruvate, ammonia and a reactive thiol. Acute nephrotoxicity induced by these conjugates is characterized by diuresis, proteinuria, glucosuria, elevated BUN concentration, and proximal tubular necrosis. The site of the lesion appears to be species dependent with most rodent models exhibiting the initial lesion in the corticomedullary region (S₃ segment of the proximal tubule). However, the initial lesion in dogs appears in the S_1 and S_2 segments of the proximal tubule. In addition, age, gender and strain differences exist for susceptibility to the nephrotoxicity induced by these conjugates.

 Table 7
 Some halogenated hydrocarbons whose glutathione and/or cysteine conjugates are nephrotoxicants and/or nephrocarcinogens

1-Chloro-1,2,2-trifluoroethylene 1,1-Dibromo-2,2-difluoroethylene Dichloroacetylene 1,1-Dichloro-2,2-difluoroethylene Hexachloro-1,3-butadiene Hexafluoropropene Tetrachloroethylene Tetrafluoroethylene Trichloroethylene 1,1,2-Trichloro-3,3,3-trifluoro-1-propene The nature of the ultimate nephrotoxicant species formed following the action of cysteine conjugate β -lyase is determined by the halogen substitution on the parent haloalkene. When the haloalkene is a geminal difloroalkene (e.g., tetrafluoroethylene), then the resulting glutathione conjugate and subsequent cysteine conjugate is a saturated or alkyl conjugate (Figure 10).

However, if the haloalkene is a geminal dichloroalkene (e.g., trichloroethylene), then unsaturated conjugates are formed (Figure 10). The saturated cysteine conjugates are bioactivated by β -lyase to form a thionoacyl fluoride which can rapidly acylate renal macromolecules to induce toxicity, while the unsaturated cysteine conjugates are bioactivated by β -lyase to the highly reactive thioketene metabolites (Figure 11). Primary targets for these reactive intermediates appear to be the renal mitochondria which contain a portion of cellular cysteine conjugate β -lyase.

The reasons why the kidney is a major target for cysteine conjugate toxicity are not completely understood, particularly since cysteine conjugate β -lyase is present in organs other than the kidney. However, the ability of the kidney to (1) accumulate metabolites formed by the glutathione conjugate pathway from blood, (2) convert glutathione and *N*-acetylcysteine conjugates to cysteine conjugates, and (3) rapidly activate nephrotoxicant cysteine conjugates to their reactive intermediate may explain the susceptibility of the kidney to these agents.

The toxicity induced by nephrotoxicant glutathione, cysteine, and *N*-acetylcysteine conjugates can be modified by a variety of compounds. Probenecid, an

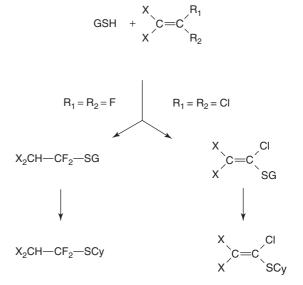


Figure 10 Glutathione conjugation with geminal difluoro- or dichloroalkenes.

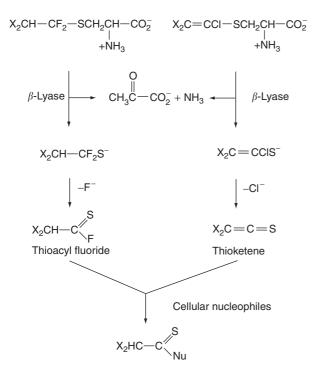


Figure 11 Cysteine conjugate β -lyase bioactivation of cysteine conjugates of haloalkenes.

inhibitor of the organic ion transporter, can reduce the nephrotoxicity induced by glutathione conjugates, presumably by decreasing the renal accumulation of the mercapturate. Acivicin (AT-125), an anticancer agent, is an irreversible inhibitor (>97%)of gamma-glutamyl transpeptidase and can attenuate the toxicity of nephrotoxicant glutathione conjugates by inhibiting conversion of the glutathione conjugate to the cysteine conjugate. In addition, aminooxyacetic acid (AOAA) inhibits the action of pyridoxaldependent enzymes (e.g., β -lyase) and blocks the conversion of cysteine conjugates to their ultimate nephrotoxicant species. These inhibitors are useful tools in the study of glutathione and cysteine conjugate nephrotoxicity, but they may not always give clear results. Probenecid has biological effects unrelated to inhibition of organic ion transport, and acivicin pretreatment can fail to protect against haloalkene (e.g., hexachloro-1,3-butadiene) nephrotoxicity even when it is known that glutathione and/or cysteine conjugates of the parent haloalkene are nephrotoxicants. Also, AOAA not only inhibits β -lyase but can inhibit oxidative enzymes and other pyridoxal-dependent pathways as well.

Facilitated Accumulation of Nephrotoxicants Bromobenzene, a chemical intermediate, is both a heptotoxicant and a nephrotoxicant. Bromobenzene-induced nephrotoxicity is characterized by

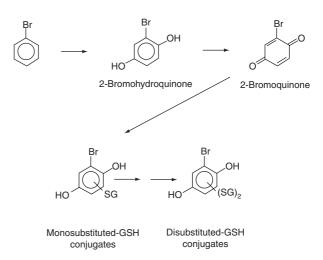


Figure 12 Bioactivation of bromobenzene to nephrotoxicant metabolites.

glucosuria, enzymuria, proteinuria, elevated BUN concentration and kidney weight, and proximal tubular necrosis with the S_3 segment exhibiting the greatest damage. Bromobenzene hepatotoxicity is due to cytochrome P450 mediated formation of a 3,4-epoxide (arene oxide) metabolite of bromobenzene that can arylate hepatic tissue. However, the formation of the ultimate nephrotoxicant species appears to require multiple biotransformation steps and possibly multiple toxicant species.

The first step in the bioactivation of bromobenzene to a nephrotoxicant is the hepatic cytochrome P450 mediated oxidation of bromobenzene to 2-bromophenol, which is further oxidized in the liver to 2bromohydroquinone. 2-Bromohydroquinone can be activated by a renal quinol oxidase to 2-bromoquinone (Figure 12) which can directly arylate renal macromolecules. Although 2-bromoquinone can undergo redox cycling to potentially generate oxidative stress and reactive oxygen species, arylation appears to be more important for the cellular toxicity induced by 2-bromoquinone.

Recent studies have also demonstrated that monoand di-glutathione conjugates of 2-bromohydroquinone can be formed *in vivo* in rats (Figure 12). The diglutathionyl conjugate of 2-bromohydroquinone (a quinol-thioether) induces nephrotoxicity which is indistinguishable from bromobenzene, 2-bromophenol, or 2-bromohydroquinone nephrotoxicity, but occurs at a dose 10–15 times lower than with 2-bromohydroquinone. The ability of acivicin but not AOAA to attenuate nephrotoxicity induced by the diglutathionyl conjugate has suggested that gamma-glutamyl transpeptidase but not β -lyase is important in activating the conjugate. Thus, entry into renal tissue via gamma-glutamyl transpeptidase is a key step for diglutathionyl conjugate nephrotoxicity. Partial protection by acivicin against 2-bromohydroquinone nephrotoxicity *in vivo* suggests that the mono- and/or diglutathionyl conjugates of 2-bromohydroquinone may be formed extrarenally, and that glutathione conjugation may play a role in the transport and selective accumulation of 2bromohydroquinone in renal tissue.

The cellular mechanism of nephrotoxicity induced by the quinol-thioethers appears to be related to the ability of the conjugates to undergo redox cycling with the concomitant formation of reactive oxygen species and oxidative stress. The cysteine conjugates of 2-bromohydroquinone are more readily oxidized than the corresponding glutathione conjugates or mercapturates, and therefore, are probably responsible for most of the conjugate-induced oxidative stress and resultant nephrotoxicity. However, the exact nature of the subcellular targets and relative contributions of the various bromobenzene metabolites to bromobenzene nephrotoxicity remains to be determined with certainty.

Mycotoxins The mycotoxins are secondary products of fungal metabolism. Numerous mycotoxins have been identified as toxicants in humans and/or animal models with several organ systems, including the kidney, being targets for these fungal products. Perhaps the two mycotoxins that have received the most attention as nephrotoxicants are citrinin and ochratoxin A. These two mycotoxins have received particular interest due to their possible role in endemic Balkan nephropathy.

Citrinin is produced by several *Penicillium* and *Aspergillus* species which may be found associated with grains (e.g., wheat, oats, etc.). Humans and animals eating the grain can experience citrinin-induced nephrotoxicity which is characterized by diuresis, decreased urinary osmolality, glucosuria, proteinuria, and elevated BUN concentration. Morphological changes included cytoplasmic vacuolization of proximal tubular cells, mitochondrial swelling and ultimately, proximal tubular necrosis. The exact site of the renal lesion may vary depending on the species studied.

The mechanism of citrinin-induced nephrotoxicity has not been completely elucidated. However, it appears that citrinin accumulates in proximal tubular cells via the organic anion transporter, and that the parent compound is the nephrotoxicant species. Mitochondria are early targets for citrinin with multiple effects on mitochondrial function observed following exposure of mitochondria to citrinin, including uncoupling of mitochondrial respiration. The subsequent lost of cellular ATP content may eventually lead to cell death.
 Table 8
 Miscellaneous nephrotoxicants

Petroleum components	Paraquat
Carbon disulfide	Lindane
Oxalic acid	Diquat
Venoms	3-Chloropropane
Crotalus venom	Glycols
Brown recluse spider venom	Maleic acid
Decalin	Ethylene glycol
Tetralin	Propylene glycol
Mushroom poisoning	Glycerol
D-Limonene	Radiation
Carbon monoxide	Physical agents
1,4-Dichlorobenzene	Crush injuries

Ochratoxin A is also produced by Aspergillus species and is one of the most widely occurring mycotoxins in food and grains. Ochratoxin A nephrotoxicity is similar to citrinin nephrotoxicity but can also include renal interstitial fibrosis and glomerular changes. Like citrinin, ochratoxin A accumulates in proximal tubular cells via the organic anion transporter and appears to induce nephrotoxicity without bioactivation to a toxic metabolite. Mitochondria are also a target for ochratoxin A with proximal tubular ATP content significantly decreased in the presence of as little as 10^{-8} mol l⁻¹ ochratoxin A. However, ochratoxin A also inhibits renal gluconeogenesis and lowers mRNA levels, and it has been suggested that these events may also contribute to ochratoxin A nephrotoxicity. While each of these cellular effects might contribute to the renal toxicity induced by ochratoxin A, the precise cellular mechanism of toxicity remains to be determined.

Miscellaneous Nephrotoxicants There are numerous additional agents that have been identified as nephrotoxicants, and some of these chemical and physical agents are shown in Table 8. In addition to the compounds listed, there are hundreds of chemicals whose effects on the kidney are either unknown or are poorly characterized, and unquestionably this list will continue to grow with the ongoing development of newer drugs, agricultural agents, and industrial intermediates.

See also: Carbon Tetrachloride; Cephalosporins; Chloroform; Cisplatin; Glutathione; Metals; Penicillin.

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Killer Lakes

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Killer Lakes is the name given to the disasters involving rapid releases of massive volumes of carbon dioxide (CO_2) from two lakes in Cameroon. Humans and other animals were asphyxiated, with reports of 37 victims in the Lake Monoun disaster in 1984, and 1800 victims in the Lake Nyos disaster in 1986. The lakes occupy the crater of a supposedly extinct volcano, in a region known for numerous gaseous water springs, a common feature of old volcanic areas. These disasters began when a cloud of dense gas erupted from each lake, covering the surrounding areas under a layer of gas many meters thick, for an unknown amount of time. The source of the gas was determined afterwards, with the evidence including damage to the shores by waves and strong winds, and having the normally clear waters turn reddish.

Investigations found indications that much, if not all, of the CO₂ released was stored in the lakes prior to the events, and that a volcanic eruption was not thought to be associated with either disaster. Accumulation of CO₂ in the lakes started from CO₂-rich gas of magmatic origin rising to the earth's surface and contacting groundwater; the CO₂-charged groundwater is then discharged into the bottom of the lakes in springs. Before the gas events, these lakes were strongly stratified, that is, the surface and bottom waters did not mix, which allowed the gas that was being discharged from the groundwater to build up in the bottom waters of the lakes. The sudden release to the surface of CO₂ trapped at the bottom of the lakes has been called 'lake overturn'.

The triggers responsible for the gas releases from either lake are unknown, but one cause could have been a large landslide entering the lake and causing the lake stratification to be disrupted and allowing local oversaturation to initiate the gas release. Both disasters occurred in August, when stratification is Basic Science of Poisons, 6th edn., pp. 491–514. New York: McGraw-Hill.

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weakest as the surface water loses heat during the monsoon season. Further, that both disasters occurred in the mid-1980s could be related to lower than normal temperatures and higher than normal rainfall during those years in Cameroon.

Both lakes Nyos and Monoun still contain appreciable levels of CO_2 . Steps to reduce the hazards since the 1984 and 1986 disasters have included the use of pipes inserted into each lake to pump the gas-rich bottom waters to the surface, where the gas can be discharged harmlessly to the atmosphere. An international advisory committee is coordinating this effort, with major funding from the Cameroonian Government, The US Office of Foreign Disaster Assistance (part of the US Agency for International Development), and The French Embassy in Cameroon. Researchers involved in the degassing project have been funded in part by their home institutions. Further, private donations have helped to support CO_2 early-warning systems.

A third lake, Kivu in Rwanda, has been highlighted in a survey of deep lakes in Africa and Indonesia as another location where this type of disaster could happen from a massive geological event, that is, an earthquake or volcanic eruption. Lake Kivu was found to have high concentrations of dissolved CO_2 in its bottom water, and is one of the largest and deepest lakes in Africa with two million people living on its shore.

See also: Carbon Dioxide.

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Lanthanide Series of Metals

Charles E Lambert

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- REPRESENTATIVE CHEMICALS: Cerium (Ce); Dysprosium (Dy); Erbium (Er); Europium (Eu); Gadolinium (Gd); Holmium (Ho); Lanthanum (La); Lutetium (Lu); Neodymium (Nd); Prometheum (Pm); Praseodymium (Pr); Samarium (Sa); Terbium (Tb); Thulium (Tm); Ytterbium (Yb); Yttrium (Y)
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Cerium (CAS 7440-45-1); Dysprosium (CAS 7429-91-6); Erbium (CAS 7440-52-0); Europium (CAS 7440-53-1); Gadolinium (CAS 7440-54-2); Holmium (CAS 7440-60-0); Lanthanum (CAS 7439-91-0); Lutetium (CAS 7440-94-3); Neodymium (CAS 7440-00-8); Prometheum (CAS 7440-12-2); Praseodymium (CAS 7440-10-0); Samarium (CAS 7440-19-9); Terbium (CAS 7440-27-9); Thulium (CAS 7440-30-4); Ytterbium (CAS 7440-64-4); Yttrium (CAS 7440-65-5)
- SYNONYMS: Rare earths, rare-earth metals
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Transition metals
- CHEMICAL FORMULAS: Common compounds:
 Cerium oxide: Ce₂O₃
 - Gadolinium oxide: Gd₂O₃
 - Lanthanum oxide: La_2O_3
 - Yttrium oxide: Y_2O_3
 - Cerium nitrate: Ce(NO₃)₃
 - Cerium Intrate: Ce(1(03))
 Cerium chloride: CeCl₃

Uses

Most of the industrial uses of the lanthanides require compounds (e.g., oxides) rather than pure elements. Most of these are cerium compounds or mixtures of lanthanides as they occur in ores (e.g., lanthanum concentrate). Some of the major uses are:

- *Carbon-arc lighting*: The US Army, Navy, and Coast Guard searchlights all use lanthanide-cored carbons.
- *Lanthanide alloys*: These alloys are used in cigarette lighter flints, magnesium alloys, and ferrous alloys.

- *Glass industry*: Important uses for the coloring and decoloring of glass, the polishing of spectacle and optical instrument lenses, the surface preparation of mirror glass and other glass specialties.
- *Medicine*: Gadolinium diethylenetriamine pentaacetic acid (DTPA) is routinely used as an intravenous contrast agent for magnetic resonance imaging.

Other applications for the lanthanides include phosphors for X-ray screens and television tubes, catalysts, lasers, powerful magnets, and high-temperature superconductors.

Background Information

The lanthanide series of metals includes the 15 elements with atomic numbers 57-71, plus yttrium (atomic number 39). The lanthanides occur in the earth's crust at concentrations exceeding some commonly used industrial elements making the term 'rare earths' something of a misnomer. For example, yttrium, cerium, lanthanum, and neodymium are present in the earth's crust at higher concentrations than lead. Of the 15 lanthanides, only promethium does not occur in nature - it is a man-made element. All of the lanthanides have similar physical and chemical properties. Because of similarities in their chemistry and toxicity, the characteristics of the lanthanides are often described as a group. Within the lanthanide group, however, there are differences between the toxicity of the individual lanthanide elements and their compounds.

Exposure Routes and Pathways

Direct eye or skin contact with the powder or liquid forms of the soluble lanthanide compounds including the chlorides and acetates can cause irritation. These forms of the lanthanides are the most likely to cause damage at the point of contact. The highly insoluble lanthanide oxides and carbonates may cause mild abrasive irritation upon dermal contact.

Occupational exposure may occur through inhalation of dust and dermal contact. The general population may be exposed to naturally occurring concentrations of the oxides in soil or through medical procedures such as the intravenous administration of magnetic resonance imaging (MRI) contrast agents.

Toxicokinetics

Different forms of lanthanide differ in their toxicity. There are three forms of lanthanides: soluble (chlorides, nitrates, acetates), insoluble (oxides, carbonates), and chelated compounds (DTPA). Most of the available information on lanthanide absorption and toxicity comes from the soluble lanthanide salts. In one study, rats given DTPA (chelating agent) 1 or 2 days after oral administration of cerium chloride were found to have significantly reduced whole body retention of soluble cerium (from 40% to 2%).

Different forms of lanthanide have different organ distribution and excretion rates. Intravenously injected chelated lanthanide is transiently accumulated in the kidney and most of the injected dose is excreted in the urine. However, intravenously injected soluble salt is taken up by the reticuloendothelial cells, with most of the dose accumulating in the liver and spleen. The result of this intravenous exposure is liver necrosis.

The lanthanide oxides and carbonates have been shown in *in vitro* bioaccessibility studies to have a very low gastrointestinal bioaccessibility of $\sim 6\%$.

Mechanism of Toxicity

The soluble lanthanide salts (e.g., chlorides, nitrates, and acetates) can be severely irritating to the skin, eye, and mucous membranes. The irritation appears to be a result of exposure to the anion (e.g., nitrate) and not the lanthanide cation.

Effects from oral exposure to the soluble lanthanides include eosinophil infiltration of the submucosa, hyperkerotosis of the stomach, and gastric hemorrhages. As with the irritation that occurs after skin or eye exposure, the hyperkerotosis and gastric hemorrhages seen in the stomach appears to be the result of the acidic environment produced by the anion.

Acute and Short-Term Toxicity (or Exposure)

The lanthanides have historically been characterized as low toxicity metals and therefore have not been the subject of significant toxicological investigation. The data that have been gathered are primarily from acute and chronic animal studies. Because human exposures have rarely reached toxic levels, few instances of human toxicity have been observed, despite their widespread industrial use. The following discussion of toxicity focuses on the soluble forms and the oral route of exposure.

Animal

Most of the LD_{50} s for the lanthanides are high. For example, the most recent data for lanthanum oxides, carbonates, and concentrates tested show LD₅₀s in excess of 5000 mg lanthanum per kg animal body weight. This LD_{50} range is generally regarded as 'practically nontoxic'. However, some lanthanides appear to be more toxic than the oxides and carbonates - with both compound solubility and the form of the anion playing a role in toxicity. For example, the lanthanum chlorides, nitrates, and acetates have LD_{50} s in the 1600–5000 mg kg⁻¹ body weight range, putting them in the 'slightly toxic' range. Common symptoms of acute toxicity seen after these very high doses included writhing, ataxia, slightly labored and depressed respiration, arched back, stretching of limbs on walking, and lacrimation. Table 1 includes a summary of current oral LD50, eye and skin irritancy data for individual lanthanide compounds and some commonly used mixtures.

Subchronic A number of studies have been completed in which the soluble lanthanide chlorides are given orally to animals over the course of a month. In several studies rats were given the hydrated chloride forms of lanthanum, yttrium, and europium by oral gavage at doses of 0, 40, 200, or $1000 \,\mathrm{mg \, kg^{-1} \, day^{-1}}$ for consecutive 28 days. Those animals administered lanthanum chloride demonstrated a slight decrease in body weight due to decreased food intake at the 200 and $1000 \,\mathrm{mg \, kg^{-1}}$ doses. Changes in serum transaminase activity were also observed at the higher dose $(1000 \text{ mg kg}^{-1})$. This activity is suggestive of liver toxicity, though no corresponding histopathological changes were observed in the liver. At the higher dose, stomach lesions were also observed. This is not surprising given the chloride form of the lanthanides

 Table 1
 Acute toxicity of select lanthanides and common mixtures

Compound	Eye irritation	Skin irritation	Oral LD ₅₀ (g kg ⁻¹)
Cerium concentrate Cerium chloride Cerium nitrate Lanthanum	Moderate Severe Severe Minimal	Nonirritant Severe Mild Nonirritant	>5 2.8 4.2 >5
concentrate Lanthanum oxide	Mild	Nonirritant	>5

and its potential for irritation. Similar effects were observed for the other lanthanides tested.

In another series of studies of the soluble lanthanide chlorides, rats were fed gadolinium, samarium, terbium, thulium, ytterbium, praseodymium, neodymium, lutetium, europium, dysprosium, holmium, and erbium chloride in their diet at doses of 0, 5, 50, and $500 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 12 weeks. Only ytterbium chloride caused any significant effect, with the 500 mg kg^{-1} dose causing gastric hemorrhages. The other lanthanides caused no adverse effects at the maximum 500 mg kg^{-1} dose.

Human

In one of the best documented human lanthanide exposure studies to date, the toxicity of gadolinium in patients with impaired kidney function was assessed. One hundred fifty-one patients with compromised kidney function were assessed after a dose of 0.1 mmol gadolinium DTPA per kg body weight was administered as a contrast agent for MRI examinations. A retrospective analysis of physician and nursing records, radiology reports, laboratory data, and autopsy records for 3 days prior to the MRI and 30 days after was completed. No significant adverse effects were observed after intravenous gadolinium exposure in this sensitive subpopulation.

Chronic Toxicity (or Exposure)

Animal

In one of the only drinking water studies of the lanthanides, mice consumed yttrium nitrate at 5 ppm for 18 months. Based on this 5 ppm drinking water concentration, the calculated dose was 0.95 mg kg^{-1} day⁻¹. A decrease in body weight was observed in the animals over the course of the study. However, survival of the animals compared to controls was not affected following lifetime exposure.

In a reproductive toxicity study, a diet containing a number of heavy metals including the lanthanides (as oxides) was fed to mice over three generations. The highest calculated lanthanide dose in the diet was a combination of the following: 156 mg dysprosium kg⁻¹ day⁻¹, 5 mg europium kg⁻¹ day⁻¹, 52 mg lanthanum kg⁻¹ day⁻¹, 104 mg samarium kg⁻¹ day⁻¹, 156 mg terbium kg⁻¹ day⁻¹, 16 mg ytterbium kg⁻¹ day⁻¹, and 10 mg thulium kg⁻¹ day⁻¹. After three generations of exposure no reproductive or other health effects were observed in the treated animals.

None of the lanthanide toxicity data gathered to date indicates that the lanthanides cause long-term health effects such as reproductive or carcinogenic toxicity.

Human

Current literature on long-term (20 year) occupational exposure to high levels of lanthanide dust suggests that the lanthanide oxides may cause benign pneumoconiosis (the deposition of material in the lung, visible on X-ray, without any impairment of lung function). This conclusion is further supported by animal studies.

Clinical Management

The soluble forms of the lanthanides may cause severe eye and skin irritation. If direct eye contact immediately hold eyelids apart and flush the affected eye(s) with clean water for several minutes. If skin contact, cleanse affected area(s), thoroughly washing with mild soap and water.

Ingestion of the soluble lanthanides may cause gastric irritation. The stomach contents can be diluted by drinking copious amounts of water. Because of potential gastrointestinal irritation, vomiting should not be induced.

The lanthanide oxides and carbonates have a low degree of toxicity by ingestion; however, dusts may be abrasive and irritating to the eyes and skin.

Lanthanides, because of their high density, may produce striking abnormalities on chest X-rays. However, lanthanides are generally not believed to be fibrogenic and the lesions typically have little or no clinical significance. Occasional cases of suspected pneumoconiosis have been reported.

Environmental Fate

The lanthanides can be found in the earth's crust at a wide range of concentrations. For example, thulium is present at only 0.5 ppm, whereas lanthanum and cerium are present at 30 and 60 ppm, respectively. The mineralized forms of the lanthanides that are of greatest commercial and mining interest are monazite, bastnaesite, and cerite. The most common commercial forms of the lanthanides are the oxides and carbonates, which have low solubility and mobility. In contrast, the lanthanide chlorides, nitrates, and acetates, because of their high solubility, are more likely to leach into groundwater and surface water.

Ecotoxicology

There is little information available on the ecotoxicology of the lanthanides. Lanthanides do not appear to be essential elements for plants and animals. In general, plants do not absorb lanthanides from soil due to discrimination against their absorption by the roots. This negligible accumulation of lanthanides by plants effectively blocks the dietary transfer of lanthanides from the soil to wildlife. In mammals the gastrointestinal absorption of simple lanthanide salts is poor.

One study assessed the toxicity of lanthanides in soil invertebrates. In this study native microflora were exposed to soils treated with 57 ppm lanthanum chloride for 23 days. Reduced respiration, which can be directly related to reproductive success, was observed at 57 ppm lanthanum chloride.

No water quality objectives or other water quality standards were found for the lanthanide metals. Aquatic toxicology data were only found for the lanthanide soluble salts. These soluble salts are known to have high chronic toxicity in fish, moderate chronic toxicity in green algae, and low acute toxicity in daphnids based on exposures in moderately hard water in terms of lanthanide per liter.

Other Hazards

Always refer to the appropriate Material Safety Data Sheet (MSDS) for detailed information on handling and disposal. The soluble lanthanides may be corrosive. The insoluble lanthanide oxides and

Law and Toxicology

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Introduction

Courts, legislatures, and administrative agencies in the United States frequently rely upon toxicologists to assist them in legal proceedings. The role of the toxicologist varies with the forum. When agencies develop regulations, for example, toxicologists typically are asked only to review documents and advise regulators on technical issues, either as a matter of personal knowledge, or as a matter of expertise. Similarly, in court cases, the toxicologist's role most often is limited to reviewing documents and advising litigants. Occasionally, however, both in regulatory and in judicial proceedings, the toxicologist contributes further by offering oral or written testimony on behalf of the parties, or at the request of the agency or tribunal. Rarely, toxicologists may be granted opportunities to testify before committees of Congress or state legislatures.

carbonates are expected to be stable indefinitely under most conditions.

Exposure Standards and Guidelines

Of the lanthanides, only yttrium has occupational exposure standards. The other lanthanides have low levels of toxicity similar to or less toxic than yttrium and therefore the exposure limits set for yttrium are generally used for the other lanthanides.

See also: Kidney; Metals.

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Thoughtful toxicologists seeking to make significant contributions to legal process should develop a basic understanding of the law of evidence as it applies to 'experts'. At the outset, toxicologists should appreciate that many of the rules governing their participation in legal proceedings reflect a compromise between two fundamental principles that determine the competency of scientific or medical evidence. One principle holds that problematic or deficient evidence should never be admitted; the other holds that any problem or deficiency in evidence should influence only the weight, and not the admissibility, accorded that evidence.

Recent Developments in American Law of Experts

Historically, the law of experts in the United States has focused on two fundamental questions. First, is the subject matter of the expert's opinion appropriate to the matter at hand? Second, is the expert sufficiently qualified to render the proffered opinion? During the last century, three watershed events – a federal court decision in 1923, a federal enactment taking effect in 1975, and a federal court decision in 1993 – provided basic answers to these questions.

From 1923 to the present, a decision from the US Court of Appeals for the District of Columbia in Frye v. United States, 293 F. 1013, has provided the most recognized standard for admissibility of expert evidence and testimony. In an opinion refusing to admit the results of a 'lie detector' test, the court announced that "just when a scientific principle or discovery crosses the line between the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential force of the principle must be recognized, and while courts will go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs." By the early 1970s, Frye had been approved not only in the federal courts but also in 46 states.

From 1975 to the present, Rules 403 and 701 through 706 of the Federal Rules of Evidence (FRE) have provided an alternative touchstone for determining the requirements of admissibility of expert testimony. As of 2004, at least 41 states pattern their evidence codes directly after the Federal Rules.

Revised FRE 702 states "if scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise, if (1) the testimony is based upon sufficient facts or data, (2) the testimony is the product of reliable principles and methods, and (3) the witness has applied the principles and methods reliably to the facts of the case." By contrast, Revised FRE 701 states "if the witness is not testifying as an expert, the witness' testimony in the form of opinions or inferences is limited to those opinions or inferences which are (a) rationally based on the perception of the witness and (b) helpful to a clear understanding of the witness' testimony or the determination of a fact in issue, and (c) not based on scientific, technical, or other specialized knowledge."

Revised FRE 703 states "the facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to the expert at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence in order for the opinion or inference to be admitted. Facts or data that are otherwise inadmissible shall not be disclosed to the jury by the proponent of the opinion or inference unless the court determines that their probative value in assisting the jury to evaluate the expert's opinion substantially outweighs their prejudicial effect."

FRE 704 states "(a) except as provided in subdivision (b), testimony in the form of an opinion or inference otherwise admissible is not objectionable because it embraces an ultimate issue to be decided by the trier of fact; (c) no expert witness testifying with respect to the mental state or condition of a defendant in a criminal case may state an opinion or inference as to whether the defendant did or did not have the mental state or condition constituting an element of the crime charged or of a defense thereto. Such ultimate issues are matters for the trier of fact alone." (Importantly, FRE 704 does not permit expert witnesses to offer legal conclusions, or to directly express opinions about the credibility of other witnesses.)

FRE 705 states "the expert may testify in terms of an opinion or inference and give reasons therefore without first testifying to the underlying facts or data, unless the court requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross examination."

FRE 706 states "the court may on its own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The court may appoint any expert witnesses agreed upon by the parties, and may appoint expert witnesses of its own selection. An expert witness shall not be appointed by the court unless the expert witness consents to act. An expert witness so appointed shall be informed of duties by the court in writing or at a conference in which the parties shall have the opportunity to participate. A witness so appointed shall advise the parties of the witness' findings, if any; the witness' deposition may be taken by any party; and the witness may be called to testify by the court or any party. The witness shall be subject to crossexamination by each party, including a party calling the witness."

FRE 403 provides "although relevant, evidence may be excluded if its probative value is substantially outweighed by the danger of unfair prejudice, confusion of the issues, or misleading the jury, or by considerations of undue delay, waste of time, or needless presentation of cumulative evidence."

In 1993, an opinion from the Supreme Court of the United States in *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 US 579, held that the adoption of the Federal Rules impliedly overturned the decision in *Frye*. Importantly, the text of the FRE does not mention the Frye test or any need for scientific evidence to be generally accepted as a precondition to admissibility. Rather, the FRE replaced the general acceptance test with a validation (reliability) standard derived from the language of FRE 702. According to the Daubert Court, to be admissible, the subject of an expert's testimony must be based on reliable 'scientific...knowledge'. To qualify as 'scientific knowledge', a theory or technique must be validated by the scientific methodology of research and experimentation. However, the approach to scientific knowledge is 'flexible', and "its overarching subject is the scientific validity – and thus the evidentiary relevance and reliability – of the principles that underlie a proposed submission." The Daubert Court also observed that inquiry must be directed to the principles and methodology used by the expert in reaching his or her conclusions, and not to the conclusions themselves.

Factors to be considered in assessing the adequacy (reliability) of the methodology include whether: (1) the theory can be falsified by empirical testing, (2) the documentation supporting a theory or technique has been subjected to peer review and publication, (3) a known or potential rate of error has been determined, and (4) the theory or technique has been accepted in the relevant scientific community. Regarding the modern role of the general acceptance test in determinations of reliability of evidence, the Daubert Court stated "a reliability assessment does not require, although it does permit, explicit identification of a relevant scientific community and an express determination of a particular degree of acceptance within that community. Widespread acceptance can be an important factor in ruling particular evidence admissible, and a known technique that has been able to attract only minimal support within the community may properly be viewed with skepticism."

Standards governing the role of the toxicologist as 'expert' most likely will continue to be defined by the dynamic and evolving interplay of Frye, Daubert, and the FRE. Federal courts currently must rely on the language of the FRE and the teachings of Daubert, with 'general acceptance' providing only one of several factors that may be considered in determining whether the subject matter of the expert's testimony is appropriate for the matter at hand. Confirmation of this point can be found in Kumho Tire Co. v. Carmichael, 526 US 137 (1999), where the Supreme Court stated that the factors enunciated in Daubert are not meant to be exhaustive and do not necessarily apply in every case. The Kumbo Court also said that "whether Daubert's specific factors are, or are not, reasonable measures of reliability in a particular case is a matter

that the law grants the trial judge broad latitude to determine."

Potential toxicology experts should note that predictions of the demise of the *Frye* "general acceptance" test have yet to be realized. As of the fall of 2002, state courts in at least 17 jurisdictions (Alabama, Arizona, California, Colorado, Florida, Illinois, Kansas, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nevada, New Jersey, New York, Pennsylvania, and Washington) remain committed to *Frye*. Importantly, three-quarters of these jurisdictions fall within the 25 most populated states, and twothirds fall within the 25 most litigious states. Consequently, a majority of state trials are conducted in *Frye* jurisdictions that may or may not recognize or incorporate *Daubert* indicia (factors) of validity and reliability into their *Frye* jurisprudence.

In retaining the 'general acceptance' standard, some state supreme courts have explicitly or implicitly refused to follow Daubert. Proffered explanations for this phenomenon include: (1) simple coincidence with random distribution of case outcomes, (2) lack of perceived need, especially in more populous states, to follow the lead of federal courts, (3) satisfaction with the status quo, (4) desire to prevent 'inappropriate relaxation' of the standards for introducing scientific testimony, (5) perception that *Frye* 'general acceptance' is a more rigorous, cautious, conservative, or higher standard of admissibility than the more liberal, lenient, or relaxed standard of 'validity-reliability' articulated in Daubert, and (6) perception that the Daubert standard requires state trial judges to make scientific judgments that exceed the typical judge's competence.

Critics of *Frye*, however, maintain that the scope of application of the 'general acceptance' standard is severely limited in many states. These critics argue that *Frye* applies (and often is applied) only to novel theories and techniques of 'hard science', and does not permit scrutiny of traditional techniques, 'soft science', and nonscientific expertise. By contrast, most *Daubert* Courts are compelled to examine all types of expert testimony, especially after *Kumho* made it clear that whether the proponent characterizes the proffered expertise as scientific, technical, or specialized, the proponent nevertheless must make a showing of the reliability of the expertise.

Critics of *Frye* also maintain that most state and federal trial judges can and do marshal the resources needed to increase their competence and confidence in performing the 'gatekeeping' responsibilities mandated by the *Daubert* decision. Examples of such resources include use of court-appointed experts under FRE 706, use of 'special masters' under Rule 53 of the Federal Rules of Civil Procedure, use of the

Federal Judicial Center's *Reference Manual on Scientific Evidence* (2nd edn, 2000), and attendance at forensic science and continuing legal education courses, such as those offered by the National Judicial College in Reno, Nevada.

Finally, critics of *Frye* point out that whether the evidence sought to be admitted has gained general acceptance in the appropriate field can depend on whether the 'field' is defined narrowly or broadly. Although courts have recognized that *Frye* does not require unanimity of view, courts have not provided functional definitions of 'general acceptance'. Consequently, a clear standard has not emerged for measuring 'general acceptance' in the relevant scientific community.

Subject Matter of the Expert's Opinion

Most American courts insist that three basic requirements be met before an individual will be permitted to offer testimony as an 'expert' witness. First, the testimony must be composed of scientific, technical, or other specialized knowledge. Second, the testimony must assist the factfinder in understanding the evidence or in resolving a factual dispute in the case. Third, the witness must be qualified to render the opinion.

Regarding scientific knowledge, the Daubert Court explained that "the adjective 'scientific' implies a grounding in the methods and procedures of science. Similarly, the word 'knowledge' connotes more than subjective belief or unsupported speculation. The term applies to any body of known facts or to any body of ideas inferred from such facts or accepted as truths on good grounds. Of course, it would be unreasonable to conclude that the subject of scientific testimony must be 'known' to a certainty; arguably, there are no certainties in science. But, in order to qualify as 'scientific knowledge,' an inference or assertion must be derived by the scientific method. Proposed testimony must be supported by appropriate validation (i.e., 'good grounds') based on what is known. In short, the requirement that an expert's testimony pertaining to 'scientific knowledge' establishes a standard of evidentiary reliability."

Regarding assistance to the factfinder, the expert's specialized knowledge must be 'helpful'. Courts do not agree, however, on the meaning of 'helpful'. Some courts believe that "[w]here the subject matter is within the knowledge or experience of laypeople, expert testimony is superfluous," and therefore not helpful. Others, by contrast, hold that there is no requirement that expert testimony be 'beyond the jury's sphere of knowledge' before that testimony can be deemed helpful. Regarding qualifications, many courts attempt to characterize the nature of an expert's opinion before deciding on admissibility. Opinions offered by physicians, for example, may address causation, diagnosis, treatment, identity, prognosis, standard of care for diagnosis, and standard of care for treatment. Importantly, American courts are split on the issue of the competency of nonphysicians to testify against physicians regarding diagnosis, treatment, prognosis, or standards of care. Conversely, American courts also are split on the competency of physicians to testify against nonphysician practitioners. By contrast, nonphysicians, as well as physicians, frequently are permitted to testify regarding causation and identity.

In malpractice cases, most courts do not require physician experts to practice in, or to be board-certified in, precisely the same specialty as the defendant practitioner. Furthermore, in jurisdictions that rely on local or state-wide standards of care, it is not necessary that an expert actually live and practice in the locale where alleged substandard care was provided. Some courts allow experts to assert knowledge of local practice through professional contacts, while others allow experts to assert that national standards of care apply equally in every location.

Foundation of the Expert's Opinion

Prior to enactment of the Federal Rules of Evidence in 1975, American courts held that the facts underlying an expert opinion had to be admitted into evidence before the expert could state an opinion. As noted previously, however, in jurisdictions that have adopted FRE 703, an expert now can base an opinion on personal knowledge, on facts made known or admitted into evidence, and on facts that have not been admitted into evidence and that are themselves inadmissible. FRE 703 "is designed to broaden the basis for expert opinions beyond that current in many jurisdictions and to bring the judicial practice into line with the practice of the experts themselves when not in court. Thus a physician in his own practice bases his diagnosis on information from numerous sources and of considerable variety, including statements by patients and relatives, reports and opinions from nurses, technicians, and other doctors, hospital records, and X-rays. Most of them are admissible in evidence, but only with the expenditure of substantial time in producing and examining various authenticating witnesses. The physician makes lifeand-death decisions in reliance upon them. His validation, expertly performed and subject to crossexamination, ought to suffice for judicial purposes."

Regarding the level of scrutiny of facts or data 'reasonably relied upon', by experts in a particular discipline, "courts have adopted two judicial approaches to Rule 703: one restrictive, one liberal. The more restrictive view requires the trial court to determine not only whether the data are of a type reasonably relied upon by experts in the field, but also whether the underlying data are untrustworthy for hearsay or other reasons. The more liberal view...allows the expert to base an opinion on data of the type reasonably relied upon by experts in the field without separately determining the trustworthiness of the particular data involved." In re "Agent Orange" Product Liab. Litig., 611 F.Supp. 1223, 1244 (E.D.N.Y. 1985), aff'd, 818 F.2d 187 (2d Cir. 1987), cert. denied, 487 US 1234 (1988).

In the Agent Orange cases, the court had to determine the trustworthiness of symptom checklists completed by plaintiffs in preparation for litigation. Are these checklists a type of 'data' reasonably relied upon by physician and nonphysician experts in offering conclusions about diagnosis and causation in the fields of toxicology and epidemiology? The court said 'no', such checklists "are not material that experts in this field would reasonably rely upon and so must be excluded under Rule 703." According to Judge Weinstein, "the court may not abdicate its independent responsibilities to decide if the bases meet minimum standards of reliability as a condition of admissibility. If the underlying data is so lacking in probative force and reliability that no reasonable expert could base an opinion on it, an opinion which rests entirely upon it must be excluded."

Toxicologists should understand that modern courts can, and frequently do, analyze expert opinions from two perspectives. Under FRE 702, courts determine whether an opinion is derived from scientific knowledge. Under FRE 703, courts determine whether an expert opinion has an adequate factual foundation. Whether one or both perspectives are applied, in courts that follow *Daubert*, the critical focus of inquiry is 'reliability'. By contrast, in courts that follow *Frye*, the focus remains 'general acceptance'.

Unfortunately, the Supreme Court in *Daubert* could not, and did not, resolve all the difficult issues regarding admissibility of expert testimony. One question left unanswered by *Daubert* was: does the validation (reliability) standard apply only to traditional 'scientific' evidence, or does it also apply to other 'technical', 'specialized', or 'social science' evidence? A second question was: does the validation (reliability) standard apply only to the *methodology* underlying the expert's evidence and opinion, or does it also apply to the *reasoning process* used by the expert in extrapolating or drawing inferences from the underlying scientific evidence to reach his or her conclusion?

In the *Kumho Tire* decision, 526 US 137 (1999), the Supreme Court held that the 'reliability' standard does apply to 'less scientific' or 'nonscientific' evidence. In *General Electric Co. v. Joiner*, 522 US 136 (1997), the court told federal judges that both methodology and reasoning should be scrutinized. According to the court, "[n]othing in either Daubert or the [FRE] requires a district court to admit opinion evidence which is connected to existing data only by the ipse dixit of the expert. A court may conclude that there is simply too great an analytical gap between the data and the opinion proffered." In other words, reliability and consequent admissibility requires a 'good fit' between the expert's methodology and conclusion.

In Downs v. Perstorp Components, Inc., No.00-5507, 01-04-02, the United States Court of Appeals for the Sixth Circuit affirmed summary judgment for the defendant and approved a federal district court's extensive scrutiny of a toxicologist's methodology and reasoning. The facts indicate that in 1995, defendant purchased Rubiflex SI 30690, a chemical product used in the production of foam insulation. Plaintiff, who was contracted to deliver the Rubiflex, found that the packaged product was too large for transport by chartered airplane. Defendant's representative suggested repackaging the Rubiflex in smaller containers. During repackaging, Rubiflex splashed out of the containers and onto plaintiff's arms and face. Plaintiff experienced a burning sensation but was told by defendant's representative that exposure to the chemical was safe. Plaintiff experienced neurological symptoms, and a physician toxicologist diagnosed chemical encephalopathy caused by exposure to Rubiflex.

The federal magistrate judge excluded the toxicologist's expert testimony, finding it failed to meet the Daubert admissibility standard. Although the expert identified Rubiflex as an epoxy and determined that it contained two toxic substances, he did not identify the components of Rubiflex that were responsible for plaintiff's condition. Furthermore, the expert did not know the amount of Rubiflex to which plaintiff was exposed and did not attempt to independently identify what dose of Rubiflex is necessary to cause the conditions, he observed in the plaintiff. Moreover, the expert could not point to any scientific literature suggesting that Rubiflex could lead to neurologic problems, and he did not conduct any testing to determine the potential effects of exposure to Rubiflex. Consequently, the Sixth Circuit agreed with the lower court that the expert's opinion should not be admitted because his "methodology

primarily involved reasoning backwards from [plaintiff's] condition, and through a process of elimination, concluding that Rubiflex must have caused it."

As the twenty-first century unfolds, the toxicologist expert witness still needs to distinguish *Frye* jurisprudence from *Daubert* jurisprudence. *Frye* jurisdictions remain divided on whether the 'general acceptance' test applies to technical, specialized, psychological, or other social science types of evidence. *Frye* Courts also disagree on whether 'general acceptance' applies not only to an expert's general methodologies, but also to his or her conclusions.

The future, however, may bring a melding of analytical principles. Recent state appellate decisions suggest a desire by some courts to adopt a 'Frye plus reliability' standard for admission of some types of scientific evidence. For example, in Harris v. Cropmate, 706 N.E.2d 55 (1999), the Illinois Court of Appeals said "Illinois utilizes a 'Frve plus reliability' standard for admission of novel scientific evidence... in applying the Frye standard the trial court must determine that (1) the scientific test is reliable; and (2) the test's reliability is generally accepted in the particular scientific field to which the test belongs." Furthermore, state trial courts "must not delegate their authority to the scientific community...in serving as gatekeepers to keep out scientific evidence that constitutes nothing more than 'junk science' or mere speculation, trial courts should constantly be asking, does the proffered witness have sufficient information, based upon the evidence in this case, to render a *reliable* opinion? Courts should remember that they need not - and should not - accept an expert's opinion on the basis of ipse dixit, that is, such a thing is so because I say it is so."

In DuPont v. Castillo, the Florida District Court of Appeals (Fifth District) said "it is the function of the court to not permit cases to be resolved on the basis of evidence for which a predicate of reliability has not been established. Reliability is fundamental to issues involved in the admissibility of evidence... Novel scientific evidence must also be shown to be reliable on some basis other than simply that it is the opinion of the witness who seeks to offer the opinion." Finally, in Slay v. Keller Industries, Inc., No. 1001091, Ala. (2001), the Alabama Supreme Court concluded that "mere assertion of belief, without any supporting research, testing, or experiments, cannot qualify as proper scientific testimony under either the 'general acceptance' standard enunciated in Frye or the 'scientifically reliable' standard of Daubert."

Regarding the admissibility of clinical medical testimony, courts have taken different approaches under *Daubert*. In Moore v. Ashland Chemical Company (5th Cir. 1998) (en banc), the Fifth Circuit excluded the conclusion of the plaintiff's pulmonary and environmental medical specialist that the cause of his reactive airways distress syndrome was his workplace exposure to toluene. The 'expert's' opinion was based on his training and experience, the physical examination, laboratory test results, an MSDS warning that inhalation of toluene could cause lung injury, onset of symptoms shortly after 'exposure' to toluene, and existing scientific studies on the association between toluene and respiratory illness. Nevertheless, the court of appeals held that the district court reasonably concluded that this basis was insufficient to meet the requirements of *Daubert*. The court said the studies contained self-doubts and qualifiers, and the MSDS was of limited value because the types of tests underlying the MSDS were unknown and there was no information regarding the airborne concentrations required to sustain the injuries described in the warning. The court also discounted the proximity of onset of the injuries to exposure as inadequate without other studies to demonstrate a scientifically validated causal connection. Furthermore, the court concluded that the plaintiff's lifestyle and medical history were inconsistent with the specialist's opinions because the plaintiff was a smoker, experienced asthma as a child, and had recovered from pneumonia shortly before his alleged inhalation of toluene. Three judges in dissent stated that the majority had improperly interpreted the Daubert factors to require all expert testimony to meet a standard within the 'hard science community'. According to the dissenting judges, 'generally accepted clinical medical methodology' could not meet this standard.

By contrast, in Westberry v. Guslavad Gummi AB (4th Cir. 1999), the Fourth Circuit was less hostile in its assessment of clinical medical methodologies. The plaintiff alleged that he developed severe sinus infections following industrial exposure to talc. The plaintiff's physician concluded that the plaintiff's condition was caused by his inhalation of talc. The defendant argued that plaintiff's physician could not and did not rely on epidemiological studies, peerreviewed published studies, animal studies, or laboratory data to support his conclusions. Moreover, plaintiff's expert could not show that tissue samples from the sinuses contained talc. According to the defendant, plaintiff's expert merely relied on a differential diagnosis and the temporal proximity of the 'exposure' to the onset of symptoms.

The court of appeals recognized that the technique of differential diagnosis includes physical examination, review of the medical history, and review of various laboratory tests prior to any determination of the most probable cause of an illness or condition. The differential diagnostic process includes generation of a list of possible causes for the symptoms, and elimination of those that can be ruled out. The court viewed as irrelevant not only the physician's lack of knowledge of the precise amount of talc that may have been inhaled by the plaintiff, but also the physician's lack of any means to assess the intensity of exposure that may have been sufficient to produce plaintiff's sinus irritation. According to the court, while such information may be 'beneficial', it was not essential to a determination of causation. On the other hand, the court found the MSDS to be highly relevant because it stated that inhalation of talc dust 'in high concentrations irritates mucous membranes'. Importantly, the court found sufficient evidence of anecdotal proof of 'high concentrations' in the plaintiff's workplace. Furthermore, the court stated that evidence of temporal proximity of the exposure to the onset of the plaintiff's symptoms 'can provide compelling evidence of causation'.

The juxtaposition of Moore and Westberry highlights the significant differences in interpretation of Daubert and its progeny by lower courts. Westberry has been frequently cited for the proposition that clinical medical differential diagnosis, if properly undertaken, can satisfy the standards of Daubert independently. The Fourth Circuit stated that while "[a] differential diagnosis that fails to take serious account of other potential causes may be so lacking that it cannot provide a reliable basis for an opinion on causation," no requirement exists that the physician must rule out each and every possible alternative cause.

Use of Literature as Evidence

Traditionally, learned treatises and articles could not be admitted as substantive evidence because they were viewed as prohibited forms of hearsay. Such literature could be used, however, during cross-examination to impeach or contradict expert testimony. Modern courts typically require that the treatise or article: (1) must have been relied upon by the expert in reaching his or her conclusions, or (2) must be acknowledged by the witness to be an 'authoritative source' or a 'recognized authority' in the relevant field. Some courts also permit treatises and articles to be used for impeachment even if the witness does not acknowledge the source as a recognized authority, as long as authoritativeness can be established by judicial notice or through testimony of other witnesses. FRE 803(18), which is best read in conjunction with FRE 703 (discussed above), states "the following are not excluded by the hearsay rule, even thought the declarant is available as a witness: (18) Learned Treatises - To the extent called to the attention of an

expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits."

In both federal and state forums, the proponent of substantive admissibility of medical and scientific literature will argue one or more of the following points: (1) the author of an article or treatise does not have an interest in the outcome of a particular case; (2) the scrutiny of the peer review process increases the reliability of opinions or conclusions published in peer-reviewed literature; (3) treatises may be more 'up to date' than live, testifying experts; (4) attorneys can attempt to prevent confusion, selective presentation, or presentation out of context; and (5) crossexamination is not necessary when a live expert is available to explain the article or treatise.

By contrast, the opponent of substantive admissibility of medical and scientific literature will argue one or more of the following points: (1) the author is not available for cross-examination; (2) treatises quickly outdate because medical and scientific knowledge change rapidly; (3) the trier of fact may be unable to understand complex technical passages that may be presented out of context; and (4) the literature is unnecessary as substantive evidence when live expert witnesses are available.

Conclusion

Toxicologists can make meaningful and significant contributions to legal proceedings. To function effectively in judicial, legislative, or regulatory forums, however, the toxicologist must be willing to do the following: (1) dress appropriately; (2) prepare properly and extensively; (3) leave his or her ego at the door; (4) resist the temptation to elaborate, pontificate, or volunteer information; (5) frequently answer 'yes', 'no', 'I don't know'; 'I don't recall'; and 'I don't understand the question'; (6) avoid bringing documents unless specifically asked to do so; (7) expect to be verbally 'attacked'; (8) think and react calmly under pressure; (9) recognize the 'hypothetical' question; (10) avoid overstatement and use of the words 'always' and 'never'; (11) avoid hasty answers, so as to enable objections; (12) listen to objections carefully; (13) avoid argument with the examiner; (14) refuse to answer if counsel instructs not to answer; (15) appreciate that, in legal forums, one is an expert only if the tribunal so states; (16) ask

to review entirety of a document before answering questions about parts of it; (17) assert the right to read a transcript of testimony before signing it; and (18) use only those methodologies and state only those conclusions that can be defended before peers in the field of toxicology. See also: Toxic Torts.

Further Reading

Smith FM (ed.) (2002) Reference Manual on Scientific Evidence. Collingdale, PA: Diane Publishing Company.

LD₅₀/LC₅₀ (Lethal Dosage 50/Lethal Concentration 50)

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Introduction

The 50% lethal dose (LD₅₀ or LD50) is the statistically calculated dose (or concentration) of a material (generally expressed as the amount of material per unit of body weight) that would be expected to cause the death of half the members of the target species receiving it. The 50% lethal concentration (LC_{50}) is the equivalent statistical projection for inhalation. Until the mid-1980s, these figures were considered perhaps the basic component of a toxicity profile for any chemical or drug. However, after many years of controversy and debate on a number of fronts, including objections from animal rights advocates, three alternative animal tests have been developed to replace the LD₅₀. They are the fixeddose procedure, the acute toxic class method and the up and down (or 'up/down') method, and their use has led to significant improvements in animal welfare. These new tests have undergone revision and refinement to improve their scientific performance and to increase their regulatory acceptance. Further, research into replacements for test animals, such as cellular cultures, organs harvested from slaughterhouses, in silico (computer) modeling, and physical/ chemical systems, has been extensive. While these approaches will not be able to completely replace the use of animals in the foreseeable future, they have a bright future.

The *in vitro* cytotoxicity tests that have been developed can already help reduce the number of animals used in acute oral toxicity testing. For example, cytotoxicity data are being used to determine the starting dose for *in vivo* testing by applying a standard regression between cytoxicity and acute oral LD_{50} values. A database of correlations between cytotoxic responses and the acute oral LD_{50} of rats or mice has been determined for hundreds of chemicals. Using this approach, it has been proposed that a

tiered *in vitro/in vivo* testing process will reduce animal use in the up/down method. For example, the *in vitro* cytotoxicity of a new chemical is determined as the first step, and the LD_{50} value (mg kg⁻¹) is predicted from the cytotoxicity data. The predicted LD_{50} dose is then used as the starting dose in the up/down protocol.

In silico models (or 'expert systems') have also been developed. These are computer software-based structure-activity relationship and quantitative structure-activity relationship analyses of data libraries of acute toxicity data developed for use in evaluating and predicting the acute oral and inhalation toxicity potential of a chemical or drug.

While most of the focus has been on the potential for oral toxicity, in vitro testing and computer modeling for the evaluation and prediction of respiratory toxicity are also being developed. For example, one strategy consists of checking the existing data available for the test material itself, or on related substances, followed by acquiring knowledge on the physicochemical properties of the test material. These steps are followed by the use of computer modeling techniques to try to predict the likely toxic effects and target sites. In vitro tests could then follow to identify likely target cells and evaluate the specific effects on the cells (e.g., morphology could be determined and assessments of the cellular energy status). A further phase of *in vitro* tests could then be conducted on the basis of results obtained in the first phase of in vitro testing, choosing from tests using various types of respiratory tract cells.

When did modern Western society become concerned with lethality testing? For what reasons were protocols developed for describing lethality in animals in quantitative terms for the purposes of making scientific, regulatory, or marketing decisions? Interestingly, in this age of genetic engineering, few people realize that biologically derived materials were the subject of regulations well before the passage of the Pure Food and Drug Act in the United States in 1906. In 1901, a diphtheria epidemic broke out in St. Louis, MO, because of improperly manufactured antidiphtheria toxin. In response to the resulting public outcry, the US Congress passed the Virus Act of 1902. It regulated all viruses, serums, toxins, antitoxins, and other such products sold for the prevention or cure of disease in man. Among other things, the bill eventually established consistent potency criteria. In fact, by World War II the US FDA was requiring batch-to-batch certification and release for biologicals, a policy that remains in effect for certain drugs. Hence, the earliest lethality testing was for the purpose of establishing consistent potencies of biologicals, such as diphtheria toxin, and not for evaluating synthetic chemicals.

One of the earliest publications discussing lethality testing was an investigation into the lethality of diphtheria toxin in guinea pigs. The publication described lethality empirically in terms of percentage of dead animals at each dosage because methods for calculating lethality curves and the median lethal dosage had not yet been developed. The authors reported that lethal response to a given dosage of toxin varied with the time of the year. Hence, years before the term LD_{50} came into parlance, supposedly as an exact indicator of toxicity, data had been published attesting to the volatility and imprecision of this calculated parameter.

Because the first use of lethality testing was in describing the potency of biologicals, it only makes sense that the same methods were soon applied to extracted botanicals. (Note: There is no doubt that both the Germans and the English tested in animals the various poison gases employed during World War I. Little of this work, however, appears to have been published in the open scientific literature, although portions of it have recently been made public.) In 1926, de Lind van Wijngaarden published on the lethality of digitalis extracts. Interestingly, he did not plot his data as mortality versus dose. He delivered his extracts intravenously and titrated the dosage until he achieved complete heart stoppage. He was thus able to determine the precise lethal dosage for each animal and noted that these followed a bellshaped or Gaussian distribution. His experiments took 5 years and used more than 500 cats, an effort that would have been excessive and expensive by today's standards. However, he did conclude that no more than nine cats would normally be required to 'calibrate' an extract of digitalis. Trevan, in a pivotal paper (1927), described the lethality of strophanthin, cocaine, and insulin. Modern reviews have focused a great deal of attention on the large number of frogs used by Trevan. Most of the data he discussed, however, were derived from experiments in mice using cocaine or insulin. Perhaps so little attention was given to this aspect of Trevan's paper, even though it comprised the major portions of his work

(which ran to 31 pages and contained 11 figures and six tables of data), because it has never been replicated. For some of the lethality curves reported by Trevan, well over 900 mice were dosed. Again, such efforts would be excessive and expensive by today's standards but were necessitated, in part, by the less rigorous method of deriving lethality curves and calculating the median lethal dosage (LD₅₀). Modern methods of data transformation and statistical analysis were, at that time, still in their infancy. He also recognized that it was not necessary to describe an entire dosage-response curve to calculate an LD_{50} . He, in fact, recommended that lethality determinations start with small groups of two or three animals each and that larger groups be used for confirmatory purposes.

Behren confirmed the observations of both de Lind van Wijngaarden and Trevan. It is clear from his article that the use of animals for standardizing digitalis extracts was accepted to the point of being incorporated into the German and Dutch pharmacopoeias. The objective of his paper was to compare the cat and frog methods and develop a basis for using fewer animals. He concluded that the frog method was superior and that no more than 44 frogs needed to be used, which was considerably less than the 100–200 frogs prescribed in the German pharmacopoeia of that period. Interestingly, these early papers are often criticized with regard to the numbers of animals used, but the objectives and conclusions are often ignored.

Both Trevan and Behrens noted that when the percentage of animals that died at specific dosages was plotted against the logarithm of the dosage, the resulting curve (the lethal dosage curve) had a sigmoidal shape slope and range that was 'characteristic' for the species and the test substance. Shackell (1925) first pointed out that such curves are integrated or cumulative frequency curves (or ogives) and coined the term 'dose-response ogive' (curve). Trevan noted that these curves owe their shape to the fact that different individual animals require different quantities of poison for death to occur. It was also Trevan who identified the midpoint on this curve as being the dosage that would kill 50% of the animals exposed. He designed that point as the median lethal dose, or LD₅₀, and, thus, is widely credited with having developed the classical LD₅₀. Trevan and Behrans essentially read the LD₅₀ directly from their mortality dose-response curves.

Lethality testing of biologicals and botanicals was essentially a response to governmental regulation. It was only natural that similar methods would be applied to synthetic chemicals. Major chemical companies started establishing toxicity or industrial health laboratories during the 1930s; the lethality testing of synthetic chemicals was established by the 1930s. However, there were no regulatory requirements for such tests. In fact, there was no premarketing toxicity testing of synthetic chemicals required at all. In 1937, an elixir or sulfanilamide dissolved in ethylene glycol was introduced into the market. Over 100 people died as a result of ethylene glycol toxicity. The public response to this tragedy helped prompt the US Congress to pass the Federal Food, Drug, and Cosmetic Act of 1938. It was this law that mandated the premarket testing of drugs for safety in experimental animals. By the mid-1940s, most chemical and pharmaceutical companies were routinely testing new chemicals for lethality. In fact, until the 1960s, preclinical or premarketing toxicity data packages normally consisted of little more than acute lethality data. Recently, new laws, increased scientific sophistication, and greater societal concern over sublethal chronic toxicity has led to more extensive and expensive preclinical or premarketing toxicity testing packages, where acute lethality is a small, but still real, concern.

The protocols used to assess lethality have changed considerably since the 1920s. While the principles originally described by Trevan have never been questioned, the methods for calculating the LD_{50} have become more sophisticated and the need for a high degree of precision has been questioned. The practical result is that by using modern protocols, relatively few animals are species (generally rats and mice) are employed and only two routes of administration are used. At least one route must be the intended or the most probable human exposure route. Hence, such protocols generally result in the generation of eight lethal dosage curves (one/route/ sex/species). In the drug industry (where this approach is common), the two routes are generally oral and intraperitoneal for an oral drug and oral and intravenous for an intravenous drug.

Protocol Design Considerations

Whatever type of experimental protocol one chooses to use in a lethality test, there are certain principles and criteria that should be universally applied. The principles are especially relevant in studies in which small numbers of animals are used.

First, a wide variety of intrinsic and extrinsic factors can influence the outcome of a lethality test. These include species, strain or substrain, age, weight, and sex of the animals; husbandry practices (e.g., type of bedding and cage population); environmental conditions; feed and water quality; nutritional state; and volume and vehicles of test substance delivery. The point to be made here is that the criteria for all these factors should be specified in detail in the protocol and strict adherence to the protocol observed. Otherwise, intrastudy comparisons are invalid. Small differences in protocols can cause large differences in the LD_{50} and are probably the major cause of the considerable laboratory-to-laboratory variation in the LD_{50} s.

Second, because the animals will generally receive a single exposure, great care must be given to the preparation and delivery of the test articles. In a chronic study, occasional miscalculations or misdelivery of the dosage would not generally greatly affect the study outcome but would clearly have a greater effect on the conclusions of a lethality screen. One should always include appropriate safeguards.

Third, one must make sure that all animals are successfully dosed and that accidental deaths are identified as such. In acute rodent studies, we routinely assign spare animals to a dosing group. Permanent numbers are not assigned until we are certain that the dose has been successfully delivered (e.g., Was the supposedly intraperitoneal dose accidentally delivered intravenously? Was there reflux from the site?). Spare animals not dosed are returned to the pool of animals available for the study. Animals found dead should be examined for evidence of accidental trauma. For example, it is not uncommon for a rat to suddenly move while being gavaged. This may result in a torn esophagus that may take 1 or 2 days to become evident. Depending on the administration route, one must pay close attention to dosing techniques and the volume limitations imposed by these techniques. For example, 20 ml kg^{-1} is the maximum volume that should be given orally to a rodent. Deaths that are clearly accidental should not be considered in the final conclusions.

Fourth, lethality protocols, by the nature of the question they address, do not specify all dosages. This can sometimes result in a study in which absurdly high dosages are administered. Hence, all protocols should clearly state what the ceiling or limit dosage will be and the reasons for selection.

Classical (Traditional) Designs

The classical or traditional methods of determining the lethality of a substance have been established since the 1920s. In discussing this type of study design, it is assumed that what are desired are an LD_{50} and the slope of the lethality curve. In general, these are only necessary for meeting specific regulatory guidelines. If less precise information will suffice (which is generally the case), other protocols can be used. Briefly, this type of protocol specifies that animals (of the same species/strain, sex, and age) be divided into groups. All the animals are treated via the same route. The animals are then held and observed for a set and consistent period of time, usually 14 days.

Mortality in each group is calculated on the basis of the number of animals that die during the observation period and is normally presented in percentage terms: (number dead/number dosed) × 100. If mortality at each dosage is plotted against dosages, a sigmoidal dose–response curve is obtained. The LD₅₀ is simply the dosage, either observed or calculated, that yields 50% mortality. Seldom are such curves reported as such because the LD₅₀ is difficult to read off a curvilinear plot and the small number of dosages normally used makes drawing an accurate lethal dosage curve difficult. It is most common to prohibit transforming the data to obtain a rectilinear plot.

Traditionally, because of US FDA and foreign regulatory guidelines, protocols have frequently been designed as batteries, including both sexes of two species (generally rats and mice) and two routes of administration. At least one route must be the intended or the most probable human exposure route. Hence, such protocols generally result in the generation of eight lethal dosage curves (one/route/sex/ species).

While such extensive data packages may still be required for regulatory purposes, scientifically they are of little value. First, there is no reason to assume that either the rat or the mouse is the better predictor for humans - or for each other. The only general correlation between the rat and mouse LD₅₀ is that when one is high, so is the other. Obtaining lethality data from two different rodents, rather than a single species, does not generally change our conclusions or improve our understanding of the toxicity of a drug or chemical or the potential hazard to humans. It is recommended that a simple preliminary screen be performed to pick out the most sensitive species and a rigorous protocol applied only to that species. Because the slope of the fitted line in these assays has a very large uncertainty, in relation to the uncertainty of the LD₅₀ itself (the midpoint of the distribution), great caution must be used with calculated lethal doses other than $LD_{50}s$. It is quite possible to calculate values for other points along the lethality curve, such as the LD₃₅, a value close to the LD₅₀, but these are not precise or statistically 'stable' values due to the shape of the curve in areas away from the center point.

Note: The Registry of Toxic Effects of Chemical Substances (http://www.cdc.gov/niosh/rtecs.html) is among the largest single collections of LD_{50} and LC_{50} values.

See also: Animal Models; Dose–Response Relationship; Food, Drug, and Cosmetic Act; Levels of Effect in Toxicological Assessment; Maximum Allowable Concentration (MAC); Maximum Tolerated Dose (MTD); Toxicity Testing, Alternatives; Toxicity Testing, Modeling.

Further Reading

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Relevant Websites

- http://ecvam.jrc.cec.eu.int European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods.
- http://oacu.od.nih.gov (US) National Institutes of Health, IRAC Recommendation on LD50 Testing (from NIH's Interagency Research Animal Committee).

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7439-92-1
- SYNONYM: C.I. Pigment Metal 4
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Heavy metals
- CHEMICAL FORMULAS: Pb²⁺, Pb⁴⁺

Uses

Lead and its compounds are widely used throughout industry. They are found in lead acid storage batteries, paints, sheet metal, bearings, solder, piping, and ammunition. Lead arsenate is used in insecticides and herbicides. Lead chromate is used as a yellow pigment in paints, rubber, plastics, and ceramic coatings. House paints must have less than 0.05% lead.

Various arts and hobbies involve lead-containing materials. Lead is found in artist's paints (certain pigments), ceramic glazes (particularly reds), solder used in stained-glass windows, and linings in containers used for distilling homemade whiskey. Certain Mexican, Middle Eastern, and Asian folk remedies and cosmetics contain lead.

In the past, lead solder was used to seal canned foods and lead pipes were used to carry drinking water. Tetraethyl lead was once routinely added to gasoline as an antiknock agent; certain vehicles may still use leaded gasoline (e.g., agricultural vehicles). Lead was commonly used in paint, with certain formulations containing up to 50% lead.

Background Information

Lead was one of the first metals used by man. It has a wide variety of uses, but its well-established bioaccumulation and chronic toxicity with low exposure levels has led to the enactment of strict limits on use and potential exposure.

Exposure Routes and Pathways

Exposure to lead and its compounds may occur through ingestion, inhalation, or dermal contact. The specific characteristics of a lead compound influence how exposure is to occur through a particular route and the degree of absorption into the body through that route. Most inorganic forms of lead are not well absorbed through the skin, whereas organic forms (e.g., tetraethyl lead) are more likely to be absorbed through the skin.

For the general population, ingestion of contaminated water and food is the primary source of exposure to lead. The average adult ingests $\sim 300 \,\mu g$ of lead each day in food. Inhalation is the most significant route of exposure to lead in the workplace.

Respirable particulate or gaseous forms of lead may be inhaled. Sources include cigarette smoke; vehicle exhaust; emissions from municipal waste incinerators, iron and steel plants, smelting and refining operations, lead acid battery manufacturing facilities, and sandblasting and burning of surfaces coated with lead paint. Particulate air emissions may eventually deposit and contaminate the soil.

Direct release of lead-containing industrial wastewater into surface water or ground-water may ultimately impact drinking water. Lead may also be present in drinking water because of leaching from old pipes, solder, water coolers, or faucets. Some historians attribute the fall of the Roman Empire to the effects of lead leaching from drinking-water pipes and wine casks.

Food may contain low levels of lead due to uptake from the environment or higher levels due to lead leaching from containers (e.g., lead crystal or leadcontaining glazes on earthenware). An important cause of lead poisoning in young children is ingestion of peeling and chipping lead-based paint in older homes.

Toxicokinetics

Lead is readily absorbed through the digestive tract. Absorption becomes less efficient with age. Children absorb between 30% and 50% of ingested lead, whereas adults absorb less than one-third of that amount (between 5% and 15%). This absorption is enhanced by diets deficient in iron, zinc, and calcium. Absorption is generally greater for organic forms. Lead is well absorbed from the lung (from 50% to 70% of respirable lead particulate). Generally, inorganic forms of lead are not absorbed through the skin, while organic forms (e.g., tetraethyl lead) can be absorbed.

Once absorbed, lead is distributed throughout the body tissues via the blood. Almost all ($\sim 95\%$) of the lead in the blood is found in red blood cells. There are two primary sites in the red blood cell

where lead forms complexes: the membrane and the hemoglobin. The concentration of lead in the blood is used as an indicator of recent lead exposure.

Lead tends to accumulate in the kidneys, the brain (i.e., the gray matter and various nuclei), and the skeleton. Lead can cross the placenta and has been shown to accumulate in the developing child. Prolonged exposure to lead (>4 weeks) in young children can lead to the accumulation of lead in the growth plates at the end of the long bones.

The total body burden of lead is a function of the balance between the amount being taken in (all routes combined), the amount distributed throughout the tissues, and the amount being excreted. Most of the body burden of lead is sequestered in the bones and teeth: over 70% in children and over 90% in adults. The remainder of the body burden is distributed between soft tissue and the blood. Lead is stored in the bone for the greatest length of time. The estimated half-lives of lead range from 10 to 30 years in bone, are 40 days in soft tissues, and range from 28 to 36 days in blood (in adults). Children tend to retain approximately five times more absorbed lead than adults.

In young children (<3 years) the blood-brain barrier (an anatomical barrier that limits access to the brain) is not fully developed. Inorganic lead circulating in the blood is much more likely to reach the brain in an infant or a very young child.

Chronic high-level exposure to lead can result in the accumulation of large stores of lead in the bone, which can be slowly released over many years after the initial exposure has stopped. The release of lead from bone may be accelerated under certain conditions, including high stress, certain metabolic fluctuations, and pregnancy.

Lead is excreted from the body in bile (into feces), and in urine, sweat, sloughed-off skin cells, and lost hair.

Mechanism of Toxicity

Lead can affect most organs and systems in the body. It can interfere with certain cellular signaling processes, the generation of action potentials in certain nerve cells, and the function of a number of enzymes. Lead interferes with the sodium–potassium ATPase pump on cell membranes, the metabolism of vitamin D, heme synthesis, certain enzymes involved in oxidative phosphorylation (cytochromes), and calcium uptake and metabolism. In addition, lead can interfere with signal transmissions in nerve cells, including dopaminergic transmissions and signaling processes at the postsynaptic and presynaptic junctions. Lead can depress the function of the adrenal glands and the thyroid.

Lead binds certain active groups on protein (e.g., sulfhydral groups) and therefore may change the structure and function of certain proteins and enzymes. Lead interferes with the biosynthesis of heme in at least two steps in the multistepped process. Heme proteins are important to the structure and function of hemoglobin in red blood cells. Lead binds with 8-aminolevulinic acid dehydratase and depresses its activity. This biochemical block explains the occurrence of anemia found in chronic lead poisoning. Measurement of the blood levels of this enzyme is used as a test for lead intoxication. Lead also interferes with the incorporation of ferrous iron into the porphyrin ring. If iron is not attached to heme, then zinc will occupy the iron-binding site. The concentration of zinc protoporphyrin also can be used as a diagnostic tool for lead poisoning.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute lead exposure can lead to renal toxicity. The acute intraperitoneal LD_{50} for lead acetate in rodents is $100-200 \text{ mg kg}^{-1}$. Lead acetate is considerably less toxic by the oral route $(LD_{50} > 4 \text{ g kg}^{-1}$ in rats). The acute oral LD_{50} of tetraethyl lead in rodents is $10-100 \text{ mg kg}^{-1}$. Acute organolead exposure can sensitize dopaminergic neurotransmission in the central nervous system.

Human

Anorexia, vomiting, malaise, and convulsions (due to increased intracranial pressure) are most commonly seen in children. Sources of childhood exposures are typically environmental such as to paint chips, pottery, drinking water, and dust. Acute exposure in adults may cause gastrointestinal effects, pain in arms and legs, and hypertension. Exposure to very high levels may cause tremor, memory loss, confusion, stupor, renal failure, convulsions, and coma.

Chronic Toxicity (or Exposure)

Animal

Lead is a tumorigen, mutagen, reproductive and developmental toxicant. Neural, renal, and hematologic toxicity are all possible with chronic exposures. Oral exposure to certain lead compounds has been shown to induce tumors in kidneys of rats and mice in more than 20 studies. Based on such animal data, lead is classified as a probable human carcinogen by several agencies including the US Environmental Protection Agency (EPA).

In dogs, sheep, goats, and cattle, there have been reports of toxicity resulting from contact with environmental lead. Sources of exposure may include lead salts, lead-based paints, and waste oils.

Lead can disrupt learned behavior in certain adult animals and has been shown to disrupt learning and memory in certain young animals. Young animals tend to be more susceptible to the effects of lead than older animals. Studies on monkeys have shown the abnormalities and effects after lead exposure. Effects included encephalopathy and offspring that exhibited neurological and behavioral symptoms at maturity.

Human

Lead can disturb cellular and molecular processes in the body and affect many organs and physiological functions. The probability of adverse health effects occurring is related to the level of exposure, duration of exposure, and total body burden. The adverse effects associated with exposure to lead are a function of dose and are usually the same regardless of the route of exposure. The primary targets for toxicity are the nervous system, the blood, and the kidneys. Reproductive effects can also occur and include male infertility, abortion, and neonatal morbidity and mortality. Lead may damage sperm and parts of the reproductive tract. Chromosomal effects have been observed in lead-exposed workers. Other effects include impairment of the immune system, which has been associated with joint pains (lead arthralgia) related to gout, myocarditis, cardiac fibrosis, weight loss, and anemia.

The most sensitive and vulnerable target for lead appears to be the nervous system. Exposure to high concentrations of lead can cause either encephalopathy or peripheral neuropathy. Encephalopathy is rare in adults but is more likely to occur in significantly exposed children. It has been observed in young children (1 and 3 years) following chronic lead poisoning due to ingestion of significant amounts of lead-based paint. Typically, there is gastrointestinal distress (e.g., colic), disorientation, stupor, seizures, and coma.

Significant early childhood exposure (above 0.05 mg%, and arguably lower) has been associated with certain neuropsychiatric changes, including learning disorders, decreased IQ, behavioral abnormalities (e.g., hyperactivity), and deficits in vocabulary. In addition, decreased growth, loss of hearing acuity, deficits in reaction time, fine-motor dysfunction, developmental abnormalities, deficits in hand/ eye coordination, anemia, and death have been associated with exposure in children. Symptoms of

acute exposure in young children include anorexia, vomiting, and irritability. In cases of very high levels of exposure, symptoms may also include slurred speech, peripheral neuropathy, paralysis, convulsions, and coma.

Exposure to lead in adults has been associated with hypertension, nephropathy, decreased hearing acuity, anemia, peripheral neuropathy, and encephalopathy. Onset of symptoms may be slow with chronic exposure. Anemia, common in chronically exposed adults and children, tends to be more severe in children. The life span of red blood cells decreases when lead concentrations in blood increase. In the past, the morphology of various blood cells was used to diagnose lead poisoning. Zero content is allowed in food (Food and Drug Administration).

Clinical Management

The decision to actively treat a patient exposed to lead is made based on a number of criteria, including patient history, symptomology, blood lead levels, and other indicators of level of exposure. It is common to screen for exposure to lead based on blood lead levels (μ g dl⁻¹). Certain exposure criteria are expressed in terms of an acceptable blood lead level. The Centers for Disease Control and Prevention in Atlanta defines above 9μ g dl⁻¹ as a trigger of concern in young children. The concentration of erythrocyte protoporphyrin (EP; a heme-containing protein) in red blood cells is also used to indicate exposure levels. The higher the concentrations of EP, the higher the exposure. X-ray techniques may be used to estimate concentrations of lead in bones and teeth.

Chelation therapy is usually the treatment of choice. Both CaNa₂-EDTA (calcium disodium salt of ethylenediaminetetraacetic acid) and British Antilewisite compound (BAL; 2,3-dimercaptopropanol) are commonly used to remove lead from the body. Both are administered via intramuscular injection. BAL binds lead to sulfhydral groups and chelates metal from both inside and outside the cellular space. Lead removal through the bile and urine is increased within 30 min of administration. BAL is the common choice when there is known toxicity to the kidney, but it is contraindicated if there is liver failure or glucose-6-phosphate dehydrogenase deficiency. BAL treatment has produced a number of adverse reactions, including nausea, vomiting, tachycardia, and fever.

CaNa₂-EDTA binds extracellular lead. After administration, excretion of lead through the kidneys may be increased 20- to 50-fold. If there is renal dysfunction, use of CaNa₂-EDTA may enhance toxicity. Blood lead levels may rise after the administration of CaNa₂-EDTA alone. BAL is usually given with CaNa₂-EDTA to reduce toxicity associated with the mobilization of lead stored in soft tissues. CaNa₂-EDTA is usually not used for patients with known low zinc stores. Sodium-EDTA is not used to treat lead poisoning because it will also chelate and reduce calcium in the body.

D-Penicillamine, a chelating agent that can be administered orally, is currently used to chelate lead on an experimental basis. Individuals who are allergic to penicillin may experience adverse reactions to this agent; toxic effects have been reported in as many as 20% of the patients treated with this compound.

2,3-Dimercapto-1-propanesulfonic acid and dimercaptosuccinic acid have mechanisms of action similar to BAL. Both are water-soluble analogs of BAL that can be administered orally, are less toxic and have fewer unpleasant side effects than BAL. They have been found to be effective in removing lead via the kidneys. Treatment regimens may also include removal from the source of exposure and changes in the patient's diet.

Environmental Fate

Lead occurs naturally in the environment. However, most of the lead dispersed throughout the environment comes from human activities. Before the use of leaded gasoline was limited, most of the lead released into the US environment came from car exhaust. Since the EPA has limited the use of leaded gasoline, the amount of lead released into the air has decreased. Other sources of lead released into the air include burning fuel, such as coal or oil, industrial processes, and burning solid waste.

The release of lead to air is now less than the release of lead to soil. Most of the lead in inner city soils comes from landfills and leaded paint. Landfills contain waste from lead ore mining, ammunition manufacturing, and other industrial activities such as battery production. Very little lead goes directly into water.

Higher levels of lead from car exhausts can be measured near roadways. Very low levels of lead from car exhausts are found at distances of 25 m (\sim 80 ft) from the road edge. However, once lead goes into the atmosphere, it may travel thousands of miles if the lead particles are small or if the lead compounds are volatile. Lead is removed from the air by rain as well as by particles falling to the ground or into surface water. Once lead deposits on soil, it usually sticks to soil particles. Small amounts of lead may enter rivers, lakes, and streams when soil particles are displaced by rainwater. Lead may remain stuck to soil particles in water for many years. Movement of lead from soil particles into underground water or drinking water is unlikely unless the water is acidic or 'soft'.

Some of the chemicals that contain lead are broken down by sunlight, air, and water to other forms of lead. Lead compounds in water may combine with different chemicals depending on the acidity and temperature of the water. The lead atom cannot be broken down.

The levels of lead may build up in plants and animals from areas where air, water, or soil are contaminated with lead. If animals eat contaminated plants or animals, most of the lead that they eat will pass through their bodies. It is the small amount absorbed that can cause harmful effects.

The amount of lead in paints sold for consumer use may not exceed 0.06%.

Releases from lead-based paints are frequently confined to the area in the immediate vicinity of painted surfaces, and deterioration or removal of the paint can result in high localized concentrations of lead in indoor air and on exposed surfaces. Sandblasting procedures to remove paint may disperse lead in the local environment.

The largest volume of organolead vapors released to the atmosphere results from industrial processes such as primary and secondary nonferrous metal smelting, and from the use of leaded gasoline which contains tetraethyl lead as an antiknock additive. These vapors are photoreactive, and their presence in the local atmosphere is transitory. Halogenated lead compounds are also formed and, ultimately, oxides and carbonates. Tetraalkyl lead compounds have been found to contribute 5–10% of the total particulate lead present in the atmosphere. Organolead vapors are most likely to occur in occupational settings (e.g., gasoline transport and handling operations, gas stations, and parking garages) and hightraffic areas.

Although aquatic releases from industrial facilities are expected to be small, lead may he present in significant levels in drinking water. In areas receiving acid rain (e.g, northeastern United States) the acidity of drinking water may increase, thus increasing the corrosivity of the water, which may, in turn, result in the leaching of lead from water systems, particularly from older systems during the first flush of water through the pipes. Fish in more acidic waters accumulate more lead than fish in a more alkaline environment.

The grounding of household electrical systems to the plumbing can increase corrosion rates and the subsequent leaching of lead from the lead solder used for copper pipes. Areas where the pH of the water is less than 8.0 may have higher lead drinking water levels as well. Canning foods in lead-soldered cans may increase levels of lead by 8- to 10-fold; however, the impact of canning appears to be decreasing as a result of a decrease in the use of lead-soldered cans. Additional exposure to lead through dietary intake by people living in an urban environment is estimated to be $\sim 28 \text{ mg} \text{ day}^{-1}$ for adults and 91 mg day⁻¹ for children, all of which can be attributed to atmospheric lead (dust). Atmospheric lead may be added to food crops in the field or garden (through uptake from soil and from direct deposition onto crop surfaces), during transport to market, processing, and kitchen preparation.

Lead may leach from lead crystal decanters and glasses into the liquids they contain. Flaking paint, paint chips, and weathered powdered paint, which are most commonly associated with deteriorated housing stock in urban areas, are major sources of lead exposure for young children residing in these houses, particularly for children with pica (i.e., the compulsive, habitual consumption of nonfood items). Lead concentrations of 1000–5000 mg cm⁻² have been found in chips of lead-based paint, suggesting that consumption of a single chip of paint would provide greater short-term exposure than any other source of lead.

Ecotoxicology

The impact of environmental lead on wildlife and ecosystems has been a subject of study and concern. Water fowl may become poisoned from ingesting lead shot. Poisoning may lead to anorexia, lethargy, coma, and death. Other birds have also been shown to be impacted by environmental lead. Water fowl can also be contaminated with lead by swallowing fishing sinkers, in particular the small 'split shot' type.

Exposure Standards and Guidelines

Most adult exposure is occupational. The Immediately Dangerous to Life or Health value for lead is 100 mg m^{-3} . The recommended exposure limit is 0.1 mg m^{-3} and the permissible exposure limit/ threshold limit value for lead is 0.05 mg m^{-3} .

See also: Behavioral Toxicology; Developmental Toxicology; EDTA (Ethylenediaminetetraacetic acid); Kidney; Metals; Neurotoxicity; Occupational Toxicology; Pollution, Air; Pollution, Water; Psychological Indices of Toxicity; Reproductive System, Male; Skeletal System; Toxicology, History of; Veterinary Toxicology.

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Relevant Websites

- http://www.osha.gov Toxic Metals: Lead (from the US Occupational Safety and Health Administration).
- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lead.

Lethal, Dosage or Concentration See LD₅₀/LC₅₀ (Lethal Dosage 50/Lethal Concentration 50)

Levels of Effect in Toxicological Assessment

Michael Dourson

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Level of Effect terminology varies somewhat from country to country, and from one organization to another. Terms are used to identify specific locations along a severity spectrum, moving from doses with no toxicologically recognizable effect, to negligible effects, to doses with more profound toxicity. A list of relevant terms is given below.

• *Acceptable daily intake (ADI)*: The daily intake of a chemical, which, during a lifetime, appears to be

without appreciable risk on the basis of all the known information at the time.

- *Acute toxicity*: The older term used to describe immediate toxicity. Its former use was associated with toxic effects that were severe (e.g., mortality) in contrast to the term 'subacute toxicity' that was associated with toxic effects that were less severe. The term 'acute toxicity' is often confused with that of acute exposure.
- Adaptive effect: An adaptive effect enhances an organism's performance as a whole and/or its ability to withstand a challenge. An example of an adaptive effect is an increase in hepatic smooth endoplasmic reticulum, but only if hepatic metabolism reduces the chemical's toxicity.
- *Adverse effect*: A biochemical change, functional impairment, or pathological lesion, which impairs performance and reduces the ability of the organism to respond to additional challenge.
- *Allergic reaction*: An adverse reaction to a chemical resulting from previous sensitization to that chemical or to a structurally similar one.
- *Chronic toxicity*: The older term used to describe delayed toxicity. However, the term 'chronic toxicity' also refers to effects that persist over a long period of time whether or not they occur immediately or are delayed. The term 'chronic toxicity' is often confused with that of chronic exposure.
- *Compensatory effect*: This effect maintains overall function without enhancement or significant cost. Increased respiration due to metabolic acidosis is an example of a compensatory effect.
- *Critical effect*: A chemical often elicits more than one toxic effect, even in one species, or in tests of the same or different durations. The critical effect(s) is the first adverse effect(s) or its known precursor(s) that occurs as dose rate increases. The critical effect(s) may change among toxicity studies of different durations, may be influenced by toxicity in other organs, and may differ depending on the availability of data on the shape of the dose–response curve.
- *Idiosyncratic reaction*: A genetically determined abnormal reactivity to a chemical.
- *Immediate versus delayed toxicity*: Immediate effects occur or develop rapidly after a single administration of a substance, while delayed effects are those that occur after the lapse of some time. These effects have also been referred to as acute and chronic, respectively.
- Local versus systemic toxicity: Local effects refer to those that occur at the site of first contact between the biological system and the toxicant; systemic effects are those that are elicited after

absorption and distribution of the toxicant from its entry point to a distant site.

- Lowest-observed-adverse-effect-level (LOAEL): The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.
- *Minimum risk level (MRL)*: An estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure.
- No-observed-adverse-effect level (NOAEL): An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse, nor precursors to specific adverse effects. In an experiment with several NOAELs, the regulatory focus is primarily on the NOAEL seen at the highest dose. This leads to the common usage of the term NOAEL to mean the highest exposure without adverse effect.
- *Reference dose (RfD)*: An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime.
- *Reversible versus irreversible toxicity*: Reversible toxic effects are those that can be repaired, usually by a specific tissue's ability to regenerate or mend itself after chemical exposure, while irreversible toxic effects are those that cannot be repaired.
- *Tolerable concentrations* (TCs, often expressed in mg m⁻³): TCs are generally airborne concentrations to which it is believed that a person can be exposed continuously over a lifetime without deleterious effect. They are based on noncarcinogenic effects.
- Tolerable daily intake (TDI): An estimate of the quantity of a chemical contaminant in food or water, which can be ingested daily over a lifetime without posing a significant risk to health. 'Contaminants' are different from 'Residues' in this context: a contaminant is a chemical whose presence in food or water does not serve, and never has served, any useful purpose. TDIs are thus distinct from ADIs, which relates to residues of chemicals that have been deliberately added to a product, for example, residues of pesticide sprays or antifungal agents.
- Uncertainty factor (UF)/safety factor (SF): One of several, generally 10-fold, factors used in

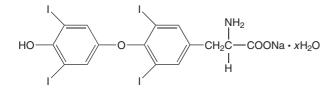
operationally deriving an RfD or ADI from experimental data. Among other things UFs are intended to account for (1) the variation in sensitivity among the members of the human population; (2) the uncertainty in extrapolating animal data to the case of humans; (3) the uncertainty in extrapolating from data obtained in a study that is of less-than-lifetime exposure; (4) the uncertainty in using LOAEL data

Levothyroxine

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 51-48-9
- SYNONYMS: Eltroxin; L-Thyroxine; Levo-T; Levotec; Levothyroid; Levoxyl; Synthroid; T4; Thyroxine
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Synthetic thyroid hormone
- CHEMICAL STRUCTURE:



Uses

Levothyroxine is used for thyroid hormone replacement.

Exposure Routes and Pathways

Ingestion is the most common route of accidental and intentional exposure to levothyroxine (T4). It is also available in an intravenous dosage form.

Toxicokinetics

T4 oral bioavailability varies from 30% to 90%. Peak serum T4 levels occur 2–6 h after therapeutic dosing. Approximately 99% of T4 is protein bound. T4 has a volume of distribution of $8-101 \text{ kg}^{-1}$. From 75% to 85% of T4 is deiodinated in the liver, kidney, muscles, heart, and brain. Half of this is converted to active T3. Approximately 20% of T4 is excreted in the feces

rather than NOAEL data; and (5) the inability of any single study to address adequately all possible adverse outcomes in man.

See also: Acceptable Daily Intake (ADI); Dose–Response Relationship; Reference Dose (RfD); Toxicity, Acute; Toxicity, Chronic; Uncertainty Factors.

intact after oral dosing and 10% of conjugated T4 is excreted in the urine. The half-life of T4 is 5–9 days in euthyroid patients taking therapeutic doses.

Mechanism of Toxicity

Thyroid compounds are necessary for metabolism, growth, and development. T4's primary action is related to calorigenesis and protein synthesis. Thyroid hormones potentiate the effects of catecholamines. About half of T4 is converted to T3. T3 is three to five times more potent than T4.

Acute and Short-Term Toxicity (or Exposure)

Animal

Both dogs and cats are at risk for thyroid toxicity. Signs of toxicity in animals include vomiting, diarrhea, tachycardia, tachypnea, decreased level of consciousness, and restlessness.

Human

In general, adults and children can tolerate acute overdoses of T4. Ingestion of less than 4 mg of T4 is unlikely to produce symptoms. In acute exposures that become symptomatic, clinical effects are generally mild. Symptoms may develop days after ingestion when T4 is converted to the more potent T3. In cases of intentional overdose with large amounts of T4 ingested, acute clinical effects may include tachycardia, hypertension, tachydysrhythmias, flushing, diaphoresis, nausea, vomiting, diarrhea, restlessness, confusion, headache, mydriasis, and fever that can persist for days.

Chronic Toxicity (or Exposure)

Animal

When administered to young, pregnant rats during the 9th to 20th day of pregnancy, cataracts developed in the offspring.

Human

Chronic exposure to high doses of T4 may cause thyrotoxicosis. The development of thyrotoxicosis in an acute exposure is rare. Thyrotoxicosis is characterized by tachycardia, cardiac arrhythmias, hypertension, hyperpyrexia, tremors, and seizures. In patients with severe toxicity, coma and circulatory collapse can result.

In Vitro Toxicity Data

Studies of *in vitro* and *in vivo* models of hyperthyroidism have documented substantial impact on rat liver function. Recent developments have suggested that these findings are likely due to induction of apoptosis via a mitochondrial mediated pathway or pathways.

Clinical Management

Most patients after acute overdose can be managed on an outpatient basis. Gastric decontamination may be considered in patients presenting early after large ingestions. The absence of clinical effects within the first 24 h does not preclude the later development of significant toxicity. In patients manifesting toxicity, cardiac and blood pressure monitoring should be performed. Cooling methods should be employed to decrease hyperpyrexia. Intravenous fluids should be administered in dehydrated and/or hypotensive patients. Adrenergic hyperactivity can be treated with propranolol. Propylthiouracil (PTU) may be administered to decrease conversion of T4 to T3. Forced diuresis and extracorporeal methods are not effective in levothyroxine overdose.

See also: Endocrine System; Thyroid Extract.

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Lewisite

Harry Salem*

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: CAS 541-25-3 (Lewisite 1: 2-chlorovinyldichloroarsine); CAS 40334-69-8 (Lewisite 2: (2-chlorovinyl)chloroarsine); CAS 40334-70-1 (Lewisite 3: Tris(2-chlorovinyl)arsine)
- SYNONYMS: Arsine; Arsonous; Dichloride; Arsine, L
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Blister agent/Vesicant class of chemical warfare agents
- CHEMICAL FORMULA: C₂H₂AsCl₃
- CHEMICAL STRUCTURE: $ClCH = HC-AsCl_2$

Uses

Lewisite was synthesized in 1918 by Dr. Wilford Lee Lewis as a vesicant for chemical warfare. Its production was too late to use in World War I. It can be used with mustard to lower the freezing point of the mixture for ground dispersal and spraying.

Background Information

Other organic arsenical chemical warfare agents are methyldichlorarsine (MD), phenyldichloroarsine (PD), and ethyldichloroarsine (ED). These plus lewisite (L), mustard agents, and phosgene oxime make up the vesicant class.

Exposure Routes and Pathways

Lewisite is an oily, colorless liquid that can appear amber to black in its impure form. It has the odor of geraniums. It is more volatile than the mustard agents. Lewisite in the air can cause damage to the eyes, skin, and airways by direct contact. Lewisite in water can lead to exposures from drinking the water or from skin contact, and lewisite-contaminated food can be ingested. Lewisite remains as a liquid under a wide range of environmental conditions, from below freezing to very hot temperatures.

^{*}The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Toxicokinetics

Although the exact mechanism of biological activity is unknown, the trivalent arsenic in lewisite combines with the thiol groups in many enzymes.

Mechanism of Toxicity

Lewisite is readily absorbed from the skin, eyes, and respiratory tract, as well as after ingestion and through wounds. It causes blistering on the skin and mucous membranes on contact. After absorption, it causes an increase in capillary permeability, which produces hypovolemia, shock, and organ damage. Unlike the mustard agents, lewisite vapor or liquid causes immediate pain or irritation although lesions require up to 12 h to become full-blown cases.

Human Toxicity

Nasal irritation by lewisite begins at $\sim 8 \text{ mg min m}^{-3}$ and its odor is detected at $\sim 20 \text{ mg min m}^{-3}$. Vesication and death from lewisite inhalation is caused at the same Ct as mustard, which is 1500 mg min m⁻³. The immediately dangerous to life health (IDLH) value of lewisite is 0.003 mg m⁻³. Lewisite causes vesication at $\sim 14 \text{ mg}$ and the LD₅₀ is 2.8 g on the skin.

Within 5 min after contact with liquid lewisite, a grayish area of dead epithelium is produced. Erythema and blister formation follows more rapidly that it does with mustard even though the full-blown lesion does not develop for 12–18 h. The lesion has more tissue necrosis and tissue sloughing than does a mustard agent lesion.

On the eyes, lewisite causes pain, tearing, and blepharospasm on contact. Edema of the conjunctiva and lids follows and the eyes may be swollen shut within an hour. Iritis and corneal damage may also occur. Within minutes, liquid lewisite causes severe eye damage on contact. Upon inhalation, the airway mucosa is the primary target and the damage progresses down the airways with pseudomembrane formation. Pulmonary edema may complicate exposure to lewisite. Runny nose, sneezing, hoarseness, bloody nose, sinus pain, shortness of breath, and cough also occur on inhalation. Lewisite causes an increase in permeability of systemic capillaries resulting in intravascular fluid loss, hypovolemia, shock, and organ congestion. This has been termed 'Lewisite shock' or hypotension. This also leads to hepatitis or renal necrosis with more prominent gastrointestinal effects of diarrhea, nausea, and vomiting.

The long-term effects of lewisite exposure do not include extensive skin burning as is seen with the mustard agents, but chronic respiratory disease may occur. Also, unlike the mustard agents, suppression of the immune systems does not occur, but extensive eye exposure may cause permanent blindness.

Animal Toxicity

Vapor exposure - Inhalation

Species	LCt_{50} (mg min m $^{-3}$)	
Mouse	900	
Rat	1500	
Rabbit	1000	
Guinea pig	1200	
Dog	1400	
Goat	1250	

Liquid percutaneous

Species	LD ₅₀ (mg kg ⁻¹)
Mouse	15
Rat	20
Rabbit	5
Guinea pig	12
Dog	70
Goat	10

Clinical Management

To prevent or lessen lewisite damage, early decontamination within minutes after exposure must be instituted. Unlike mustard, lewisite does not cause damage to the hematopoietic organs, but fluid loss from increased capillary permeability necessitates careful attention to fluid balance.

British antilewisite (BAL) or dimercaprol was developed as an antidote for lewisite. It is used in medicine as a chelating agent for heavy metals. Although BAL can cause toxicity itself, evidence suggests that BAL in oil administered intramuscularly will reduce the systemic effects of lewisite. BAL skin and ophthalmic ointment decrease the severity of skin and eye lesions when applied immediately after early decontamination, but neither of these ointments is currently manufactured.

See also: BAL (British Antilewisite); Blister Agents/Vesicants.

Relevant Websites

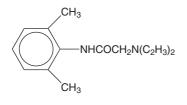
- http://www.bt.cdc.gov US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.
- http://sis.nlm.nih.gov US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Lidocaine

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 137-58-6
- SYNONYMS: Dilocaine; Lidoderm; Lidoject-1; Lignocaine; Nervocaine; Nulicaine; Octocaine; Solarcaine; Xylocaine; Xylocard
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Amidetype local anesthetic; Class IB antiarrhythmic
- CHEMICAL STRUCTURE:



Uses

Lidocaine is used for local anesthesia and in the management of ventricular arrhythmias.

Exposure Routes and Pathways

Lidocaine is administered either topically or by the parenteral route. Ingestion of topical lidocaine products can occur and result in toxicity.

Toxicokinetics

Lidocaine is absorbed from the gastrointestinal tract but undergoes significant first-pass metabolism (60–70%). Absorption from local sites is dependent on the dose and vascularity of the site. It is well absorbed from mucosa. Lidocaine is widely distributed to tissues. The volume of distribution is $\sim 11 \text{ kg}^{-1}$. Protein binding is 60–80%. Lidocaine is dealkylated by hepatic CYP3A4 to the active metabolite monoethylglycinexylide, which is then inactivated to glycine xylidide. These metabolites and $\sim 5\%$ of unchanged lidocaine are renally excreted. The elimination half-life is 1–2 h.

Mechanism of Toxicity

Lidocaine combines with fast voltage-gated sodium channels and inhibits recovery after repolarization. As a result, cellular conduction is blocked by lidocaine's inhibition of permeability of excitable membranes to sodium that normally is produced by membrane depolarization.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects of lidocaine in animals are similar to those observed in humans.

Human

Therapeutic lidocaine serum concentrations range from 1 to 5 mg ml^{-1} . Signs of toxicity are usually seen above $6-10 \text{ mg ml}^{-1}$, and death has been associated with serum concentrations above 15 mg ml^{-1} . Nausea, vomiting, lightheadedness, euphoria, dizziness, drowsiness, confusion, visual changes, increasing agitation, and muscle fasciculations may be seen with lidocaine toxicity. Severe lidocaine toxicity can result in seizures and coma. Cardiovascular effects of lidocaine include a variety of arrhythmias and hypotension. Arrhythmias include sinus arrest, sinus bradycardia, heart block, and asystole. Hypotension may be due to vasodilation or decreased cardiac output.

Chronic Toxicity (or Exposure)

Animal

Chronic dosing of rats starting 2 weeks before mating and continuing through delivery at $100-250 \text{ mg kg}^{-1} \text{ day}^{-1}$ by continuous intravenous infusion resulted in no serious adverse effects.

Human

Small children treated with topical lidocaine 2% for teething five to six times daily for a week developed seizures. Patients being managed for several days with lidocaine for control of acute arrhythmias may accumulate lidocaine and its metabolites if they have changes in blood flow (e.g., shock, circulatory collapse). Decreased clearance and accumulation of lidocaine and desmethyllidocaine may result in the development of drowsiness, tinnitus, muscle twitching, and may eventually lead to seizures, coma, and arrhythmias.

In Vitro Toxicity Data

Ames *Salmonella* assays of the mutagenicity of lidocaine have been inconclusive.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Decontamination with syrup of ipecac is contraindicated due to the likelihood for developing rapid onset seizures. Gastric decontamination with activated charcoal may be considered for substantial recent ingestions. Fluid and electrolytes need to be frequently monitored and replaced as necessary.

Treatment is symptomatic and supportive after decontamination. Bradycardia is usually responsive to atropine. For hypotension, intravenous fluids should be administered and if unsuccessful, vasopressor therapy should be initiated. Arrhythmias can be refractory to drug management; however, treatment should be guided by electrocardiographic changes. Sodium bicarbonate may be used to reverse the effects of sodium channel blockage caused by lidocaine. There are case reports of patients developing methemoglobinemia secondary to lidocaine. Those with elevated methemoglobin concentrations should be managed with intravenous methylene blue.

See also: Poisoning Emergencies in Humans.

Further Reading

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Mofenson HC, Caraccio TR, and Miller H (1983) Lidocaine toxicity from topical mucousal application. *Clinical Pediatrics* 22: 190–192.

Life Cycle Assessment

David W Pennington and Tomas Rydberg

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The provision of goods and services (collectively, products) contributes to environmental impacts. Life cycle assessment (LCA) is a tool for comparing product options and for identifying opportunities for reducing related impacts. LCA provides insights that are complimentary to those of many regulatory and more site- or process-oriented risk and impact assessments.

The focus of an LCA is typically on contributions to regional or global scale impacts, including the consumption of resources. All stages in a product's life cycle can result in the generation of wastes, in emissions, and in resource consumption. These environmental exchanges contribute to impacts, such as climate change, stratospheric ozone depletion, photooxidant formation (smog), eutrophication, acidification, toxicological stress on human health and ecosystems, the depletion of resources, and noise, among others. An LCA helps decision makers take into account the contributions to these impacts, as well as the trade-offs, that occur at the many stages in a product's life cycle, that is, during the extraction of raw materials, energy acquisition, production, manufacturing, use, reuse, recycling, through to ultimate disposal.

Structure of LCA

LCA practitioners tabulate the wastes, the emissions, and the resources consumed, for example, at every relevant stage in a product's life cycle, from its 'cradle to grave'. The compilation, tabulation, and preliminary analysis of these data are termed life cycle inventory (LCI). After LCI, it is generally necessary for practitioners to calculate indicators of the contributions to (potential) impacts that are associated with these inventory data. This is life cycle impact assessment (LCIA).

The standards and reports in the International Organization for Standardization (ISO) 14000 series provide, in general, an accepted framework and terminology for LCA (although not practical insights).

ISO 14040:1997	Life cycle assessment – Principles and framework
ISO 14041:1998	Life cycle assessment – Goal and scope definition and inventory analysis
ISO 14042:2000	Life cycle assessment – Life cycle impact assessment
ISO 14043:2000	Life cycle assessment – Life cycle interpretation
ISO/TR 14047	Life cycle assessment – Examples of application of ISO 14042
ISO/TS 14048:2002	Life cycle assessment – Data documentation format
ISO/TR 14049:2000	Life cycle assessment – Examples of application of
	ISO 14041 to goal and scope definition and inventory analysis

Figure 1 distinguishes the three phases within an LCA. Interpretation is vital at each stage and an assessment will typically be refined iteratively.

In the goal and scope definition of an LCA, the practitioner defines the product system in terms of the system boundaries of the study and a functional

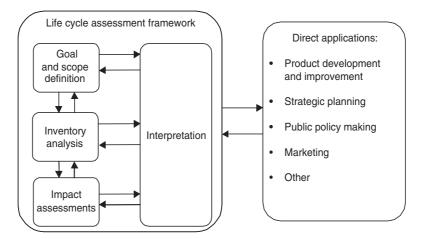


Figure 1 Phases and applications of an LCA. (Based on ISO14040, 1997.)

unit. The functional unit is vital. The functional unit facilitates the direct comparison of alternative goods or services.

A functional unit is not just a quantity of material. Practitioners may compare, for example, alternative types of packaging on the functional unit basis of $1m^3$ of packed and delivered product. The comparison is then in terms of the service that the packaging provides. The quantity of packaging material required to provide this functional unit, termed the reference flow, will vary in the studies depending on the packaging option selected (e.g., paper, plastic, metal, and composite).

LCI is the methodology for estimating the consumption of resources, the quantities of wastes, the emissions, the traffic accidents, the noise, etc., that are associated with each stage in a product's life cycle. The material and energy flows are modeled between the processes within a life cycle. The overall models provide mass and energy balances for the product system, its total inputs and outputs into the environment, on a per functional unit basis.

The design of the product system model, especially with respect to the system boundaries and what processes are included within these boundaries, can be decisive for what data are appropriate and the outcome of an LCA study. As one example different types of life cycle models can be designed to describe:

- a product system and its environmental exchanges considering, for example, the average energy mixes needed to generate electricity (termed an attributional model), or
- how the environmental exchanges within the system might be expected to change as a result of actions taken, for example, accounting for which type of fuel source could be reduced as a result of reduced electricity requirements due to product

design modifications (termed a consequential model).

LCIA provides indicators for the interpretation of the inventory data in terms of contributions to different impact categories. The indicator results of an LCIA facilitate the evaluation of a product, and each stage in its life cycle, in terms of climate change, toxicological stress, noise, land use, water consumption, etc. The scope of the evaluation is, with some exceptions, limited to impacts at a regional and global scale.

The overall indicator results of an LCIA reflect cumulative contributions to different impact categories that are summed over time and space. Unlike some other assessment approaches, these indicator results usually do not reflect risks or impacts at any particular location or point in time. The consumption of resources and the generation of wastes, emissions, etc., often occur in a product's life cycle:

- at multiple sites and in multiple regions around the world,
- as different fractions of the total emissions at any one site (the fraction required to provide the specified functional unit, with allocation amongst related and unrelated coproducts in a facility such as a refinery, for example),
- at different times (the use phase of a car compared to dismantling), and
- over short and long time periods (e.g., multiple generations in the case of emissions of persistent chemicals and from landfills).

Interpretation occurs at every stage in an LCA. If two product alternatives are compared and one alternative has a higher consumption of each resource, for example, an interpretation purely based on the LCI can be conclusive. In other studies, drawing conclusions will require at least an LCIA, a sensitivity analysis, and consideration of the statistical significance of differences in each impact category.

Some category indicators can be further crossaggregated and compared on a natural science basis. Further aggregation can be to calculate the overall sum of years of human life lost, for example, the years of life lost that are attributable to climate change, potential carcinogenic effects, noise, traffic accidents, etc.

A practitioner may also want to compare across other impact categories, particularly when there are trade-offs between product alternatives or if it is desirable to prioritize within a product's life cycle. This is often termed valuation or weighting. For example, emissions of CO_2 equivalents in one life cycle may result in a higher climate change indicator than for another, but the alternative involves the use of more pesticides. These pesticides may result in a higher contribution to the risks of regional toxicological impacts. A stakeholder may therefore want more information to help guide which difference is of a higher priority.

Resolving such trade-offs between impact categories draws not only on natural sciences but also often relies on social science and economics. In some applications, particularly for policy support and as one example, this results in the monetization of externalities (the impact indicators) to provide results for different impact categories in terms of Euros, Yen, or similar.

Assessing Impacts in LCA

LCIA consists of both mandatory and optional elements, as described, for example, in ISO 14042:

- Selection of the impact categories of interest, the indicators for each impact category, and, although often implicitly considered by most practitioners, the choice of the underlying models (a procedure also considered in the initial goal and scope phase of an LCA study).
- Assignment of the inventory data to the chosen impact categories (*classification*).
- Calculation of impact category indicators using characterisation factors (*characterisation*).
- Calculation of category indicator results relative to reference value(s) (*normalisation*; optional).
- *Grouping* and/or *weighting* the results (optional, weighting not being allowed when strictly adhering to ISO14042 in comparative assertions disclosed to the public).
- Data quality analysis (mandatory in comparative assertions disclosed to the public, according to

ISO 14042, but receiving little attention in current practice).

In practice, these elements are often supported using available databases and tools.

Toxicological Impact Characterization in LCA

Equation (1) provides a simple example of how impact indicators can be calculated from inventory data using generic *characterization factors*. These factors are generally the output of *characterization models*. The factors are often available to practitioners in a precalculated format in the literature, in the form of databases, as well as in available support tools.

Category Indicator =
$$\sum_{s}$$
 Characterization Factor(s)
× Emission Inventory(ies) (1)

where the lower limit of the summation, s, denotes the chemical.

For wastes and emissions, the inventory data in eqn (1) are in terms of the mass of each substance that is released into the environment associated with the functional unit. For example, the mass of the different chemicals released into the environment that are associated with all of the life cycle stages related to providing packaging for 1 m^3 of packaged and delivered product. The characterization factors in eqn (1) therefore linearly express the contribution to an impact category of releasing a unit mass (e.g., 1 kg) of an emission into the environment.

As one example of a characterization factor, the relative contributions of different gases to climate change are commonly compared in terms of carbon dioxide equivalents using global warming potentials (GWPs). A GWP₅₀₀ of 100 implies that 1 kg of the substance has the same cumulative climate change effect as 100 kg of carbon dioxide during, in this case, a 500 year time period.

A number of methodologies and associated interpretations have been proposed for calculating characterization factors for toxicological risks and the potential impacts in LCA. Score-based factors initially helped to rank emissions in terms of selected fate, exposure, and toxicity parameters. These were often similar to the ordinal persistence, bioaccumulation, and toxicity (PBT) scores used in other applications. Approaches now rely to a greater extent on the use of mechanistic models and, to a lesser extent, on epidemiological data.

To be consistent, but to provide broad chemical coverage, there has been a common reliance in LCIA

on the use of multimedia chemical fate models, human exposure correlations for organic chemicals, as well as the toxicological methodologies/data that were designed for chemical risk screening in a regulatory context. The results of these early modeling approaches can be interpreted in terms of 'policy-based hazard equivalents'. For example, indicators are presented in terms of 'kg equivalents of benzene' for cancer effects. The characterization factors reflect the ratio of the hazard (exposure/regulated effect level) of a unit emission (kg or kg day⁻¹) of one chemical relative to the hazard ratio for the same emission of benzene.

Some methodologies are emerging to estimate characterization factors for toxicological effects that are designed more specifically for use in relative comparison applications like LCA. These are more consistent with the underlying basis of commonly adopted approaches for the other impact categories, as well as for the assessment of substances such as radionuclides. The emerging factors reflect the cumulative risk attributable to a unit emission (e.g., 1 kg) of a chemical into the environment. Cumulative risk, in this case, is defined as the risk integrated over time and space, as well as over the entire exposed population, that is associated with the unit mass of a chemical emitted. This definition, and the associated data, required differ from those adopted in many regulatory contexts for chemical screening assessments in the context of toxicological effects.

In estimating the cumulative risk of a chemical in LCA, dose-response extrapolations can be based on toxicological benchmarks. Such a benchmark approach is considered more appropriate for use in comparative assessment contexts, such as in an LCA study. Benchmarks are an exposure measure associated with a consistent change in response, such as the 10% or even the 50% effect level. Regulatory-based measures do not necessarily provide a consistent risk basis for comparison, as they were often never developed for use in such a comparative context or to facilitate low dose-response extrapolation. Other data differences include the use of median, rather than extreme, data in the fate and exposure modeling, as well as the consideration of safety factors only as part of the uncertainty assessment and not as an integral part of the toxicological effects data.

It is important to additionally account for differences in potential toxicological consequences (severity, damage, or impact) in the comparison. Characterization factors can be expressed in terms of metrics such as DALYs (disability adjusted life years) for human health, for example. The results for toxicological impacts can be directly cross-compared with those of DALY-based indicators for other impact categories, such as for climate change. Metrics such as DALYs are derived from statistics for mortality. These are dominant for cancer effects. Equivalent numbers of DALYs, calculated using social science techniques, are provided for the years of life lost for morbidity (nonfatal) effects. Further developments remain necessary for noncancer toxicological effects. Although some DALY-based characterization factors are available from epidemiological data for respiratory illness, including for secondary particulate matter (nitrates and sulfates).

Similar approaches exist for impacts on ecosystems, although at this time the consequences of ecotoxicological effects are primarily addressed in terms of cumulative risks or hazard indicators multiplied by the area or volume affected.

Concluding Remarks

LCA provides insights that are complimentary to those of many regulatory and more site, or process, orientated risk and impact assessments. LCA is a tool for comparing product options and for identifying opportunities for reducing related impacts.

An LCA study can include the consideration of contributions to potential regional and global scale toxicological impacts. These contributions are calculated using characterization factors. Characterization factors linearly express the cumulative contribution to the risk of a potential toxicological impact and the relative consequences that are attributable to releasing a unit mass (e.g., 1 kg) of a chemical into the environment.

A number of researchers continue to assess the statistical importance of the influences of many of the underlying modeling options on the characterization factors and on the overall results of LCA studies. These include when, and how, differentiation is required in terms of the modes of entry and the times/ locations of chemical releases when considering cumulative regional and global effects, the duration over which these effects are likely to occur, as well as the influence of distinguishing between short- and long-term effects by using time horizons like 50 or 500 years.

See also: Risk Assessment, Ecological; Risk Assessment, Human Health.

Further Reading

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Lily of the Valley

Amanda Lofton

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- SYNONYMS: Convallaria; *Convallaria majalis*; Convall-lily; Jacob's Ladder; Ladder-to-Heaven; Lilje-konvall; Lily Constancy; Lily convalle; Male Lily; May Lily; Our Lady's Tears
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Cardiac glycosides

Uses

Lily of the valley is used in bouquets or cut flower arrangements and as a garden perennial.

Background Information

A member of the family Liliaceae, lily of the valley is an herbaceous perennial arising from upright root stocks with pairs of simple broad oval leaves and a flowering stock. The stock bears a one-sided row of scented, waxy, bell-shaped flowers. These fragrant flowers are usually white; however, pale pink, pink, and yellow varieties also exist. The plant also bears a red-orange berry, ~ 0.5 in. in diameter, which is filled with seeds. Lily of the valley is commonly cultivated in North America, the United Kingdom, and throughout Europe.

Exposure Routes and Pathways

Ingestion of the fragrant flowers or bright berries is the most common route of exposure. (LCA). Environmental Toxicology & Chemistry 23: 1796–1807.

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Relevant Website

http://www.iso.ch – International Organization for Standardization (ISO).

Toxicokinetics

Limited pharmacokinetic data are available on these plants. It is expected that absorption from plant extracts (e.g., tea brewed from the plant) would be faster and greater than absorption from the raw plant.

Mechanism of Toxicity

The whole plant contains convallarin and convallamarin, both cardiac glycosides. With significant exposure, the sodium–potassium ATPase pump in the heart is disrupted, which results in markedly high-grade atrioventricular (AV) conduction block. As intracellular potassium concentrations decline, progressive electrical changes occur. These events may progress to complete loss of normal myocardial electrical function and asystole. The myocardium may lose its ability to respond to electrical pacing.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals are susceptible to toxicity following *Convallaria majalis* exposure. In one case report, a dog that ingested an unknown quantity of lily of the valley leaves suffered seizures and death. Autopsy findings revealed severe hepatic congestion and caudal vena cava distention. Gross and microscopic lesions consistent with cardiac shock were also evident. Leaves of the plant were found in the middle section of the jejunum. Animals exposed to

Convallaria majalis may receive activated charcoal, digoxin-specific immune Fab fragments, and supportive care as required.

Human

Poisoning by *Convallaria majalis* is clinically indistinguishable from digoxin toxicity. Following an acute exposure, patients experience nausea and vomiting within a few hours. Clinical effects later worsen and progress to lethargy, dizziness, hyperkalemia, cardiac conduction delays, hypotension, bradycardia, and tachydysrhythmias. After chewing on a leaf from lily of the valley, a 4-year-old boy was administered activated charcoal and admitted to the hospital for observation and cardiac monitoring. He experienced four episodes of grade II AV-block. A 5-year-old developed only vomiting after ingesting 15 berries from lily of the valley.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion of *Convallaria majalis* may also result in clinically significant cardiac effects, although such exposures have rarely been reported. Patients chronically exposed to the plant may be less likely to present with gastrointestinal distress as their initial symptom of toxicity.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. With significant ingestion, decontamination is advised. Activated charcoal will adsorb plant toxins. Patients should be managed similar to those with digoxin poisoning. Continuous EKG monitoring is indicated. Diagnostic digoxin radioimmunoassays may cross-react with convallarin and convallamarin, but these tests cannot specifically quantify or rule out exposure. Monoclonal antibody assays will not detect convallarin or convallamarin. The administration of digoxin-specific immune Fab fragments may be beneficial in severe intoxications; however, the required dose is unknown. Digoxinspecific immune Fab fragments have been administered to patients poisoned by oleander and foxglove, both cardiac glycoside-containing plants.

See also: Aminoglycosides; Digitalis Glycosides.

Further Reading

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Limonene

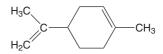
Samantha E Gad and Pertti J Hakkinen

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- CHEMICAL NAME: (*R*)-1-Methyl-4-(1-methylethenyl) cyclohexene
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 5989-27-5 (Closely related to D-Limonene are: L-Limonene (CAS 5989-54-8), Chemical Name: (S)-1-Methyl-4-(1-methylethenyl) cyclohexene; D,L-Limonene (commonly known as Dipentene) is a mixture of the above two isomers. The isomers are chemically identical except that their molecular structures are mirror images of one another (optical isomers) (CAS 138-86-3), Chemical Name: 1-Methyl-4-(1-methylethenyl) cyclohexene)
- SYNONYMS: D-Limonene; 4-Isopropenyl-1-methylcyclohexene; (R)-(+)-Limonene; (+)-Limonene;
 D-(+)-Limonene; (D)-Limonene; Limonene;

(*R*)-1-Methyl-4-(1-methylethenyl)cyclohexene; D*p*-Mentha-1,8-diene

- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Terpene, closely related to isoprene
- CHEMICAL FORMULA: C₁₀H₁₆
- Chemical Structure:



Uses

The monoterpene D-limonene is a naturally occurring chemical which is the major component in oil of orange and other natural oils including lemon, grapefruit, berry, leaf, caraway, dill, bergamot, peppermint, and spearmint oils. D-Limonene may be obtained by steam distillation of citrus peels, from pulp resulting from the production of juice, as cold-pressed oils, and from deterpenation of citrus oils. Any citrus-based cleaning product, air freshener, perfume, essential oil, or fragrance product will likely contain D-limonene.

D-Limonene is also synthetically produced, including as a by-product in the manufacture of terpineol. It is used in flavors and fragrances, as a solvent (e.g., as a natural replacement for petroleum-based solvents in paints and cleaning products), and for numerous other commercial uses. D-Limonene is also used as an inert ingredient in pesticides, and is used in resin manufacture.

Terpenes such as D-limonene have been used as penetration enhancers (also called sorption promoters or accelerants) for improving transdermal drug delivery and work by penetrating into skin to reversibly decrease the barrier resistance.

Commercial mixtures of the limonene molecules can also contain other terpenes, and related compounds such as *p*-cumene.

Exposure Routes and Pathways

Inhalation

D-Limonene may be inhaled when products such as citrus-scented air fresheners, perfumes and candles, cleaners and paints, are used indoors, especially without adequate ventilation. Terpenes are used in products for their solvent properties and, in some cases, for their odor. Limonene is a commonly identified indoor pollutant with time-averaged indoor concentrations in the range of 5-10 ppb, and much higher concentrations (>80 ppb) can occur during the use of limonene-containing products.

Dermal

D-Limonene can be absorbed through the skin after application of citrus oils, perfumes, soaps, and other fragranced personal care products, and through skin contact with citrus-based cleaning products.

Oral

D-Limonene is a compound found in many natural oils, including orange, lemon, grapefruit, berry, leaf, caraway, dill, bergamot, peppermint, and spearmint, and is used as a flavor additive in some foods, beverages, and chewing gum.

Workplace and Occupational Exposures

Inhalation and dermal contact may occur during production, formulation, transport, or use.

Natural Environmental

Exposure to the general population may occur by inhalation due to its presence in the atmosphere as a result of its release from natural sources. Studies have measured levels in both outdoor air and in the indoor air of residences. D-Limonene emissions to the environment have been associated with many plants, for example, wax myrtle, sweet acacia, oranges, tomatoes, grasses, and California western sagebrush.

Environmental Pollution

Found in cigarette mainstream smoke. D-limonene may be released to the environment as a fugitive emission during its production, use, or transport. Limonene has been measured in 'open burning' emissions, and studies have shown that gas phase reactions between ozone and terpenes can be a significant source of secondary organic aerosols. The simultaneous occurrence of ozone and terpenes in an indoor environment is relatively common. Ozone is routinely transported from outdoors to indoors, and indoor sources include photocopiers, electrostatic precipitators, and ozone generators. Limonene has been found as a contaminant in washed and dried shredded polyethylene terephthalate (PET, flake) obtained from curbside collection.

Toxicokinetics

The toxicokinetics of D-limonene were studied in human volunteers exposed by inhalation in an exposure chamber. The relative pulmonary uptake was $\sim 70\%$ of the amount supplied. About 1% of the total uptake was eliminated unchanged in the expired air after the end of exposure, while $\sim 0.003\%$ was eliminated in the urine. A long half-life in blood was observed in the slow elimination phase, which indicates accumulation in adipose tissues.

The major urinary metabolites of D-limonene were identified as perillic acid-8,9-diol in rats and rabbits, perillyl- β -D-glucopyranosiduronic acid in hamsters, *p*-menth-1-ene-8,9-diol in dogs, and 8-hydroxy-*p*menth-1-ene-9-yl- β -D-glucopyranosiduronic acid in guinea pigs and humans. D-Limonene in male rat kidney is associated with a protein fraction, α -2uglobulin. The major metabolite associated with α -2uglobulin was D-limonene-1, 2-oxide, and parent D-limonene was also identified as a minor component in the α -2u-globulin fraction.

Mechanism of Toxicity

The renal toxicity of D-limonene results from the accumulation of a protein, α -2u-globulin, in male rat

kidney proximal tubule lysosomes. This protein is synthesized exclusively by adult male rats, and the nephrotoxicity of D-limonene in male rats is attributed to its ability to bind to α -2u-globulin. Other species, including humans, synthesize proteins that share significant homology with α -2u-globulin; however, none of these proteins, including the mouse equivalent of α -2u-globulin, can produce this toxicity. This indicates a unique specificity for α -2u-globulin. With chronic exposure to D-limonene, the hyaline droplet nephropathy progresses and the male rat kidneys show tubular cell necrosis, granular cast formation at the corticomedullary junction, and compensatory cell proliferation.

The tumorigenic activity of D-limonene in male rats has been concluded to be nonrelevant to humans because of the (1) male rat specificity of the nephrotoxicity and carcinogenicity, (2) role that α -2u-globulin plays in toxicity, as evidenced by the complete lack of toxicity in other species despite the presence of structurally similar proteins, and (3) lack of genotoxicity of both D-limonene and D-limonene-1,2-oxide, supporting the concept of a nongenotoxic mechanism, that is, sustained renal cell proliferation. Both D-limonene and cis-D-limonene-1,2-oxide (the major metabolite involved in this toxicity) are negative in *in vitro* mutagenicity screens. Therefore, the toxicity-related renal cell proliferation is believed to be integrally involved in the carcinogenicity of D-limonene as persistent elevations in renal cell proliferation may increase fixation of spontaneously altered DNA, or serve to promote spontaneously initiated cells.

D-Limonene has chemopreventive and chemotherapeutic activity against many rodent solid tumor types. The chemopreventive activity of limonene during initiation is attributed to the induction of phase I and phase II enzymes, with resulting carcinogen detoxification. The chemopreventive activity of limonene during promotion/progression may be due in part to inhibition of the post-translational isoprenylation of growth-controlling small G proteins, such as p21ras. The complete regression of mammary carcinomas by limonene appears to involve tissue redifferentiation. The multiple antitumorigenic effects of limonene are attainable at a high therapeutic ratio, suggesting that limonene and related monoterpenes may be efficacious in the chemoprevention and chemotherapy of human malignancies.

The dermal allergenic potential of D-limonene oxidation products has been studied. Air-exposed Dlimonene was a strong sensitizer in guinea-pig studies and oxidation of D-limonene is necessary for its sensitizing potential, producing potent allergens such as limonene oxide and carvone.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse oral LD_{50} is 5600 mg^{-1} (males) and $6600 \text{ mg} \text{ kg}^{-1}$ (females), and the rat oral LD_{50} is $4400 \text{ mg} \text{ kg}^{-1}$ (males) and $5200 \text{ mg} \text{ kg}^{-1}$ (females). The rabbit dermal LD_{50} is greater than $5000 \text{ mg} \text{ kg}^{-1}$. Application of undiluted D-limonene, under a covering, to intact or abraded rabbit skin caused moderate irritation after 24 h; in other studies, application of 0.5 ml of D-limonene caused mild to moderate irritation.

Abnormalities in bone formation were seen in offspring of rats fed $591-2869 \text{ mg kg}^{-1} \text{ day}^{-1}$ and mice fed $591-2363 \text{ mg kg}^{-1} \text{ day}^{-1}$ of D-limonene, respectively, over several days during pregnancy. Maternal toxicity was seen at high doses. Teratogenic effects were not observed in offspring of rabbits fed 250– 1000 mg kg⁻¹ day⁻¹ for several days during pregnancy. The high dose was toxic to the females. Repeated administration of high concentrations of D-limonene adversely affected female reproductive organs in rats and male reproductive organs in dogs.

As discussed above, D-limonene causes nephropathy in male rats characterized by hyaline droplet formation with degenerative intracellular changes.

Strong upper airway irritants can be found in reaction mixtures of limonene, other terpenes, and ozone. The identified products included aldehydes, ketones, and carboxylic acids. These identified chemicals and some unidentified reaction products have been studied in upper airway irritation studies in mice, with reduction of the respiratory rate as a key end point.

Limonene and essential oils containing limonene have been tested for anti-inflammatory activity in the mouse model of pleurisy induced by zymosan and lipopolysaccharide (LPS), and assayed for immunoregulatory activity by measurement of the inhibition of nitric oxide (NO) and production of the cytokines, interferon- γ and IL-4. Limonene and the oils, when administered orally, were able to inhibit the LPS-induced inflammation including cell migration. Limonene was also effective in inhibiting production of NO, and produced a significant inhibition of interferon- γ and IL-4 production.

Inhibition of cholesterol biosynthesis occurred in the small intestine of rats after administration of D-limonene for 7 days, but no significant effect on the secretion of radiolabeled cholesterol into bile and feces was observed. D-Limonene increased the perfusion pressure of the sphincter of Oddi in dogs when injected IV or directly into the common bile duct.

Human

Limonene is a mild skin and eye irritant. Ingestion of 20 g of D-limonene caused diarrhea and a temporary increase in protein in the urine (proteinurea) in five male volunteers. These data, in addition to the low acute toxicity in animal tests, suggest that D-limonene is not very toxic by ingestion.

Air levels of D-limonene may irritate the eyes and airways of some people, especially when the levels build up indoors (see above for discussion about gas phase reactions between ozone and terpenes which can be a significant source of secondary organic aerosols).

D-Limonene has been used successfully for the postoperative dissolution of retained cholesterol gallstones.

Chronic Toxicity (or Exposure)

Animal

D-Limonene causes skin sensitization; however, see sections on Mechanism of Toxicity and Chronic Toxicity – Human for discussion and data useful for assessing human sensitization risks.

In 2 year gavage studies, there was clear evidence of carcinogenic activity of D-limonene for male rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. There was no evidence of carcinogenic activity of D-limonene for female rats. There was no evidence of carcinogenic activity of D-limonene for male or female mice. The nephrotoxicity of D-limonene was studied in rats and mice. Kidney sections taken from male rats that had been part of a 91 day oral dosing study of limonene in rats and mice were examined by light microscopy. The study showed that renal alterations were induced only in male rats. As discussed above, the mechanism by which D-limonene caused the tumors in male rats is irrelevant to humans.

Two studies have been reported where a dog and cats were exposed dermally to insecticidal dips containing 78.2% D-limonene. Severe dermatitis was observed in the dog and, at five and ten times the recommended dose, excess salivation, incoordination, and muscle tremors (signs typical of organophosphate poisoning) were observed in the cats. The effects were reversible within 5 h; in the absence of complete ingredient information, these effects cannot be completely attributed to D-limonene.

The effects of D-limonene and citrus oils on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced neoplasia of the lungs and forestomach of female A/J mice has been investigated. D-Limonene and the citrus fruit oils given orally 1 h prior to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, also administered orally, inhibited pulmonary adenoma formation and the occurrence of forestomach tumors. In an additional experiment, D-limonene given orally 1h prior to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone administered IP again showed pronounced inhibition of pulmonary adenoma formation. The anticarcinogenic effects of monocyclic monoterpenes such as limonene have also been demonstrated when given during the initiation phase of 7,12-dimethylbenz(a)anthracene-induced mammary cancer in rats. The regression of rat mammary tumors caused by limonene has been associated with a marked increase in the mannose-6-phosphate (M6P)/insulin-like growth factor II receptor (M6P/ IGF-II receptor) and TGF- β level. TGF- β becomes active when it is released from its latent complex mediated by the M6P/IGF-II receptor.

Human

Low levels of D-limonene in the diet have not been reported to cause adverse effects in humans. D-Limonene can be a dermal sensitizer (see above for discussion about D-limonene oxidation products); however, an 8% solution of D-limonene in petrolatum did not cause an allergic skin response in any of 25 volunteers tested. D-limonene is also a recommended 'quencher' in that it can decrease the sensitizing effect of cinnamic aldehyde when used at a 1:1 ratio with cinnamic aldehyde (International Fragrance Association, that is, IFRA, guidelines developed and used by the fragrance and consumer product industries). There is inadequate evidence in humans for the carcinogenicity of D-limonene, and the overall conclusion by experts is that, as discussed above, D-limonene produces renal tubular tumors in male rats by a non-DNA reactive α -2-globulin-associated response. Therefore, the mechanism by which D-limonene increased the incidence of renal tubular tumors in male rats is not relevant to humans.

In Vitro Toxicity Data

D-Limonene was not mutagenic in four strains of *Salmonella*, did not significantly increase the number of trifluorothymidine-resistant cells in the mouse L5178Y/TK^{+/-} assay, and did not induce chromosomal aberrations or sister chromatid exchanges in cultured CHO cells. All assays were conducted in the presence and absence of exogenous metabolic activation.

Limonene has been among the known chemopreventive substances tested as a reference compound in a battery of cell- and enzyme-based *in vitro* marker systems relevant for prevention of carcinogenesis *in vivo*. This battery of tests included systems assessing modulation of drug metabolism, determination of radical scavenging and antioxidant effects, antiinflammatory mechanisms, and antitumor-promoting activities (e.g., inhibition of phorbol ester-induced ornithine decarboxylase (ODC) activity in murine keratinocytes).

Monoterpenes such as limonene are effective in both preventing and treating a large variety of organspecific rodent cancers. Based on this, clinical testing of the monoterpene-perillyl alcohol (POH) has been conducted in advanced cancer patients. Mechanistically, similar cellular and molecular mechanisms form the basis of both the cancer-preventive and therapeutic activities of the monoterpenes. These include the inhibition of proliferation and the induction of apoptosis, and the effects are confined to premalignant and malignant tissue and do not affect normal tissue.

Clinical Management

Mild irritation and skin sensitization may occur from dermal exposures. Hematuria and albuminuria might occur from ingestion of large amounts, and other symptoms following limonene ingestion could include a burning pain in the mouth and throat, abdominal pain, nausea, vomiting, diarrhea, transient excitement, ataxia, delirium, stupor, coughing, choking, dyspnea, cyanosis, fever, and tachycardia. In addition, pulmonary edema and pneumonitis may occur with limonene aspiration or systemic absorption, and dizziness and suffocation may be observed following limonene inhalation.

Milk should be given to mitigate gastric irritation for acute ingestions. Fluids should be given to maintain maximum urinary output to expedite elimination of limonene from the body. For inhalation exposures, patients should be moved to fresh air and monitored for respiratory distress. Exposed eyes should be treated with copious amounts of room temperature water. For skin exposures, any contaminated clothing should be removed and the exposed skin washed thoroughly with soap and water.

Environmental Fate

Terrestrial

D-Limonene is expected to have low to slight mobility in soil. It will rapidly volatilize from both dry and moist soil to the atmosphere.

Aquatic

It may bioconcentrate in fish and aquatic organisms, and it may significantly adsorb to sediment and suspended organic matter. It is expected to rapidly volatilize from water to the atmosphere.

Atmospheric

If released to the atmosphere, D-limonene is expected to rapidly undergo gas-phase oxidation reactions with photochemically produced hydroxyl radicals, ozone and, at night, with nitrate radicals. Limonene can react with ozone, forming submicron particulates that could impact asthmatics and those with other respiratory ailments.

Ecotoxicology

Calculated bioconcentration factors based on the water solubility of D-limonene and its estimated log octanol/water partition coefficient indicate that D-limonene may bioconcentrate in fish and aquatic organisms. In addition, D-limonene has been studied for general lethality as well as neurotoxic effects in earthworms. Generally, the chronic and acute intoxication of earthworms involved a rapid and predictable cascade of behavioral and morphologic symptoms. Chronic D-limonene exposures induced significant weight loss, but there was no effect on median giant nerve fiber and lateral giant nerve fiber conduction velocities. Acute exposures, however, induced significant decreases in conduction velocity that were reversible once D-limonene exposure ceased.

Exposure Standards and Guidelines

D-Limonene is regulated as an air pollutant in Florida when released from citrus processing plants, because it can combine with NO to form ozone, contributing to smog, on hot days. The legislation establishes that 65% of the citrus oil (D-limonene) be captured for all plants. Most plants now capture between 40% and 45% of the oil.

A time-weighted average (TWA) or other occupational exposure limit is unavailable for D-limonene. The Workplace Environmental Exposure Level (WEEL) for limonene (terpenes) is 30 ppm as an 8 h TWA.

See the discussion above about International Fragrance Association (IFRA) guidelines that D-limonene is a recommended 'quencher' in that it can decrease the sensitizing effect of cinnamic aldehyde when used at a 1:1 ratio with cinnamic aldehyde. IFRA guidelines also state that limonene and natural products containing substantial amounts of it should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 mmol peroxide per liter. The entire set of IFRA guidance and restrictions should be consulted by developers and users of perfume materials, and by interested toxicologists, physicians, and others.

See also: Carcinogen Classification Schemes; Carcinogenesis; Fragrances and Perfumes; International Fragrance Association (IFRA); Risk Assessment, Human Health; Toxicity Testing, Sensitization.

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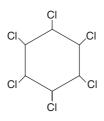
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Lindane

Benny L Blaylock

- © 2005 Elsevier Inc. All rights reserved.
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 58-89-9
- SYNONYMS: 1,2,3,4,5,6-Hexachlorocyclohexane; Agrocide; Ambrocide; Aparasin; Aphitiria; Benesan; Benexane; Benhexachlor; Benzene hexachloride; BHC; BoreKil; Borer-Tox; Exagama; Gallogama; Gamaphex; Gamma-BHC (γ-BHC); γ-Col; γ-HCH; Gammex; Gammexane; Gamasan; Gexane; Hexachlorocyclohexane; HCH; Isotox; Jacutin; Kwell; Lindafor; Lindagronox; Lindaterra; Lindatox; Lintox; Lorexane; New Kotol; Noviagam; Quellada; Steward; Streunex; Tri-6 (BHC stands for benzene hexachloride, which has been used historically but erroneously. This should never be confused with hexachlorobenzene or HCB)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organochlorine insecticide
- CHEMICAL FORMULA: C₆H₆Cl₆
- CHEMICAL STRUCTURE:



Uses

Commercially, lindane is used as an insecticide although its use is restricted. Therapeutically, it is used to treat human lice and mite infestations.

Exposure Routes and Pathways

Lindane is readily absorbed from the gastrointestinal tract, the respiratory tract, and skin. The most important human exposure routes are oral and dermal.

Toxicokinetics

Lindane is absorbed from the gastrointestinal tract, the respiratory tract, and skin. The metabolism of lindane is complex and involves a number of pathways depending on which isomer of hexachlorocyclohexane (HCH) is involved (lindane is the gamma (γ) isomer). It is nonetheless rapid. Lindane is metabolized in the liver by microsomal enzymes. The main pathways include stepwise elimination of chlorines to form tri- and tetrachlorophenols and conjugation with sulfates or glucuronides and subsequent elimination. Other metabolic pathways involve the production of mercapturates. These water-soluble products are eliminated in the urine. Lindane is bound by serum proteins in the blood. Storage is in adipose tissue and other fat-containing tissues. The γ isomer is stored in fat at a much higher rate than the other isomers.

Mechanism of Toxicity

The main site of action of lindane, unlike that of DDT, appears to be at the synapse with both excitatory and inhibitory effects. The effect of inhibition of Na⁺,K⁺-ATPases, which is slightly greater than on Mg²⁺-ATPase, is related to cation transport in nerve axons, perhaps to Ca^{2+} extrusion and therefore to the toxic action of the compound. These effects occur at concentrations of lindane greater than required for antagonism of the GABAA receptor-chloride channel complex at the picrotoxin-binding site. The resulting accumulation of intracellular free calcium ions promotes calcium-induced release of neurotransmitters from storage vesicles and the subsequent depolarization of adjacent neurons and the propagation of stimuli throughout the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the acute oral LD_{50} for lindane is 88– 190 mg kg⁻¹ while for mice it is 59–562 mg kg⁻¹. It is also moderately toxic via the dermal route, with reported dermal LD_{50} values of 500–1000 mg kg⁻¹ in rats. The effects on the nervous system are similar to that of DDT. There are also reports of liver, kidney, and immune system toxicity.

Human

Most instances of lindane poisoning following therapeutic use to control scabies or lice have involved gross misuse of the insecticide. Acute toxicity includes CNS stimulation (usually developing within 1 h), mental/ motor impairment, excitation, convulsions, increased respiratory rate and/or failure, pulmonary edema, and dermatitis. Nausea and vomiting were common.

Pulmonary edema has been reported after lindane powder was aspirated into the lungs. An acute dermal poisoning of a 2-month-old infant exposed to a whole body application of 1% γ -HCH lotion resulted in death.

Chronic Toxicity (or Exposure)

Animal

Doses of 2.6–5 mg kg⁻¹ day⁻¹ have been reported to cause convulsions and liver lesions in rats.

Reproductive toxicity includes a report in rats that doses as low as $0.5 \text{ mg kg}^{-1} \text{ day}^{-1}$ over 4 months caused observable disturbances in the rat estrus cycle, lengthened gestation time, decreased fecundity, and increased fetal mortality.

Human

Aplastic anemia was documented in a man who applied γ -HCH to his skin for 3 weeks for treatment of scabies. After 1–30 years of exposure, 60 male workers in a factory producing lindane showed no signs of neurological impairment.

International Agency for Research on Cancer lists HCHs as 2B (possibly carcinogenic for humans).

Clinical Management

Management of lindane poisoning is symptomatic. Diazepam or phenobarbital is used to control convulsions. Cholestyramine or activated charcoal has been utilized to inhibit lindane uptake after ingestion. In more severe poisonings, the serum levels of lindane may be lowered by hemoperfusion over Amberlite XAD-4.

Environmental Fate

Lindane has been shown to have a low soil binding affinity. Therefore, it may be mobile in soils with especially low organic matter content or subject to high rainfall and pose a risk of groundwater contamination. Lindane is highly persistent in most soils, with a field half-life of ~15 months.

In both fresh and salt waters, lindane has demonstrated high stability. It is resistant to photodegradation but will disappear from the water by secondary mechanisms such as adsorption on sediment, biological breakdown by microflora and fauna, and adsorption by fish through gills, skin, and food.

Ecotoxicology

The toxicity of lindane to bird species is very low to practically nontoxic. Eggshell thinning and reduced egg production has occurred in birds exposed to lindane.

Lindane is highly to very highly toxic to fish and aquatic invertebrate species. Ninety-six-hour LC_{50} values have been reported to range from 1.7 to $90 \,\mu g \, l^{-1}$ in trout (rainbow, brown, and lake), Coho salmon, carp, fathead minnow, bluegill, largemouth bass, and yellow perch. The bioconcentration factor for the compound is 1400 times ambient water concentrations, indicating significant bioaccumulation.

Other Hazards

Lindane is corrosive to metals. Lindane itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. At 102°C in steam, 0.13% hydrogen chloride was produced.

Exposure Standards and Guidelines

Acceptable daily	$0.008{ m mgkg^{-1}day^{-1}}$
intake	
Maximum Contaminant	$0.0002 \mathrm{mg}\mathrm{l}^{-1}$
Level	
Reference dose	$0.0003 \mathrm{mg kg^{-1} day^{-1}}$
Permissible exposure	$0.5 \mathrm{mgm^{-3}(8h)}$
limit	

See also: Carcinogen Classification Schemes; Diazepam; LD₅₀/LC₅₀ (Lethal Dosage 50/Lethal Concentration 50); Organochlorine Insecticides.

Relevant Websites

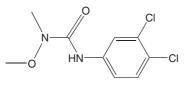
- http://www.atsdr.cdc.gov Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lindane. http://extoxnet.orst.edu – Extension Toxicology Network,
- a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho.
- http://toxnet.nlm.nih.gov TOXNET, Specialized Information Services, National Library of Medicine. Search for Lindane.
- http://www.osha-slc.gov US Department of Labor, Occupational Safety and Health Administration.

Linuron

Guangping Chen

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- CHEMICAL NAME: 3-(3,4-Dichlorophenyl)-1methoxy-1-methylurea
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 330-55-2
- SYNONYMS: Afalon; Garnitan; Linex; Linorox; Linurex; Lorox; Premalin; Sarclex; Sinuron; N'-(3,4-Dichlorophenyl)-N-methoxy-N-methyl urea
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Phenylurea herbicide
- Chemical Formula: C₉H₁₀Cl₂N₂O₂
- CHEMICAL STRUCTURE:



Uses

Linuron is a substituted urea. It is an herbicide used to control germinating and emerging annual and perennial broadleaf and grassy weeds on both crop and noncrop sites. Linuron inhibits photosynthesis in target weed plants. It is labeled for field and storehouse use in a variety of crops. Most of the linuron applied in the United States is in soybean production. Formulations include granules, wettable powders, flowable concentrates, and emulsifiable concentrates/ liquid suspensions. Direct application to water is prohibited.

Exposure Routes and Pathways

Oral exposure is the primary route from dietary sources.

Toxicokinetics

Linuron is rapidly absorbed with oral dosing. Linuron is efficiently metabolized in rat liver and does not accumulate in mammalian systems. With intravenous dosing, linuron distributes quickly and widely to peripheral tissues with rapid elimination. Primary metabolites were identified as N'-(3,4-dichlorophenyl)-N-methoxyurea, N'-(3,4-dichlorophenyl) urea, and N'-(6-hydroxy-3,4-dichlorophenyl) urea. Linuron is a liver enzyme inducer in rats.

Mechanism of Toxicity

As a reproductive and developmental toxicant, linuron works via androgen receptor antagonist activity, that is, it competes with testosterone for binding to the androgen receptor.

Acute and Short-Term Toxicity (or Exposure)

Animal

Linuron is slightly toxic by dermal, oral, or inhalation route of exposure (US Environmental Protection Agency Toxicity Category III).

Human

Linuron has little acute toxicity potential except for skin, eye, and respiratory tract irritations.

Chronic Toxicity (or Exposure)

Animal

In chronic toxicity studies with beagle dogs, linuron caused red blood cell destruction and changes in liver weight. Testicular tumors, red blood cell damage, and growth retardation were noted in long-term studies using rats. Statistically significant increases in liver tumors, reductions in body weight, and increased liver weights were noted in mice with chronic bioassays. Linuron interfered with the transmission of male hormones in a reproductive toxicity study. Linuron exposure induced malformations of the epididymis and the vas deferens. Developmental toxicity was selective to males.

Human

Relatively little is known of the long-term effects of linuron in humans. Linuron is classified in the United States as a possible (group C) human carcinogen.

In Vitro Toxicity Data

In mouse tissues, linuron competitively blocked transcription through androgen receptor induced by dihydrotestosterone in a concentration-dependent manner. Linuron is not mutagenic.

Environmental Fate

Linuron is moderately persistent (half-life ranging from 57 to 100 days) and relatively immobile. Increased mobility may occur in coarse soils and soils with low organic content. Linuron is effectively degraded by biotic processing including microbial degradation. Processes of adsorption and microbial degradation limit its migration to ground water. Runoff to surface waters can occur. Linuron has been detected in ground water in Georgia, Missouri, Virginia, and Wisconsin.

Ecotoxicology

In acute studies, linuron was slightly to moderately toxic to cold and warm water fish.

Technical linuron is highly toxic to aquatic invertebrates, while the formulated product is less toxic. Linuron was highly toxic to sheepshead minnow and moderately toxic to eastern oyster and mysid shrimp. Linuron is practically nontoxic to honeybees. Reproductive deficits have been noted in birds treated with linuron.

Exposure Standards and Guidelines

The US reference dose for linuron is $0.008 \text{ mg kg}^{-1} \text{ day}^{-1}$. No acceptable daily intake has been established.

See also: Diuron; Pesticides.

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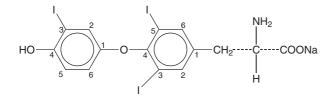
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Liothyronine

F Lee Cantrell

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 6893-02-3
- SYNONYMS: L-Triiodothyronine; T3; Cytomel; Triothyrone
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Synthetic form of triiodothyronine
- Chemical Formula: C₁₅H₁₂I₃NO₄
- CHEMICAL STRUCTURE:



Uses

Liothyronine is used in the treatment of hypothyroidism, nontoxic goiter, cretinism, and myxedema.

Exposure Routes and Pathways

Ingestion is the most common route of exposure for liothyronine, although a parenteral form is available.

Toxicokinetics

Liothyronine (T3) is 88% absorbed. Congestive heart failure can reduce absorption by half. T3 is not firmly bound to serum protein. It has a volume of distribution in the range of $41-45 \, \text{lkg}^{-1}$. T3 is metabolized to deiodinated and conjugate metabolites. From 75% to 85% of T3 is deiodinated. Conjugation takes place in the kidneys. Approximately 20% of T3 is excreted in the feces and up to 10% in the urine. T3 has a half-life of 2.5 days.

Mechanism of Toxicity

The exact mechanism of action is not well understood. Thyroid hormones are needed for metabolism, growth, and development. Most organ systems are affected by thyroid hormones. Thyroid hormones cause an increase in the basal metabolic rate, in oxygen consumption, and in the metabolism of carbohydrates. T3 increases aerobic mitochondrial function causing an increased rate of utilization of high-energy phosphates. This stimulates myosin adenosine triphosphatase and reduces tissue acetic acid. Toxicity is an extension of the pharmacologic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs and cats are at risk for thyroid toxicity. Signs of toxicity include vomiting, diarrhea, tachycardia, tachypnea, decreased level of consciousness, and restlessness. Toxic doses have not been established.

Human

Ingestion of small amounts of T3-containing products is unlikely to result in significant toxicity. Symptoms of toxicity typically occur 4–12 h following large overdoses and include tachycardia, nausea, vomiting, diarrhea, restlessness, and fever.

Chronic Toxicity (or Exposure)

Human

Thyrotoxicosis is a concern with chronic exposure. Thyrotoxicosis is characterized by tachycardia, cardiac arrhythmias, hypertension, tremors, and seizures. In severe cases, coma and cardiovascular collapse can result.

Clinical Management

Most patients after acute overdose can be managed on an outpatient basis. Gastric decontamination may be considered in patients presenting early after large ingestions. The absence of clinical effects within the first 24 h does not preclude the later development of significant toxicity. In patients manifesting toxicity, cardiac and blood pressure monitoring should be performed. Cooling methods should be employed to decrease hyperpyrexia. Intravenous fluids should be administered in dehydrated and/or hypotensive patients. Adrenergic hyperactivity can be treated with propranolol. Propylthiouracil may be administered to decrease conversion of T4 to T3. Forced diuresis and extracorporeal methods are not effective in levothyroxine overdose.

See also: Endocrine System; Levothyroxine; Thyroid Extract.

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Lipid Peroxidation

Zhengwei Cai

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The word 'lipid' is referred to a chemically heterogeneous group of substances having in common the property of insolubility in water, but solubility in nonaqueous solvents such as chloroform, hydrocarbons, or alcohols. Numerous compounds, which may have little or no chemical relationship between each other, are classified as lipids. Vegetable oil and animal fat in daily life, fatty acids, triacylglycerols, phospholipids, and sterols are good examples of lipids. It has been known since antiquity that at high temperature or under light exposure fats and oils in storage may develop unpleasant tastes and odors. This is a typical characteristic of rancidity of lipids and the consequence of lipid peroxidation.

What is Lipid Peroxidation?

Lipid peroxidation is an oxidative chain reaction in which one lipid molecule after another becomes oxidized to the maximum possible extent or so as to form a lipid peroxide (i.e., a lipid molecule containing one or more O-O bonds). At high temperatures, lipid peroxides decompose to produce a range of unpleasant-tasting and foul-smelling products such as epoxides, ketones, acids, and aldehydes. Most biological membranes are extended bilayers of amphiphilic lipids with hydrophobic moieties directed to the center and hydrophilic head groups at the two surfaces. Biological cell membranes are packed with polyunsaturated fatty acids (PUFAs), such as arachidonic and docosahexaenoic acid, in either the isolated form or the incorporated form in triacylglycerides and phospholipids. PUFAs are particularly susceptible to peroxidation. With increasing concerns about the potential adverse effects of lipid peroxidation in cellular membranes, the relevance of lipid peroxidation to biology and human diseases has been extensively explored since the 1950s.

Mechanism of Lipid Peroxidation

Lipid peroxidation is a free radical-initiated chain oxidation of lipids. Electrons in atoms occupy Gorman RL, Chamberlain JM, and Rose SR (1988) High anxiety-low toxicity: A massive T4 ingestion. *Pediatrics* 82: 666–669.

regions of space known as orbitals. Each orbital can hold a maximum of two electrons. A free radical is defined as any chemical species possessing one or more unpaired electrons and capable of independent existence. Hydroxyl (OH[•]) and superoxide $(O_2^{-\bullet})$ are examples of oxygen-centered radicals. There are also other types of radicals such as thiyl (RS[•]), trichloromethyl (CCl₃[•]), and nitric oxide (NO[•]). A free radical is marked by a dot, which designates the presence of one or more unpaired electrons.

Lipid peroxidation can be divided into three separate processes – initiation, propagation, and termination. During initiation a very small number of radicals (e.g., transition metal ions or a radical generated by photolysis or high-energy irradiation) can abstract hydrogen from lipid molecules to yield free radicals of lipids

$$X' + RH \rightarrow R' + XH$$

Propagation then allows a reaction with molecular oxygen to form lipid peroxyl radicals

$$R' + O_2 \rightarrow ROO'$$

This peroxide radical can then react with the original substrate, yielding one hydroperoxide and one new radical

$$ROO' + RH \rightarrow ROOH + R'$$

Thus, the events form the basis of a chain-reaction process. The lipid hydroperoxide decomposition produces more radicals and noxious aldehydes

$$ROOH \rightarrow RO^{\bullet}, ROO^{\bullet}, aldehydes$$

When the substrate is depleted, termination reaction begins. Two radicals combine the unpaired electrons to form a nonradical product

$$R' + R' \rightarrow R - R$$

The chain reactions are also terminated when antioxidants (A–H), which provide easily donatable hydrogen for abstraction by peroxyl radicals, combine with lipid radicals to halt further propagation

$$A-H+ROO \rightarrow ROOH+A$$

The antioxidant-derived radical (A[•]) could be dimerized harmlessly into A₂, or it could be converted back to A–H by reaction with another molecule; it might also react with another ROO[•] to become a nonradical. The most important chain-breaking antioxidant in human lipids is α -tocopherol

ROO[•](lipid peroxyl radical) +
$$\alpha$$
-tocopherol-OH
 \rightarrow ROOH + α -tocopherol-O[•]

The resultant tocopheroxyl radical is relatively stable and, in normal circumstances, insufficiently reactive to initiate lipid peroxidation itself. It has been demonstrated *in vitro* that α -tocopherol radical can be converted back to α -tocopherol by reduction with ascorbic acid at the surface of biological membranes

$$A^{\bullet} + ascorbic acid \rightarrow ascorbic acid^{\bullet} + A - H$$

However, it is uncertain that this reaction actually happens *in vivo*.

There are two types of lipid peroxidation: chemical (nonenzymic) peroxidation and enzyme-catalyzed peroxidation. The nonenzymic peroxidation has been described above. In the enzyme-catalyzed peroxidation, lipoxidases (or lipoxygenases), which are present in both plants and animals, catalyzed the following reaction:

$$R - CH \xrightarrow{cis} CH - CH_2 - CH \xrightarrow{cis} CH - R_1 + O_2 O_1$$

$$\longrightarrow R - CH \xrightarrow{cis} CH - CH \xrightarrow{ch} CH - CH - R_1$$

That is, the addition of molecular oxygen to a 1,4cis, cis-pentadiene moiety to produce a 1-hydroperoxy-2,4-trans, cis-pentadiene unit. The product of the enzymic reaction, a hydroperoxide, is similar to the products of purely chemical reaction, but the lipoxidase reaction has a number of distinguishing features. The activation energy is smaller than that for the chemical reaction, and the enzyme has very specific substrate requirements. To be a substrate, the fatty acid must contain at least two cis double bonds interrupted by a methylene group. Thus, linoleic and α -linolenic acids are good substrates for the plant enzymes while arachidonic acid, the major PUFA in mammals, is the target for the animal lipoxygenases in their tissues when it is released from complex lipids.

Reactive Oxygen Species (ROS) that Initiate Lipid Peroxidation in Cells

Initiation of lipid peroxidation is still not fully understood. Its promotion by oxygen, singlet oxygen, hydroxyl radical, superoxide anion, or some form of perferryl ion has been proposed. High-energy irradiation of aqueous solutions produces highly reactive hydroxyl radicals (OH[•]) that can attack all biological molecules, including membrane lipids. This is probably a mechanism accounting for initiation of lipid peroxidation in irradiated organisms. With the exception of such an unusual circumstance, ROS that initiate lipid peroxidation in cells are produced during normal metabolism and generally produced by electron transfer reactions.

Superoxide free radical (O_2^{-}) and hydrogen peroxide are continuously produced *in vivo*. In normal circumstances, electron 'leakage' from electron transport chains, such as those in mitochondria and the endoplasmic reticulum, to molecular oxygen can generate the superoxidation radical

$$O_2 + e^- \rightarrow O_2^{-\bullet}$$

Superoxide can also be produced by other enzymes, such as the range of flavin oxidases located in peroxisomes, and by oxidation of certain compounds including ascorbic acid, thiols, and adrenaline in the presence of transition metal ions. The autoxidation of reduced transition metal can also generate the superoxide

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-\bullet}$$

 $Cu^+ + O_2 \rightarrow Cu^{2+} + O_2^{-\bullet}$

Hydrogen peroxide is often produced in biological systems via the generation of superoxide: two superoxide molecules can react together to form hydrogen peroxide and oxygen

$$2O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$$

Although superoxide is a free radical, it is not a particularly damaging species and it does not appear to be capable of initiating lipid peroxidation. Its major significance is as a source of hydrogen peroxide and as a reductant of transition metal ion. Hydrogen peroxide is not a free radical but falls into the category of ROS. It is a source of hydroxyl radicals. In the presence of reactive transition metal ions, hydrogen peroxide can rather easily break down to produce the hydroxyl radical, the most reactive and damaging oxygen free radical that will attack most biological molecules and initiate lipid peroxidation at diffusion-controlled rates

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet} + OH^{-} + Fe^{3+}$$

In summary, the ROS that initiate lipid peroxidation include oxygen itself, superoxide, hydrogen peroxide, transition metal ions, and the hydroxyl radical. These ROS are normally produced during metabolism and in the absence of adequate defense mechanisms ROS can attack DNA, proteins, and lipids in the body. However, ROS are not always harmful. For example, they are involved in the destruction of pathogens by phagocytes.

Biological Effects of Lipid Peroxidation

Although ROS is continuously produced during normal metabolism, the integrated antioxidant systems in the body prevent lipid peroxidation. In the absence of adequate defense mechanisms, lipid peroxidation can directly damage the structure of the membrane. The occurrence of lipid peroxidation in biological membranes causes severe impairment of membrane functioning, changes in membrane fluidity, inactivation of membrane-bound receptors and enzymes, and increased nonspecific permeability to ions such as Ca^{2+} . Disruption of cellular membrane structure may further cause antioxidant systems to be ineffective. In addition, decomposition of lipid hydroperoxides produces hydrocarbon gases (such as ethane and pentane), more radical species, and cytotoxic aldehydes. Thus, lipid peroxidation can indirectly damage other cell components by these products of its decomposition.

Lipid peroxidation has been implicated in a wide range of tissue injuries, diseases, and even in the aging process. The liver toxicity of carbon tetrachloride (CCl₄) is a classic example of the destructive effects of lipid peroxidation. A very small portion of administered carbon tetrachloride is metabolized into trichloromethyl free radical (CCl₃) by the action of cytochrome P450 in the liver. This radical reacts rapidly with oxygen and gives rise to the trichloromethylperoxyl radical

$$CCl_3^{\bullet} + O_2 \rightarrow CCl_3O_2^{\bullet}$$

This trichloromethylperoxyl radical can efficiently abstract hydrogen atoms from lipids and initiate lipid peroxidation

$$R-H + CCl_3O_2^{\bullet} \rightarrow R^{\bullet} + CCl_3O_2H$$

Eventually these reactions result in the oxidative destruction of cellular membrane and serious tissue damage in the liver even though < 0.5% of CCl₄ is ever metabolized. An essential involvement of lipid peroxidation in the events leading to death of hepatocytes has been proved in the acute intoxication as well as with other haloalkanes such as bromotrichloromethane (CBrCl₃), dibromoethane, and halothane. Lipid peroxidation is also involved in the hepatotoxicity of ethanol, allyl alcohol, and some drugs like adriamycin.

Atherosclerosis is an irregular thickening of the inner wall of the artery that reduces the size of artery lumen, particularly near junctions in the arterial tree. Atherosclerosis involves the buildup of deposits in arterial walls, characterized by high concentrations of lipids that derive from plasma lipoprotein. Lipids are also involved in the formation of thrombi that may lead to the blockage of blood vessels narrowed by atherosclerosis. It is currently believed that lipid peroxidation is involved in the pathogenesis of atherosclerosis through oxidative modification of lowdensity lipoprotein (LDL) and peroxidation of the apoB100. The oxidized LDL has reduced affinity for the LDL-receptor, and instead become ligands for the family of scavenger receptors. Therefore, the macrophages may engulf large amounts of lipid in an uncontrolled manner with the development of foam cells and the initiation of the atherosclerotic lesion. The oxidized LDL deposited in the arterial wall may continuously release highly cytotoxic lipid peroxidation products, such as certain aldehydes, irritating the endothelial cell layer and causing a range of other effects that may contribute toward the development of the lesion.

Lipid peroxidation has also been suggested to be involved in the pathogenesis of several lung diseases and injuries. One of the important events for several lung diseases is arachidonic acid (AA) release induced by lipid peroxidation and metabolism of released AA to active products. Hydroperoxides have been shown to induce lipid peroxidation in the isolated perfused lung, which could lead to a perturbed plasma membrane and the activation of phospholipase A₂ (PLA₂). As a result of the activation of PLA₂, an excessive amount AA is released. The released AA and its metabolites of eicosanoids, such as prostaglandins, prostacyclin, thromboxane, and leukotriene, can lead to vasoconstriction and brochoconstriction as well as the development of edema.

Recent researches have indicated that lipid peroxidation is involved in the pathogenesis of other human diseases such as hypoxic-ischemic reperfusion injury, cancers, Alzheimer's disease, rheumatoid arthritis, renal dysfunction, and diabetes mellitus. However, it must be pointed out that in most cases increase in the bulk peroxidation of cell membrane lipids is not the cause of cell damage, but a consequence of cell damage. Rises in intracellular Ca^{2+} , protein damage, and DNA damage are more important events in causing cell injury than is the bulk peroxidation of membrane lipids. Lipid peroxidation is often a late event, accompanying rather than causing final cell death and often occurring after cell death, leading to putrefaction and added generation of products such as ethane.

Defenses against Lipid Peroxidation

Because lipid peroxidation can be very damaging, organisms have evolved antioxidant defense systems to protect against it and also repair the system to prevent the accumulation of oxidatively damaged molecules. The antioxidant defenses consist of two categories: those preventing the generation of free radicals and those intercepting the generated radicals. The preventive defenses include efficiency of electron transfer (i.e., no 'leakage' of electrons from the respiratory chain) and sequestration of transition metal ions. Iron, for example, is held tightly bound to special proteins such as transferrin and ferrin. Another type of preventive antioxidant defense is the removal of peroxides that react with transition metal ions to form reactive free radicals. Catalase and glutathione peroxidase are examples of this type of defense. Catalase is mainly located in peroxisomes and acts with hydrogen peroxide; glutathione peroxidase is found in the cytosol of most cells and is active toward both hydrogen peroxide and fatty acid hydroperoxides

> $2H_2O_2 \rightarrow catalase \rightarrow 2H_2O + O_2$ ROOH + 2GSH \rightarrow GSH peroxidase \rightarrow ROH + GSSG

The intercepting defenses 'scavenge' the generated free radicals. As mentioned previously, superoxide dismutase and α -tocopherol are good examples of enzyme and nonenzyme scavengers, respectively. Some dietary minerals are essential for the function of antioxidant enzymes (e.g., the various isoforms of superoxide dismutase use copper and zinc or manganese as cofactors, whereas an isoform of glutathione peroxidase uses selenium).

The repair system removes damaged biomolecules before cell metabolism or viability has been altered due to their accumulation. Oxidatively damaged nucleic acids are repaired by specific enzymes, oxidized proteins are removed by proteolytic systems, and oxidized membrane lipids are processed by lipases, peroxidases, and acyl transferases.

Measurement of Lipid Peroxidation

The extent of lipid peroxidation can be determined by measuring (1) losses of fatty acids; (2) amounts of primary peroxidation products; (3) amounts of secondary products, such as carbonyls and hydrocarbon gases; and (4) reduction in antioxidant activity. Some of the commonly used methods are described below. Analysis of fatty acids by gas liquid chromatography (GLC) or high-performance liquid chromatography (HPLC) is used for measuring the loss of unsaturated fatty acids, a consequence of lipid peroxidation. Lipid hydroperoxides, the primary product of peroxidation product, can be directly measured by HPLC with chemiluminescence detectors. Iodine liberation and glutathione peroxidase methods are often used for measuring lipid peroxides. Lipid peroxides oxidize I^- to I_2 for titration with thiosulfate and thus consumption of thiosulfate indirectly indicates the quantity of lipid peroxides. Hydrogen peroxides and hydroperoxides oxidize reduced GSH to oxidized glutathione (GSSG) and addition of glutathione reductase and NADPH reduces GSSG back to GSH, requiring consumption of NADPH, which can be related to peroxide content. Spin traps (phenyl t-butyl nitrone) are frequently used for trapping intermediate radicals. Products of lipid peroxide decomposition, such as hydrocarbon gases and cytotoxic aldehydes, can be measured by GLC or HPLC. Lipid peroxidation products may cause damage to DNA and formation of 8-oxo-2'-deoxyguanosine (80xodG) is a marker of oxidative damage to DNA. Contents of 80xodG in DNA can be quantified by HPLC with EC detector and by an immunochemical method (enzyme-linked immunosorbent assay).

The commonly used assays for measurement of lipid peroxidation are the thiobarbituric acid (TBA) test and diene conjugation determination. In the TBA test, lipid samples are heated with TBA at low pH and malondialdehyde (MDA), a lipid peroxidation product, reacts with TBA to develop a pink color. Darkness of the color is related to the extent of lipid peroxidation. Because of its simplicity and economy, this method is very popular. During the process of lipid peroxidation, diene conjugations (a double bond-single bond-double bond structure) are formed (see enzyme catalysed peroxidation) which absorb ultraviolet (UV) light in the wavelength range of 230-235 nm. The absorption of UV light at this wavelength range can be related to contents of diene conjugates in lipid extracts of tissues and, thus, to extent of lipid peroxidation.

One approach to determination of 'whole-body' lipid peroxidation has been measurement of exhaled hydrocarbons by GLC, especially ethane. Hydrocarbon gases are, however, minor end-products of peroxidation and their formation depends on the decomposition of peroxide. Recent studies have demonstrated that isoprostane is a good biomarker of lipid peroxidation in the human body. Isoprostanes are specific products arising from the peroxidation of unsaturated fatty acid residues in lipids and detection of them and their metabolites in urine is a useful assay of 'whole-body' lipid peroxidation. Isoprostanes can be accurately and sensitively measured by mass spectrometric techniques.

Since radical-scavenging antioxidant molecules are consumed in the process of protecting against oxidants, changes in antioxidant activity may also reflect previous or ongoing oxidative stress. Therefore, measurement of antioxidant activity under controlled oxidative stress has been also used to estimate extent of lipid peroxidation. Because all these methods measure only one stage of lipid peroxidation, and not the whole process, caution must be taken in interpreting the results from these measurements. In addition, each method has its own limitation. The simple TBA test and diene conjugation methods without previous calibration against more sophisticated assays may result in incorrect estimate of lipid peroxidation.

See also: Acetaminophen; Carbon Tetrachloride; Ethanol; Kidney; Liver; Paraquat; Respiratory Tract.

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Lithium

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 7439-93-2
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alkali earth metals
- Chemical Formula: Li⁺

Uses

The most widely known use of lithium is the use of lithium carbonate in treating manic-depressive affective disorders; the mechanism by which it alleviates depression in some people is not known. Industrially, lithium and its compounds are used in metal alloys, lubricants, nuclear reactor, coolant, ceramics, alkaline storage batteries, and electronic tubes. It is also used as a catalyst and as a reducing agent.

Background Information

Lithium was discovered as a salt in 1817. It does not occur in nature as a free metal. Lithium was used as a

salt substitute and as a major constituent of the soft drink 7-Up before 1950.

Exposure Routes and Pathways

Ingestion is the most common exposure pathway; lithium carbonate is administered orally. Occupational exposures to lithium rarely produce toxicity. Lithium occurs in breast milk of nursing women.

Toxicokinetics

Lithium is readily absorbed when administered orally and is widely distributed in the body, mainly intracellularly. It is carried into the red blood cells by the sodium transport carrier and enters the central nervous system. Lithium can penetrate the placental barrier. It is excreted in the urine – excretion depends on sodium and water balance and the glomerular filtration rate.

Mechanism of Toxicity

The exact mechanism of lithium toxicity is unknown. It may be related to lithium's displacement of potassium, producing an unusual equilibrium within the cells. Another hypothesis is that lithium is competitive with sodium for binding sites in various organs such as the kidney and the central nervous system.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD₅₀ of lithium chloride following subcutaneous administration in mice was ~17–19 mmol kg⁻¹ (~700–800 mg kg⁻¹). The acute oral LD₅₀ in rats is ~500 mg kg⁻¹. Acute lithium exposure elicits excessive urination and polydipsia.

Human

Lithium hydride rapidly converts to lithium hydroxide in contact with water. Lithium hydroxide is corrosive to all tissues, outer skin, as well as lung cells.

Chronic Toxicity (or Exposure)

Animal

Long-term dietary lithium can lead to renal failure, hypertension, and proteinuria. Lithium is neither mutagenic nor carcinogenic, but is toxic to the central nervous system. Interestingly, long-term exposure to lithium chloride dramatically protected cultured neurons against glutamate-induced excitotoxicity via apoptosis mediated by *N*-methyl-D-aspartate receptors. High dietary potassium can prevent lithium toxicity following repeated exposures in hamsters and rats.

Human

Lithium can cause kidney injury. The first sign of intoxication in patients is usually a fine hand tremor. Occupational lithium toxicity is rare. The use of lithium carbonate for depression may result in damage to the neuromuscular system resulting in ataxia and tremors. Toxicity often occurs after weeks of chronic intake. The first signs of toxicity are nausea, vomiting, and abdominal pain. The action on the central nervous system can result in tremors, epileptic-type seizures, impediment of speech, and even short blackout periods. The heart can also be affected resulting in hypertension and arrhythmias. Nephrotoxicity has been recorded in some patients. Long-term lithium treatment of pregnant women has been associated with fetal cardiac abnormalities. Chronic lithium treatment also appears to disrupt thyroid function and may lead to hypothyroidism.

Clinical Management

There is no specific antidote for lithium toxicity. For acute overdose, administration of syrup of ipecac followed by gastric lavage is recommended. Electrolyte replacement should follow. An infusion of mannitol or urea and increasing the alkalinity of the urine will enhance lithium excretion. For abnormal motor activity, a tranquilizer such as diazepam is helpful. High salt intake protects against lithium toxicity in the kidneys. Hemodialysis may also be used. As noted above, diets high in potassium may also afford protection against chronic lithium toxicity.

Environmental Fate

Concentrations of lithium in surface waters are typically very low ($<0.04 \text{ mg l}^{-1}$). Seepage into groundwater and surface water from storage sites (e.g., the US Department of Energy's Y-12 plant) may lead to concentrations much higher (0.15 mg l^{-1}).

Ecotoxicology

The 68 h LD₅₀ (gastric gavage) in common carp was $>400 \text{ mg kg}^{-1}$. The 96 h LC₅₀ in fathead minnows was $42 \text{ mg}l^{-1}$. The 96 h LC₅₀ in *Fundulus heteroclitus* was 8–39 mgl⁻¹. Lithium was reported to induce microcephaly in frogs and salamanders.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) – timeweighted average for lithium hydride is 0.025 mg m⁻³. The TLV for lithium chloride or lithium carbonate is not established.

See also: Kidney; Metals.

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Liver

Janet E Kester

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Introduction

The liver is the largest organ in the human body, weighing ~ 1.5 kg in human adults. Although not an elegant organ in its structure, having (and needing) no constant form, its highly complex activities are essential for life. The liver accommodates its form to its surroundings and expands into any part of the abdominal area not occupied by other viscera. Details of arrangement and lobation vary greatly from species to species and even between individuals of the same species. Ancient priests divined the future from the liver patterns of sacrificed animals; they found plenty of scope for personalized interpretations!

The liver performs four basic functions that are essential for maintenance of homeostasis and integration of metabolism:

- 1. storage and filtration of blood;
- metabolism and storage of many xenobiotics, as well as nutrients and endogenous compounds such as bile acids, fatty acids, and steroids and other hormones;
- 3. secretory and excretory activities involved in bile formation and flow (exocrine functions); and
- 4. synthesis of a variety of important constituents of blood plasma, which are secreted directly into the blood (endocrine functions).

Liver injury by chemical substances has been recognized for more than 100 years.

Review of Liver Structure and Function

Correlation of the liver's structure with its many functions, and an appreciation of the systemic consequences of toxic injury to this vital organ, depends on an understanding of its strategic location in the circulation.

The Liver Functions as an Interface Between the Digestive Tract and the Systemic Circulation

The liver's interposition between the digestive tract and the systemic circulation is responsible for both its primary role in metabolism of xenobiotics and its susceptibility to their toxic actions. It directly receives all the material (including xenobiotics) absorbed from the intestine (except the lipid chylomicrons, which are transported by lymphatic vessels). The absorbed materials are taken up and further metabolized by the liver or transformed and released to the blood for utilization or storage elsewhere.

The liver has a dual blood supply. Its principal afferent (incoming) blood vessel is the portal vein, which drains the capillary beds of the intestine and spleen at a rate of $\sim 1100 \text{ ml min}^{-1}$ ($\sim 75\%$ of the total). A smaller volume of freshly oxygenated arterial blood (\sim 350 ml, or 25%) is carried by the hepatic artery. These two vessels (and the bile duct) enter the liver together at the porta (Latin for 'gate') and branch together into a second capillary bed, the hepatic sinusoids. The liver is drained by the efferent (outgoing) hepatic veins into the inferior vena cava near the heart. This special arrangement of blood vessels - where blood collected from one set of capillaries passes through a larger vessel into a second set of capillaries before entering the venous circulation - is called a portal system. The hepatic blood vessels are accompanied throughout the parenchyma by bile ducts and lymphatic vessels.

The hepatic sinusoids – the second capillary bed in the hepatic portal system – differ from ordinary capillaries in several respects. They are larger and more variable in diameter, and their walls are lined with both endothelial cells and very large, actively phagocytic Kupffer cells. Unlike most other blood vessels in vertebrates, the sinusoids have actual discontinuities of as much as a micrometer in diameter between the endothelial cells, allowing the blood plasma, including plasma proteins (but not blood cells), to pass freely through into the space of Disse and directly contact the liver cells. The direct access of the plasma to the surface of the liver cell is a structural feature of great functional importance in the active exchange of materials between the liver and the bloodstream.

The Liver Serves as a Blood Filter and Reservoir

Blood entering the hepatic sinusoids carries many bacteria from the digestive tract. The phagocytic Kupffer cells interspersed among the typical endothelial cells lining this specialized capillary bed rapidly phagocytize more than 99% of bacteria and other foreign particles in the blood.

Because the liver is a soft, expansible structure, large volumes of blood can be stored in its vessels. The normal hepatic blood volume is ~ 450 ml, or almost 10% of the normal human total blood volume. As much as an additional liter can be stored

when blood volume is high. Likewise, the liver can supply extra blood when the circulatory volume is diminished.

The Liver Is Composed Primarily of Hepatocytes

Although the liver consists of several cell types present which are vital for its function – endothelial cells lining the blood vessels, bile ductular cells, connective tissue cells, nerve cells, and the phagocytic Kupffer cells – the hepatocytes constitute $\sim 80\%$ of the cytoplasmic mass and carry out the liver's characteristic metabolic and synthetic functions. These cells are essential for life and have an extraordinary capacity to regenerate and flexibly respond to varying metabolic demands. Indeed, they are unrivaled in their functional diversity, complexity, and flexibility.

Although the hepatocyte is a highly differentiated cell that rarely divides in adult vertebrates, it possesses a tremendous capacity for compensatory hyperplasia after injury or removal of liver tissue. It appears that hepatocytes engaged in the regenerative process undergo quantitative rather than qualitative changes in gene expression. Preferentially expressed are stress proteins, the multidrug-resistance gene, and several protooncogenes.

Protooncogenes in particular are thought to play an essential role in cell proliferation and differentiation because they are highly conserved in evolution, differentially expressed during development, and known to play a role in malignant transformation. In fact, analysis of protooncogene expression during liver regeneration provided one of the first demonstrations that the expression of these genes is regulated during normal growth.

The Basic Anatomical Unit of the Liver Is the Classical Lobule

The hepatocytes are arranged in single-cell-thick plates or sheets that appear to radiate out from terminal branches of the hepatic veins. These have traditionally been termed 'central veins' because of their location in the polyhedral units of liver parenchyma that constitute the classical 'liver lobules', typically hexagonal structures 2 or 3 mm in length and 1 or 2 mm in diameter. Neighboring plates are separated by sinusoids, which are closely applied to the sheets of liver cells and intercommunicate through fenestrations ('windows') in them to form a labyrinthine system covering a very large area of liver parenchyma. The human liver contains 50 000-100 000 individual lobules. The corners of the polygonal lobules are each occupied by portal space containing portal triads: branches of portal venule, hepatic arteriole, and bile ductule (see Figure 1 for anatomical illustration).

It was previously thought that blood flowed directly from the portal space vessels between plates to hepatocytes to be collected in the central veins. Although it is now clear that the actual flow of blood in these areas is not as previously envisioned, the lobule unit remains conceptually convenient because it exhibits morphologically distinguishable zones, for example, differential deposition of glycogen and fat, referred to as centrilobular, mid-zonal, or periportal. Furthermore, toxic agents of pathological conditions may selectively show their harmful effects in these areas.

The Basic Functional Unit of the Liver Is the Acinus

We now know that blood enters the sinusoids of the parenchyma via fine terminal branches of the afferent vessels, which leave the portal spaces at intervals, coursing perpendicular to the central vein and along the sides of the hexagons forming classical lobules. Each fine terminal afferent vessel supplies blood to only sectors of adjacent lobules. The associated mass of parenchymal tissue that they preferentially supply

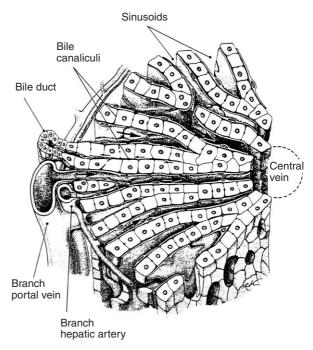


Figure 1 Diagram illustrating the basic anatomical unit of the liver, the liver lobule, showing (1) the radial disposition of the liver cell plates and sinusoids around the central vein, (2) the centripetal flow of blood from branches of the hepatic artery and portal vein, and (3) the centrifugal flow of bile (small arrows) to the small bile duct in the portal space. (Reproduced from Bloom W and Fawcett DW (eds.) (1968) *A Textbook of Histology*, 9th edn. Philadelphia: Saunders; redrawn and modified from Ham, *Textbook of Histology*. Philadelphia: Lippincott, with permission from Lippincott.)

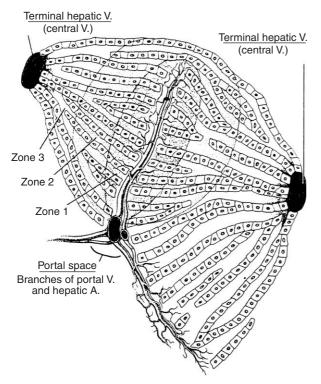


Figure 2 Diagram illustrating the basic functional unit of the liver, the acinus, consisting of the tissue centered around the terminal branches of the hepatic artery and portal vein. The cells in zone 1 nearest these vessels receive the highest concentrations of oxygen and nutrients, while those in zones 2 and 3 are exposed to progressively depleted blood. (Reproduced from Bloom W and Fawcett DW (eds.) (1968) *A Textbook of Histology*, 9th edn. Philadelphia: Saunders, with permission; redrawn from Rappaport A *et al.* (1954) *The Anatomical Record* 119: 11.)

is termed an acinus (Latin for 'berry') (Figure 2). The acinus lies between two or more terminal hepatic venules (central veins in the classical terminology), with which its vascular and biliary axis interdigitates. Acini are irregular in size and shape, and there is no physical separation between them.

As with the classical lobule, there are distinct circulatory zones within each acinus. They are typically divided into three, depending on their distance from the afferent vessels (Figure 3). Zone 1, being closest to the supply of fresh blood, is the first to receive oxygen and nutrients and the last to undergo necrosis. The more distal regions, zones 2 and 3, receive progressively depleted blood, and hence are possibly less resistant to hepatotoxicants and other vectors of hepatic injury. Furthermore, the fenestrations between sinusoidal endothelial cells gradually increase in number from the periportal to the pericentral end of the sinusoid, allowing the remaining nutrients greater access to the centrally located cells.

There is considerable evidence that position within the acinus also affects important aspects of hepatocyte

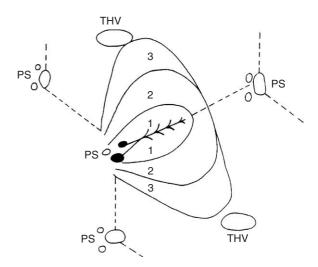


Figure 3 Schematic representation of a simple hepatic acinus. PS is the portal space, consisting of a branch of the portal vein, a hepatic arteriole, and a bile duct; THV is the terminal hepatic venule (central vein); 1, 2, and 3 represent the various zones surrounding the terminal afferent vessel. (Reproduced with permission from Klassen CD, Amdur MO, and Doull J (eds.) (1986) *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 3rd edn. New York: McGraw-Hill; © The McGraw-Hill Companies, Inc.)

 Table 1
 Predominant lobular/acinar localization of major liver functions

Periportal zone/Zone 1 Glucose release Oxidative energy metabolism Amino acid utilization Protection against oxidants Bile acid uptake and excretion Bilirubin excretion
Centrilobular zone/Zone 3 Glucose uptake Ammonia detoxification Biotransformation reactions

function, including bile secretion and metabolic activity. Thus, periportal and pericentral cells differ both structurally and functionally, as summarized in **Table 1**. For example, periportal (zone 1) cells take up more bile acids from sinusoidal blood, and hence secrete more biliary constituents. Pericentral (zone 3) cells are specialized to perform higher levels of metabolic and degradative activities.

The Liver Is an Exocrine Gland, Secreting Bile

The liver functions as an exocrine gland, with each hepatocyte continually secreting a small amount of bile into tiny bile canaliculi located between adjacent pairs of parenchymal cells. These tiny vessels form a continuous network from lobule to lobule throughout the organ. The canaliculi are smallest near the central vein, increasing in diameter with proximity to the portal triads. The canaliculi coalesce into biliary ductules, interlobular bile ducts, and larger hepatic ducts. The main hepatic duct joins the cystic duct from the gallbladder to form the common bile duct, which drains into the duodenum. The total secretion of bile by the human liver is \sim 700–1200 ml day⁻¹.

Bile is Important Both as a Digestive Secretion and as a Medium of Excretion Bile is a complex mixture of bile salts, bile pigments, phospholipids, cholesterol, inorganic electrolytes, and endproducts of metabolism.

Bile Salts Enable the Digestion of Lipids Cholesterol is the precursor of both steroids and bile salts and is an integral component of cell membranes. It is eliminated from the body via conversion to bile salts and direct secretion into the bile. In fact, the word cholesterol (from the Greek 'chole' (bile) and 'stereos' (solid)) was used originally to describe the material of which gallstones are made. In the process of degradation, it is converted to the primary bile acids cholic acid and chenodeoxycholic acid in approximately equal amounts. The salts of these acids are excreted in bile. They perform two important functions in the digestive tract:

- 1. They act as detergents, emulsifying large fat droplets into small ones. This action creates a much larger surface area for the action of lipase in the small intestine, thereby increasing lipid absorption.
- 2. They form minute complexes called micelles with the emulsified lipids. The electrical charges of the strongly ionized bile salts render these micelles highly soluble, aiding in their transport to the absorptive surfaces of the intestinal brush border. The fats readily diffuse across the membrane, leaving the charged bile salts to retrieve more fats in a 'ferrying' activity.

Bile salts are extensively metabolized to secondary bile acids by intestinal microflora in the gut. Approximately 94% of the bile salts are reabsorbed at special mucosal receptor sites in the distal ileum and reused by the liver by the process of enterohepatic circulation. In enterohepatic circulation, compounds secreted in bile are reabsorbed in the gastrointestinal tract and returned to the liver. On reaching the liver in the portal blood, almost all of the bile salts are taken up across the sinusoidal membranes of hepatocytes (predominantly in periportal regions). The bile acids are then resecreted into the bile. On average, these salts make the entire circuit ~ 18 times before being lost in the feces. Thus, enterohepatic circulation of toxicants increases their half-life in the body and hence their opportunity to exert toxic effects.

Bile Pigments are Products of Red Blood Cell Destruction Red blood cells circulate for ~ 120 days before their membranes become very fragile and they rupture in vascular 'tight spots' like the spleen. The hemoglobin released when these cells rupture is rapidly taken up by tissue macrophages in many parts of the body, but especially by the Kupffer cells of the liver. The porphyrin portion of the hemoglobin molecule is oxidized in the macrophages to biliverdin, which is rapidly reduced to the yellowgreen bile pigment bilirubin. The macrophages release bilirubin into the blood, where it binds tightly to albumin and is transported throughout the circulation to the liver. Inside hepatocytes, it associates with proteins that 'trap' it inside the liver cells. It is subsequently removed from these holding proteins and conjugated with glucuronide (80%), sulfate (10%), and other substances (10%). These conjugated forms are then actively transported into the bile canaliculi and largely eliminated with the feces.

This pigment is highly soluble in all cell membranes and also very toxic; hence, its excretion is one of the liver's most important functions. Interestingly, however, recent studies have demonstrated that bilirubin is an effective antioxidant of possible physiological importance. Along with urate and ascorbate (vitamin C), it is one of the three principal antioxidants in plasma. In membranes, its antioxidative efficacy rivals that of vitamin E. Thus, this toxic endproduct of a degradative pathway can also perform a beneficial function.

The Liver Plays a Key Role in the Integration of Metabolism

Rich in both phase I (principally the cytochromes P450, catalyzing hydrolysis, reduction, and oxidation reactions) and phase II (catalyzing conjugation of xenobiotic molecules with hydrophilic moieties) biotransforming enzymes, the liver is the metabolic center of the body. In fact, most of the field of biochemistry is concerned with its metabolic reactions. The liver essentially converts ingested food into a balanced cell culture medium via metabolic interconversion of amino acids, carbohydrates, and lipids and synthesizes many substances that are subsequently exported for use in other areas of the body. It is also a major locus of biotransformation of xenobiotic compounds to both harmless (detoxification) and toxic (bioactivation) metabolites. The balance between bioactivation and detoxification reactions determines whether adverse effects occur. Given the complex metabolic machinery of the liver and the inevitable presence of multiple chemicals (both naturally occurring and xenobiotic), it is not surprising that metabolic and toxicologic interactions among chemicals are observed.

Carbohydrate Metabolism The liver serves as an energy reservoir, storing glucose as glycogen for release on demand. It thus plays a very important role in maintaining a normal blood glucose concentration. In the event of severe glucose deficiency, the liver can convert amino acids into glucose.

Fat Metabolism The liver also plays a central role in synthesis, oxidation, storage, and distribution of lipids. It not only aids in the absorption of fats through the action of the bile salts, but also (1) both synthesizes and oxidizes fatty acids, cholesterol, triacylglycerols, and phospholipids (the major components of cell membranes); (2) synthesizes most of the plasma lipoproteins; and (3) converts carbohydrates and proteins into fat.

About 80% of the cholesterol synthesized in the liver is converted into bile salts. The remainder of the cholesterol, triacylglycerols, other lipids, and hydrophobic substances (including xenobiotics) are transported to other tissues throughout the body by plasma lipoproteins. These lipoproteins, which are classified according to density, consist of apoproteins (also made by the liver) and various combinations of fat and fat-soluble compounds. The liver also stores vitamins, especially vitamin A but also vitamins D, E and K, as well as vitamin B12, in fatstoring Ito cells, located between endothelial cells and hepatocytes.

Protein Metabolism The most important functions of the liver in protein metabolism are (1) deamination of amino acids for use as energy or conversion into fats and carbohydrates, (2) synthesis and interconversion of amino acids and other metabolically important compounds, (3) formation of urea for excretion of ammonia, and (4) formation of plasma proteins.

Approximately 90% of the plasma proteins are formed by the hepatocytes. Among these important products are albumin (involved in the maintenance of osmotic pressure), clotting and anticlotting factors, and immunoglobulins. The remaining 10% are largely γ -globulins synthesized by plasma cells.

Toxic Liver Injury

The Liver Is Often a Target of Toxic Agents

Many toxic agents enter the body via the gastrointestinal tract and after absorption are carried directly to the liver. There, the drug metabolizing enzyme systems may detoxify them or, in some cases, create toxic intermediates which injure the liver and other tissues. Even chemicals that are successfully excreted in the bile can return to the liver via the cycle of enterohepatic circulation. Furthermore, the liver has a high concentration of binding sites and therefore a tendency to accumulate certain xenobiotics. The liver's position at the interface of the digestive tract and the blood, and its central role in the metabolism and excretion of foreign chemicals, therefore, renders it especially vulnerable to toxic injury.

Toxic Liver Injury Can Take Many Forms

Many chemicals are hepatotoxic, including thousands of synthetic drugs and chemicals as well as a plethora of natural compounds such as bacterial, fungal, plant, and animal toxins. Some examples of chemicals causing liver injury are shown in Table 2.

It is clear that a variety of pathologic processes are involved in toxic liver injury, depending on causative agent and duration of exposure. The incidence of

 Table 2
 Examples of liver toxicants causing specific types of injury

Necrosis and fatty liver Carbon tetrachloride Ethanol Trichloroethylene Acetaminophen
Cholestasis Chlorpromazine Diazepam Estradiol Sulfanilamide
Hepatitis Isoniazid Halothane Indomethacin
Porphyria Hexachlorobenzene
Cirrhosis Ethanol
Carcinogenesis (in experimental animals) Carbon tetrachloride Dimethylbenzanthracene Polychlorinated biphenyls and dioxins Tri- and tetrachloroethylene Vinyl chloride

hepatotoxic injury by a given chemical differs among species and individuals; a dose–response relationship may not always be evident. The toxicity of chemicals can be significantly modified by a number of host and other variables, particularly hepatic enzyme activity. Furthermore, different biochemical alterations in the liver can lead to the same toxic effect; no single mechanism seems to govern the appearance of degenerative changes in hepatocytes or alterations in their function. Virtually all forms of chemical-induced liver injury closely resemble other forms of liver disease not presently known to be produced by chemicals.

A variety of systems have been devised to impose some order on this profusion of toxic liver lesions. Our discussion resorts to the simple device of distinguishing between generally acute and generally chronic forms of toxic liver disease, followed by consideration of the mechanisms by which selected compounds exert these effects. A few other classification systems are mentioned here to provide the reader with a further conceptual framework for organization of current knowledge. In one such system, lesions are categorized by the duration of exposure to the causal agent. Some injuries typically occur after acute exposure, while others require chronic exposure. For example, an acute effect of ethanol is fatty change, metamorphosis, or accumulation (intracellular accumulation of triacylglycerols), while in the long term cirrhosis occurs.

Some injuries may be transient, while others are irreversible. For example, fatty change is often transient and not necessarily indicative of functional compromise; under certain conditions, hepatocytes with accumulated fat function normally. Malignant transformation, on the other hand, is irreversible and seriously disrupts hepatocellular function. Necrosis (cell death) may or may not be life-threatening, depending on its extent (see previous discussion of liver regeneration).

Yet another classification system refers to the nature of the host's response to the causative agent. Some agents, referred to as intrinsic hepatotoxicants, will cause hepatotoxicity in most individuals of most species. In the case of idiosyncratic hepatotoxicants, where a chemical's toxic effects are a function of unusual susceptibility of the exposed individual, it may not be clear whether the lesion is a manifestation of the hepatotoxic properties of the substance in question or a manifestation of the individual's untoward response to the agent. This response may mean hypersensitivity (allergic) reactions or exaggerated responses to minor alterations in liver function. For example, anabolic or contraceptive steroids cause diminished biliary excretion (cholestasis) in most humans, with a few showing jaundice. It is not clear whether the occasional jaundice is the result of an allergic response or an extreme reaction to diminished biliary excretion. Likewise, many drugs, for example, isoniazid and halothane, can precipitate a potentially fatal viral-like hepatitis in susceptible subjects.

Acute Toxic Effects

Acute exposures to hepatotoxicants may result in the following:

- 1. degenerative changes (lipid or water accumulation);
- 2. necrosis;
 - a. zonal, focal, or massive,
 - b. venoocclusive disease, and
 - c. hepatitis
- 3. hepatobiliary dysfunction; and
- 4. acute porphyria

Degenerative Changes

Disturbances in cellular metabolism can cause swelling and accumulation of materials such as water or lipid. These changes are usually (but not always) reversible. Hydropic change (water accumulation) often precedes fatty metamorphosis. The fatty liver is grossly enlarged and pale yellow. Fat droplets consisting mostly of triacylglycerols and fatty acids are visible histologically using lipid stains. Fatty change may be brought about by several distinct mechanisms involving fatty acid and protein metabolism. Examples of causative agents are carbon tetrachloride, ethionine, and ethanol.

Necrosis

Cell swelling and lipid accumulation may precede cell necrosis. Necrosis can affect small groups of cells (focal), groups of cells located in discrete zones (zonal), or many cells (massive). Centrilobular necrosis refers to the death of cells surrounding the central vein of the classical lobule (zone 3 of acinar model). Typical causative agents are carbon tetrachloride, pyrrolizidine alkaloids, acetaminophen, bromobenzene, and isoniazid. An agent that causes periportal necrosis (cell death around portal spaces; zone 1 of acinus) is allyl alcohol. Mid-zonal (acinar zone 2) necrosis can be caused by beryllium and yellow phosphorus. Because of the dose-responsiveness and predictability of the lesions they cause, these agents are regarded as direct hepatotoxicants. Note, however, that many direct hepatotoxicants must undergo biotransformation to reactive forms. A classic example of enzymatic bioactivation is the cytochrome P450-mediated conversion of carbon tetrachloride to a free radical that initiates lipid peroxidation.

Most of the unpredictable idiosyncratic forms of toxic liver injury are more diffuse, consisting of necrosis with significant inflammatory reaction. Nonspecific hepatitis, which can be caused by aspirin (acetylsalicylic acid), is characterized by a few scattered foci of necrosis. A clinical syndrome indistinguishable from viral hepatitis (hence the name viral-like hepatitis) has been associated with various drugs, for example, isoniazid and halothane. This appears histologically as generalized parenchymal damage with disruption of the normal liver cell arrangement, often accompanied by fever, rash, arthralgias, and eosinophilia. The cells may be swollen and hydropic, especially in the centrilobular regions. There may be a progression to a massive necrosis typical of viral hepatitis. Granulomatous hepatitis, characterized by well-demarcated aggregates of inflammatory cells, occurs with or without other types of hepatic injury in response to several drugs. Typical examples are sulfonamides and sulfonylurea derivatives.

Hepatobiliary Dysfunction

Interference with any step in the complex sequences of hepatic uptake and secretion could affect hepatobiliary function (see previous discussion). A number of agents cause cholestasis, generally defined as reduced bile flow. This condition can arise from extrahepatic obstruction of bile flow or intrahepatic alterations that reduce secretion and excretion of solutes and water. Because their elimination is impeded, bile substances (including bile acids and pigments, cholesterol, and various endogenous and exogenous conjugated products) accumulate within the liver and in the systemic blood. Cholestasis is generally difficult to reproduce in experimental animals using the drugs that cause it in humans. However, it is possible to produce cholestatic responses in animals following administration of certain compounds, including lithocholic acid, α -naphthylisothiocyanate, anabolic and contraceptive steroids, and chlorpromazine.

Acute Toxic Porphyria

The biochemistry of the porphyrins and the bile pigments is closely related because heme is synthesized from porphyrins and iron and the products of its degradation are bile pigments and iron. Porphyrins are cyclic compounds formed by the linkage of four pyrrole rings through methenyl bridges. Iron is complexed to the nitrogen atom of the pyrrole rings. The metalloporphyrin is in turn conjugated to proteins to form hemoglobin, cytochromes, and several other hemoproteins important in biological processes. The term 'porphyrin' is derived from the Greek word porphyra (purple) because mammalian porphyrins are deep red or purple in color (due to the iron atom). They are easily detected in blood, urine, and feces due to their color and fluorescent properties.

The porphyrias are a heterogeneous group of diseases, all of which involve disorders of heme biosynthesis, which result in accumulation and increased excretion of porphyrins or porphyrin precursors. The porphyrias can be divided into two kinds: the hereditary porphyrias, some of which can be exacerbated by exposure to certain chemicals, and the toxic porphyrias, which can be produced by exposure to certain chemicals alone. The pattern of excretion of porphyrins and porphyrin precursors is characteristic for each type. Clinical symptoms consist mainly of cutaneous photosensitivity and/or neurological disturbances. Hexachlorobenzene is a chemical inducer of porphyria.

Chronic Toxic Effects

Chronic exposure to certain chemicals can cause cirrhosis, a marked alteration of the entire liver structure with both degenerative and proliferative changes observed, and neoplasia.

Cirrhosis

Cirrhosis is characterized by diffuse destruction and partial regeneration of parenchymal tissue and the formation of collagen septa distributed throughout most of the liver. Separated by these fibrous sheaths, clusters of hepatocytes appear as nodules. The pathogenesis of cirrhosis is poorly understood. In most cases, the death of scattered single cells together with defective repair mechanisms appears to lead to scarring. In humans, the single most important cause of cirrhosis is chronic ingestion of alcohol. This condition is not easy to duplicate in laboratory animals with ethanol, leading to the suggestion that other factors unique to humans may play an important role in pathogenesis. A major factor might be diet since alcoholics usually suffer nutritional deficiency.

Neoplasia

Chemically induced liver tumors may arise from hepatocytes, bile duct cells, or sinusoidal lining cells (angiosarcoma). Susceptibility to liver tumors differs significantly among species. A large number of chemicals, both anthropogenic and naturally occurring, are known to induce liver cancers in experimental rodents, acting by a variety of both DNA-damaging (genotoxic) and epigenetic (nongenotoxic) mechanisms. Although this classification is widely used and convenient, it is important to note that although nongenotoxic agents do not alter the primary sequence of DNA, they may directly or indirectly cause mutagenicity through oxidative damage. In addition, they may promote the clonal expansion of altered cells, by stimulating cell division and/or inhibiting apoptosis.

Genotoxic hepatic carcinogens include 2-acetylaminofluorene, aflatoxin, carbon tetrachloride, and vinyl chloride. The most potent known nongenotoxic rodent hepatocarcinogen is 2,3,7,8-tetrachlorodibenzo-p-dioxin ('dioxin'), the best-studied of a large group of structurally similar halogenated aromatic hydrocarbon compounds (including the halogenated furans, biphenyls, triphenyls, azo- and azoxybenzenes, and naphthalenes) that exert their effects via interaction with the aryl hydrocarbon (Ah) receptor. Peroxisome proliferators are a large family of structurally diverse compounds that exert many of their effects, including rodent hepatocarcinogenicity, via transactivation of peroxisome proliferator-activated receptor α (PPAR α). Examples of peroxisome proliferators include many drugs (e.g., fibrates, aspirin, and acetaminophen), phthalate plasticizers, pesticides, tri- and tetrachloroethylene, and natural compounds (hormones, eicosanoids, green and black tea, and omega-3 fatty acids).

The human hepatocarcinogenicity of many chemicals has not been well established. One of the difficulties in assessing the potential risks of human exposure to chemicals is the fact that the protocols typically used in chronic chemical toxicity assessment studies tend to overpredict human carcinogenicity. For example, several of the commonly used rodent species have a higher natural incidence of liver tumors than do humans, and the test procedures involve long-term administration of doses very much larger than those to which humans are likely to be exposed. Further, evolving tools of molecular biology continue to illuminate the mechanistic bases for observed differences in animal and human responses.

See also: Acetaminophen; Acetylaminofluorene; Acetylsalicylic Acid; Aflatoxin; Biotransformation; Blood; Bromobenzene; Carbon Tetrachloride; Chloroform; Dioxins; Distribution; Ethanol; Excretion; Immune System; Isoniazid; Lipid Peroxidation; Metallothionein; Peroxisome Proliferators; Tissue Repair.

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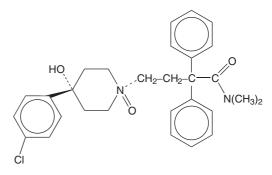
Loperamide

F Lee Cantrell

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 53179-11-6
- SYNONYMS: Imodium; Imodium AD; Imodium advanced, Imogen; Diarrid; Diar-aid; Neo-Diaral
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Antidiarrheal

- CHEMICAL FORMULA: C₂₉H₃₃ClN₂O₂
- CHEMICAL STRUCTURE:



Uses

Loperamide is a nonprescription medication for the treatment of acute nonspecific diarrhea and chronic diarrhea associated with inflammatory bowel disease. It is also used to reduce the volume of discharge from ileostomies.

Exposure Routes and Pathways

Loperamide is available in capsule and liquid forms. Ingestion is the most common route of exposure.

Toxicokinetics

Loperamide is absorbed slowly from the gastrointestinal tract reaching peak levels within 4 h. It undergoes enterohepatic circulation. Protein binding is 97%. Loperamide is primarily excreted in the urine. The half-life can range from 7 to 15 h and is dose independent.

Mechanism of Toxicity

The mechanism of loperamide toxicity is related to opioid-like activity that causes depression of the central nervous system (CNS). The abuse potential for loperamide is low.

Acute and Short-Term Toxicity (or Exposure)

Animal

In dogs $1.25-5 \text{ mg kg}^{-1} \text{day}^{-1}$ has produced vomiting, CNS depression, severe salivation, and weight loss. Amounts $> 5 \text{ mg kg}^{-1} \text{day}^{-1}$ have produced hemorrhagic enteritis and paresis.

Human

Children appear to be more susceptible than adults to the toxic effects of loperamide. These effects mimic opiate poisoning. Miosis, nausea, vomiting, and varying degrees of CNS depression exhibited by ataxia and drowsiness to prolonged coma can be seen. Loperamide is not recommended for use in children under 2 years of age. Death has occurred after misuse of loperamide in children 6.5 months of age and younger.

Chronic Toxicity (or Exposure)

Animal

Dogs dosed at $5 \text{ mg kg}^{-1} \text{day}^{-1}$ developed hemorrhagic enteritis. Doses of $1.5-5 \text{ mg kg}^{-1} \text{day}^{-1}$ produced vomiting, depression, severe salivation, and weight loss. Treatment of cerebellar symptoms with naloxone has been successful in the management of one puppy that was given 3 mg of loperamide.

Human

Loperamide has been used in the management of acute and chronic diarrhea, irritable bowel syndrome, fecal incontinence, ileostomy discharge, and Traveller's diarrhea. Adverse reactions tend to be uncommon and generally mild. Most side effects are gastrointestinal in nature (e.g., epigastric pain, nausea, vomiting, dry mouth, anorexia). Patients have reported development of hyperglycemia while taking loperamide. Volunteer studies have suggested that loperamide use may increase the likelihood for development of gallstones.

In Vitro Toxicity Data

Studies of human jejunal biopsy specimens from cystic fibrosis patients demonstrated that loperamide can restore sodium absorption to normal levels.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. In patients presenting within 1 h of ingestion, activated charcoal may be considered. Supportive care should be provided as needed. Use of the narcotic antagonist naloxone may be beneficial in patients displaying opioid symptoms.

See also: Chloroquine; Codeine; Cromolyn; Diphenoxylate; Disulfiram; Liothyronine; Loxapine; Scombroid; Shellfish Poisoning, Paralytic.

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Lotronex

Shayne C Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 1-22852-69-1
- SYNONYMS: Alosetron hydrochloride; 2,3,4,5-Tetrahydro-5-methyl-2-[(5-methyl-1*H*-imidazol-4yl)methyl]-1*H*-pyrido[4,3-*b*]indol-1-one, monohydrochloride
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Serotonin 5HT3-receptor antagonist
- CHEMICAL FORMULA: C₁₇H₁₈N₄OHCl

Uses

Lotronex is used for severe diarrhea-predominant irritable bowel syndrome (IBS).

Background Information

Animal models have shown alosetron to be active in anxiety, psychosis, cognitive impairment, emesis, and drug withdrawal, though its application in humans has been almost entirely restricted to IBS. Lotronex[®] (alosetron hydrochloride) was originally approved for IBS in the United States, and then removed from the market in 2000 due to serious gastrointestinal adverse events, some fatal. These events, including ischemic colitis and serious complications of constipation, have resulted in hospitalization, blood transfusion, surgery, and death. In 2002, the US Food and Drug Administration (FDA) approved the Supplemental New Drug Application (sNDA) for Lotronex[®] tablets under restricted conditions of use. The restrictions include a 'risk management program', the 'prescribing program' for LotrenoxTM, which is a component of the 'risk management program', and a revised indication that reflects the intent to reserve Lotronex for patients in whom the medical benefits outweigh the risks, that is, women with severe diarrhea-predominant IBS. These changes are reflective of the serious gastrointestinal adverse events that were reported with the use of Lotronex. In addition, the initial dose of Lotronex was reduced to 1 mg QD (once daily) under the 'risk management program'. Lotronex tablets for oral administration contain 1.124 mg alosetron HCl (equivalent to 1 mg of alosetron).

Exposure Routes and Pathways

Oral, as tablet.

Toxicokinetics

Absorption: Alosetron is rapidly absorbed after oral administration with a mean absolute bioavailability of ~50–60% (approximate range 30% to >90%). After administration of radiolabeled alosetron, only 1% of the dose was recovered in the feces as unchanged drug. In patients with IBS, concentrations of alosetron are influenced by gender. The plasma concentrations of alosetron are 30–50% lower in men than in women given the same oral dose. Following oral administration of 1 mg alosetron dose to young men, a peak plasma concentration of ~5 ng ml⁻¹ occurs at 1 h. In young women, the mean peak plasma concentration is ~9 ng ml⁻¹, with a similar time to peak. Efficacy has not been established in men at any dose.

Food effects: Alosetron absorption is decreased by $\sim 25\%$ by coadministration with food, with a mean delay in time to peak concentration of 15 min.

Distribution: Alosetron demonstrates a volume of distribution of $\sim 6-95$ l. Plasma protein binding is 82% over a concentration range of 20–4000 ng ml⁻¹.

Metabolism and elimination: Plasma concentrations of alosetron increase proportionality with increasing single oral doses up to 8 mg and more than proportionately at a single oral dose of 16 mg. Twice-daily oral dosing of alosetron does not result in accumulation. The terminal elimination halflife of alosetron is ~1.5 h (plasma clearance is ~600 ml min⁻¹). Population pharmacokinetic analysis in IBS patients confirmed that alosetron clearance is minimally influenced by doses up to 8 mg.

Renal elimination of unchanged alosetron accounts for only 6% of the dose. Renal clearance is $\sim 94 \text{ ml min}^{-1}$.

Alosetron is extensively metabolized in humans, by multiple cytochrome P450 (CYP) enzymes, including CYP2C9 and CYP3A4. Metabolism is rapid and extensive with N-demethylation, hydroxylation, and oxidation. The biological activity of these metabolites is unknown. A mass balance study was performed utilizing an orally administered dose of unlabeled and ¹⁴C-labeled alosetron. This study indicates that on a molar basis, alosetron metabolites reach addictive peak plasma concentrations ninefold greater than alosetron and that the additive metabolite AUCs (areas under the curve) are 13-fold greater than alosetron's AUC. Plasma radioactivity declined with a half-life twofold longer than that of alosetron, indicating the presence of circulating metabolites. Approximately 73% of the radiolabeled dose was recovered in urine with another 24% of the dose recovered in feces. Only 7% of the dose was recovered as unchanged drug. At least 13 metabolites have been detected in urine. The predominant product in urine was a 6-hydroxy metabolite (15% of the dose). This metabolite was secondarily metabolized to a glucuronide that was also present in urine (14% of the dose). Smaller amounts of the 6-hydroxy metabolite and the 6-O-glucuronide also appear to be present in feces. A bi-oxidized dicarbonyl accounted for 14% of the dose and its monocarbonyl precursor accounted for another 4% in urine and 6% in feces. No other urinary metabolite accounted for more than 4% of the dose. Glucuronide or sulfate conjugates of unchanged alosetron were not detected in urine.

Mechanism of Toxicity

Alosetron is a potent and selective 5-HT3 receptor antagonist. 5-HT3 receptors are nonselective cation channels that are extensively distributed on enteric neurons in the human gastrointestinal tract, as well as other peripheral and central locations. 5-HT3 receptor antagonists such as alosetron inhibit activation of these channels that results in the modulation of the enteric nervous system. Activation of these channels and the resulting neuronal depolarization affect the regulation of visceral pain, colonic transit, and gastrointestinal secretions, processes that relate to the pathophysiology of IBS.

Acute and Short-Term Toxicity (or Exposure)

Human

Constipation is the most frequent adverse event, with a higher incidence of transient constipation in alosetrontreated patients, typically occurring in the first month of treatment. Significant side effects noted with the use of alosetron include severe constipation, fecal

impaction, and ischemic colitis. Approximately 10% of patients on Lotronex in clinical trials withdrew because of constipation.

Chronic Toxicity (or Exposure)

Animal

In 2 year oral studies, alosetron was not carcinogenic in mice at doses up to $30 \text{ mg kg}^{-1} \text{ day}^{-1}$ or in rats at doses up to $40 \text{ mg kg}^{-1} \text{ day}^{-1}$. Reproduction studies have been performed in rats at doses up to $40 \text{ mg kg}^{-1} \text{ day}^{-1}$ and rabbits at oral doses up to $30 \text{ mg kg}^{-1} \text{ day}^{-1}$. These studies have revealed no evidence of impaired fertility or harm to the fetus due to alosetron.

Human

A small number of patients (2–3 per 1000) using Lotronex developed ischemic colitis in periods of use ranging from 3 to 6 months.

In Vitro Toxicity Data

Alosetron was not genotoxic in the Ames tests, the mouse lymphoma cell (L5178Y/TK \pm) forward gene mutation test, the human lymphocyte chromosome aberration test, and the rat hepatocyte unscheduled DNA synthesis test. Nonmutagenic in the rat micronucleus assay.

See also: Food and Drug Administration, US.

Relevant Websites

http://www.lotronex.com – GlaxoSmithKline, Prescribing Program for Lotronex[®].

http://www.fda.gov – US Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER). Lotronex Information.

Love Canal

Michael A Kamrin

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Introduction

Love Canal was an ordinary neighborhood in the City of Niagara Falls, New York until 25 years ago when it became the symbol of the dangers of hazardous wastes placed in and on the ground as a result of the boom in industrial activity during and after World War II. The national publicity devoted to this site focused public attention on the health threats to communities from buried hazardous wastes. As a result, Love Canal became the quintessential 'ticking time bomb' that citizens at contaminated sites around the country referred to when they asked the government for help with their problems. The Love Canal incident brought to light a serious environmental problem that had not been taken care of by the environmental legislation passed in the 1970s that addressed air and water pollution as well as the future generation, transport and disposal of hazardous wastes. It was the catalyst for both federal and state legislation to address this gap. On the Federal level, it led to the enactment of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), also known as the Superfund Act, in 1980. This Act provided a mechanism for assessing and remediating the worst waste disposal sites across the United States and thus ensuring that there would be no more 'Love Canals'.

It was clear as soon as it was passed that CERCLA would address only the most serious sites and that there were literally thousands of other sites containing hazardous wastes that also needed to be dealt with. In the absence of federal resources for this task, a number of states passed their own legislation to identify, assess and remediate such sites. Activities undertaken under both the federal and state statutes revealed that hazardous wastes had been buried not only in industrially owned sites but also in municipal and private landfills. Indeed, some of these hazardous wastes were the result of citizen disposal practices. Thus, the dimensions of the problems that Love Canal uncovered were revealed to be larger than first thought and the resources needed to address them much greater than anticipated. Twenty-five years later, these problems persist and many sites still await remediation.

Love Canal

The neighborhood called Love Canal was named for a partially built canal that was abandoned late in the nineteenth century. The canal was used for a variety of purposes, mainly recreational, during the early twentieth century. However, by mid-century, it became the dumping grounds for wastes from a variety of sources, most importantly for chemical wastes produced by local industries, particularly Hooker Chemical Corporation which took title to the site in the 1940s. Intensive dumping of chemicals was covered over. It is estimated that Hooker disposed of ~40 million pounds of chemicals in the canal during this time.

As a result of the housing boom following World War II, the character of the Love Canal neighborhood changed from largely rural and agricultural to mainly residential. In light of this, the City of Niagara Falls was looking for sites for new school buildings at the time the Canal was covered over. After some negotiation, the City purchased the Love Canal site from Hooker Chemical for a nominal fee. A school was placed on a small part of the canal area and other parts of the site were used for the construction of roads. This, in turn, attracted more inhabitants and the number of homes adjacent to the Canal grew over time.

Soon after the Canal was first used as a disposal site, there were complaints about odors and chemical contamination. These complaints were ongoing and did not cease even after the disposal operations were discontinued. The postdisposal problems arose because buried chemicals were brought to the surface by natural percolation of liquids upwards in the soil as well as by disturbance of the soil during school and road construction. In addition to esthetic complaints, there were incidents of burns caused by contact with contaminated soil. This situation was exacerbated in 1976–77 because of much above normal rainfall. These rains raised the water table and brought more buried chemicals, as well as some buried drums, to the surface.

By early 1978, the problems at Love Canal had attracted the attention of the local media as well as the local member of congress. This led to sampling of the site and homes around the canal by the US Environmental Protection Agency (EPA) and the State of New York. These tests revealed the presence of benzene in indoor air and seepage of a number of chemicals off-site. These discoveries led to the conduct of preliminary health studies that indicated an increased rate of miscarriages in women living near the canal and identified several cases of congenital abnormalities in children from this same population.

Release of this information to the community resulted in citizen outrage and the formation of homeowner organizations to bring pressure for swift action to be taken. Soon afterwards, in early August, the New York State Commissioner of Health decided the data required the declaration of a health emergency and the recommendation that pregnant women and families with children under two years of age who were living adjacent to the Canal move from their homes.

In light of the organized public response to these recommendations, less than a week later the Governor of New York promised that the State would purchase all of the houses in the ring closest to the Canal; a total of almost 240 homes. A few months later remedial action was begun to reduce the threat of exposure from the canal to the remaining residents in the area. However, progress was slow and in 1979, the State Health Commissioner recommended that pregnant women and families with children under the age of 2, living in the ring of houses beyond those already vacated, temporarily relocate. In the face of continuing problems in controlling the chemicals in the Canal, a few months later over 400 additional residents were temporarily relocated.

The concerns of the residents intensified in May, 1980 when the EPA announced that a study had found chromosome damage in 11 of 36 Love Canal residents tested. This study had serious scientific flaws but was widely publicized and became the focus of deep debate. The results of the chromosome study, as well as those from the studies of fetal outcomes, were evaluated by an expert panel which was established by the Governor of New York soon after the release of the chromosome damage reports. This expert group, known as the Thomas panel, came to the conclusion that no acute health effects related to exposure to the hazardous wastes had been established and that studies of chronic effects were inconclusive. Not surprisingly, the reactions to this report were mixed and reflected the positions staked out by the various actors in the Love Canal story, including a number of scientists.

Despite these findings, in October, 1980, President Carter signed a federal-state agreement for the purchase of the homes located in a ring just outside the homes that had previously been purchased by the State of New York. In December, 1980, the Superfund Act was signed into law and subsequently applied to Love Canal and thousands of other sites across the country. As part of the clean-up of the Love Canal site, the homes in the innermost ring closest to the landfill were razed and studies were conducted to decide on the habitability of the outer ring homes. The conclusion was that they were habitable and an authority was set up to rehabilitate and sell them. By 1996, almost all of these homes were sold and the neighborhood was renamed Black Creek Village.

Summary

From a societal perspective, probably the most important outcome of Love Canal was that it led to the enactment of national and state legislation, especially the federal Superfund Act, to deal with hazardous wastes that had been placed in the environment before stricter standards for waste disposal went into effect in the mid-1970s. The events at Love Canal were important because they set the tone for subsequent actions that were taken at contaminated sites around the country; in particular, these events revealed the potent role that citizen activism could play.

From a toxicological viewpoint, Love Canal illustrated the difficulties in assessing the relationship between chemical exposures and human health effects in an atmosphere of public activism and contentious policy debate. Instead of well-designed and peerreviewed studies forming the scientific basis for assessing and managing the potential risks, preliminary and poorly designed studies served this purpose. In addition, data that might have been valuable in conducting better studies were lost in the heat of the events. Unfortunately, this pattern seems common as can be seen in looking at other notorious contamination incidents; for example, Times Beach, Missouri.

Love Canal also contributed strongly to the development of risk assessment methodologies since the Superfund Act required that determinations of clean-up levels be made based on a scientific assessment of data on the toxicity of contaminants found at the hazardous waste sites and the potential for exposure to those contaminants. In light of the limitations in available knowledge, new approaches had to be devised to answer the question of 'how clean is clean?' The methodology that was adopted married scientific data to value judgments about acceptable risk and margins of safety. This approach remains in use.

See also: Comprehensive Environmental Response, Compensation, and Liability Act, US; Silent Spring; Valley of the Drums.

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Lowest-Observed-Adverse-Effect Level See Levels of Effect in Toxicological Assessment

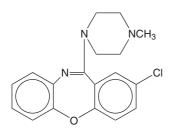
Lowest-Observed-Effect Level See Levels of Effect in Toxicological Assessment

Loxapine

F Lee Cantrell

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 27833-64-3
- SYNONYMS: Loxapine hydrochloride; Loxapine succinate; 2-Chloro-11-(4-methylpiperazin-1-yl)dibenz[1,4]oxazepine; Oxilapine; Loxitane
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Dibenzoxazepine antipsychotic
- CHEMICAL FORMULA: C₁₈H₁₈ClN₃O
- CHEMICAL STRUCTURE:



Uses

Loxapine is used to treat and control the psychotic symptoms of both acute and chronic schizophrenia. Other uses include treatment of dementia, anxiety neurosis, hostile/aggressive behavior, and psychotic depression.

Exposure Routes and Pathways

Loxapine is available in oral liquid, oral capsule, and injectable dosage forms. The principal exposure pathway is intentional ingestion by adults or accidental ingestion by children.

Toxicokinetics

Loxapine is readily but incompletely absorbed. Due to first-pass metabolism, oral bioavailability is 30% less than bioavailability after intramuscular injection. Peak blood levels occur 1 or 2 h after oral administration and 5 h after intramuscular injection. Loxapine is extensively metabolized in the liver through aromatic hydroxylation, *N*-demethylation, or *N*-oxidation. The metabolite amoxapine is active and marketed as an antidepressant. Loxapine is widely distributed throughout the body, including the central nervous system. The main metabolites are excreted both in the urine and feces, and 50% of a single oral dose is eliminated within 24 h. The mean half-life of oral loxapine is 4 h; the mean half-life of loxapine administered through intramuscular injection is 12 h.

Mechanism of Toxicity

The exact mechanism of action of loxapine is not known. It is thought to change the level of excitability in subcortical inhibitory areas of the brain by reducing the firing threshold of some polysynaptic neurons leading to seizure activity. It also appears to possess significant andrenergic and cholinergic blocking properties in overdose.

Acute and Short-Term Toxicity (or Exposure)

Animal

No specific information is available. Signs of toxicity are expected to include sedation, dullness, hypotension, respiratory depression, anorexia, colic, weakness, fever, icterus, restlessness, and seizures. Treatment consists of aggressive supportive care and gastric decontamination.

Human

Clinical signs of toxicity most frequently seen include sedation, coma, seizures, extrapyramidal effects, and rarely hypotension and cardiac arrhythmias. Coma and seizures may develop rapidly following an exposure to loxapine. Cardiac effects include prolonged QRS, Q–T intervals, and mild hypotension; however, the cardiac effects are less pronounced than those associated with tricyclic antidepressants. Anticholinergic effects, including dry mouth, blurred vision, and tachycardia, have been seen. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication. Hypokalemia has also been noted.

Chronic Toxicity (or Exposure)

Human

Adverse reactions following therapeutic use include sedation, dizziness, insomnia, agitation, tardive dyskinesia, dysphoria, dystonic reactions, tachycardia, syncope, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia. The most frequently reported dystonic reactions include akathisia, stiff neck, stiff or protruding tongue, and tremor.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. There is no antidote for loxapine exposure. In patients presenting within 1 h of ingestion, activated charcoal should be administered. Benzodiazepines are the drug of choice for seizures. Initial treatment of conduction disturbances should include electrolyte normalization and intravenous sodium bicarbonate. Antidysrhythmic class 1A agents should be avoided. Dystonic reactions respond well to intravenous benztropine or diphenhydramine. Oral therapy with diphenhydramine or benztropine should be continued for 2 days to prevent recurrence of the dystonic reaction. For patients with neuroleptic malignant syndrome, rapid external cooling, aggressive muscle relaxation with benzodiazepines or nondepolarizing neuromuscular blocking agents, and quality supportive care is the mainstay of therapy. Bromocriptine has been used in conjunction with other supportive measures. Hemodialysis and hemoperfusion have not been shown to be effective.

See also: Benzodiazepines; Diphenhydramine.

Further Reading

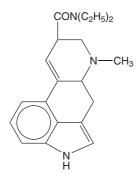
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LSD (Lysergic Acid Diethylamide)

Christopher P Holstege

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 50-37-3
- SYNONYMS: 9,10-Didehydro-N,N-diethyl-6-methylergoline-8b-carboxamide; Acid; Beast; Ben; Blotter; Blue caps; Blue drops; Brain buster; Brown caps; Cubes; Face melter; Ghost; Green caps; Hawk; Heavenly blue; Microdots; Paper acid; Pearly gates; Pink drops; Purple haze; Purple wedges; Royal Blue; Sunshine; Wedding bells; White lightning; Window Pane; Yellow caps; Yellow drops
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Lysergamides
- CHEMICAL STRUCTURE:



Uses

Lysergic acid diethylamide (LSD) is an illicit drug abused as a hallucinogen.

Exposure Routes and Pathways

The most common route of exposure is oral ingestion. Nasal insufflation or intravenous injection is also utilized.

Toxicokinetics

The absorption of LSD is described as rapid, with clinical effects within 15 min and peak concentrations within 60 min after ingestion. Protein binding is greater than 80% and the volume of distribution is 0.31 kg^{-1} . LSD is metabolized to inactive metabolites with less than 1% excreted unchanged. The drug penetrates into the central nervous system (CNS), concentrating in the visual brain areas and the reticular activating systems. The elimination half-life is $\sim 2-5$ h.

Mechanism of Toxicity

The mechanism of action of LSD is incompletely understood. LSD's hallucinogenic effects are secondary to its ability to increase central serotonin activity. LSD also stimulates both D_1 and D_2 dopamine receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats exposed to large doses of LSD have developed respiratory failure. Rabbits have also developed hyperthermia when large doses are administered.

Human

LSD produces distortion in perception. These changes in perception include sight, time, touch, odor, hearing, and sensation of body movement and image. These are usually identified by the intoxicated person as not real occurrences and may be considered pseudohallucinations. True hallucinations, which the individual believes are real, are less common. Other possible CNS effects include depersonalization, decreased ability to think and make judgments, and changes in mood and behavior. Patients are generally quiet and withdrawn, though aggression and bizarre behavior can occur. Acute panic attacks may occur, especially with unexpected use and less experienced users. Acute psychotic reactions can also occur. Seizures, hyperthermia, rhabdomyolysis, hypertension, hyperreflexia, tremors, anisocoria, hippus, and coma are associated with more severe LSD intoxication. Serotonin syndrome may occur.

Chronic Toxicity (or Exposure)

Animal

LSD has been studied as a model for schizophrenia in rats.

Human

Chronic toxic effects include flashbacks and psychosis. Flashbacks are the recurrence of the CNS changes associated with acute LSD use, which can occur up to 4 years after last use. Flashbacks occur in 15–77% of persons who use LSD and may be memory recall of the acute intoxication. Both mental and physiological stresses can precipitate flashbacks. LSD may cause chromosomal aberrations and increased risk of congenital abnormalities in the fetus when used during pregnancy.

In Vitro Toxicity Data

Leukocyte culture studies have demonstrated chromosomal breakage when exposed to LSD. Clastogenicity studies have also demonstrated suppression of mitosis. Other studies have shown LSD to have either no or only weakly mutagenic effects.

Clinical Management

The patient's airway, breathing, and circulation should be assessed and supportive care instituted as necessary. Many patients are anxious and respond to reassurance and a quiet, nonthreatening, nonstimulating environment. Benzodiazepines may be necessary for agitation or anxiety. Hyperthermia and seizures should be managed using standard therapy, a cool mist spray and fans for hyperthermia, and a benzodiazepine for seizures. Psychosis may require treatment with haloperidol.

See also: Benzodiazepines; Drugs of Abuse.

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Lubricating Oil See Oil, Lubricating

Lye

Samantha E Gad

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- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBER: CAS 1310-73-2 (Sodium hydroxide); CAS 1310-58-3 (Potassium hydroxide)
- SYNONYMS: Caustic potash; Caustic soda
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Alkali

Uses

Lye is used in household drain cleaners, ammonia, automatic dishwasher detergents, Clinitest tablets, oven cleaners, and bleaches. It is also used in the manufacture of soaps and cleaners and in chemical synthesis.

Background Information

Lye generically refers to any strong alkali, usually sodium hydroxide or potassium hydroxide.

Exposure Routes and Pathways

Exposure to lye may occur via dermal, inhalation (as a mist or spray), or oral routes.

Mechanism of Toxicity

The mechanisms of toxicity for lye are those common to alkalis, saponification of fats and solubilization of proteins, corrosion, reduction, and protein denaturation. The severity of corrosion is determined by pH, viscosity, concentration, volume ingested, and contact time.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of lye is essentially the same as of household bleaches. Lye has been shown to be a severe dermal and eye irritant.

Human

Lye is a strong eye, skin, and mucous membrane irritant and corrosive. Ingestion is followed by severe pain, vomiting, diarrhea, and collapse.

Clinical Management

Alkalis penetrate the skin slowly, making the extent of damage dependent on the duration of contact. Affected skin should be washed with running water until free of alkali as indicated by the disappearance of the 'soapy' feeling.

In cases of eye exposure, eyes should be washed with running water continuously for 15 min and then irrigated for 30–60 min with normal saline solution. Neutralizing agents should not be used.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists has a ceiling limit of 2.0 mg m⁻³ and the US Occupational Safety and Health Administration has a permissible exposure limit (PEL), 8 h time-weighted average of 2.0 mg m⁻³. In addition, the US National Institute for Occupational Safety and Health recommends a 15 min ceiling value of 2.0 mg m⁻³ and an 'immediately dangerous to life or health' value of 10 mg m^{-3} . Other occupational permissible levels include Australia (2.0 mg m⁻¹³ peak limit), Federal Republic of Germany (2.0 mg m⁻¹³ short-term level, and 4.0 mg m⁻³ ceiling limit), and the United Kingdom (10 min short-term exposure limit (STEL) of 2.0 mg m⁻³.

See also: Alkalies; Corrosives.

Further Reading

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Relevant Websites

- http://www.inchem.org Alkalis. Poisons Information Monograph from the International Programme on Chemical Safety (IPCS INCHEM) System.
- http://hpd.nlm.nih.gov US National Library of Medicine, Household Products Database (search for sodium hydroxide and potassium hydroxide).

Lyme Disease

Michael A Kamrin

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Introduction

Lyme disease is named after Lyme, Connecticut, where it was first identified in the late 1970s as the result of an investigation of a cluster of children with arthritis. The disease has now been found nationwide although it is mostly localized to the northeast, mid-Atlantic, and upper north-central regions of the United States. However, it has also been found in several northwestern counties of California. In 1999, over 16 000 cases were reported to the Centers for Disease Control and Prevention and over 90% of these occurred in northeastern, mid-Atlantic, and north-central states. The State of New York had the greatest number of cases from 1989 to 1998 while the incidence was highest in Connecticut.

In the early 1980s, the vector for the disease – blacklegged ticks – and the causative agent – the bacterium *Borrelia burgdorferi* – were identified. These ticks, also known as deer ticks, are much smaller than ticks commonly found in pets and farm animals. Most often, they become infected after feeding on rodents, which serve as reservoirs for the *Borrelia* bacteria.

Lyme Disease

In general, the incubation period in humans after tick exposure is 7–14 days. The characteristic symptom seen in most infected individuals is a red, slowly expanding, 'bulls-eye' rash. This is accompanied by general malaise, fever, headache, muscle aches, and joint pain. If the infection is not treated, the exposed individual may develop arthritis, neurological symptoms; e.g., facial palsy, nerve, and/or brain inflammation; and, rarely, cardiac abnormalities.

Diagnosis is usually based on clinical signs and known, or putative, exposure. Serological testing may be performed to support the diagnosis and also to assess the severity of the disease. However, because antibodies may persist for months or years after successful treatment, serological endpoints cannot be used as markers of active disease.

Early disease is treated with antibiotics, such as doxycycline or amoxicillin, over a 3–4 week period. If the disease has progressed significantly, it may be necessary to administer intravenous antibiotics for four or more weeks. In some cases, there may be relapses and retreatment may be necessary. In a limited number of cases, Lyme disease may lead to chronic, disabling effects. Even more rarely, it may be fatal.

While Lyme disease is treatable, it can cause serious health effects and the best way to avoid these is prevention - minimizing the possibility of being infected by the deer tick. There are several ways to accomplish this. First is to recognize those areas that are likely to be tick infested and avoid them as much as possible. A second step is to use insect repellents, such as DEET, to discourage ticks from attaching to skin. Third is to understand that clothing can be an effective barrier if it is worn properly; e.g., pants tucked into socks or high rubber boots. A fourth and very important step is to check for ticks after being in areas where exposure is likely. The checks should be done soon after every possible exposure since it generally takes \sim 36 h after a tick bite for infection to occur. Embedded ticks should be removed with tweezers and the area cleansed with an antiseptic.

In the mid-1990s, an effective vaccine against Lyme disease was developed. However, soon after it was brought to market there were claims that the vaccine caused serious side effects including a form of arthritis. While the merits of this claim were debated, sales of the vaccine decreased significantly and the manufacturer withdrew it from the market in 2002. Research into a new Lyme disease vaccine is continuing and a new vaccine may be in the market in the near future.

It has also been suggested that antibiotics be administered prophylactically to individuals who think they might have been exposed to the infectious agent. However, there is one serious drawback to this approach; it would likely expose a large number of individuals to antibiotics unnecessarily. This, in turn, could contribute to the development of antibiotic resistant organisms and thus compromise the health of others who depend on these same drugs. On balance, it appears that the risks of routine use of antibiotics outweigh the benefits.

Summary

Lyme disease is a serious problem in certain areas of the United States and can lead to serious long-term effects. However, preventive steps can significantly reduce the risks and the disease can be treated effectively, especially when detected early. No vaccine against Lyme disease is currently available but researchers are working at filling this gap.

See also: DEET (Diethyltoluamide); Public Health Service, US.

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Relevant Website

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