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WATER QUALITY CRITERIA FOR HEXACHLOROETHANE

FINAL REPORT

**Kowetha A. Davidson
Patricia S. Hovatter
Robert H. Ross**

March 1988

SUPPORTED BY

**U.S. ARMY MEDICAL
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Fort Detrick, Frederick, MD 21701-5012
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Oak Ridge, TN 37831-6050**

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90 to 95°C in water under aerobic conditions), and adsorption to organic matter and mineral surfaces (50 percent of total sorption in <2 hr with equilibrium reached in 3 days).

Hexachloroethane is slightly more toxic to freshwater fishes than to freshwater invertebrates. Bluegill sunfish and rainbow trout are the most sensitive species, with 96-hr LC₅₀ values ranging from 0.86 to 1.18 mg/L, and Daphnia pulex is the least sensitive species, with a 48-hr EC₅₀ of 13.0 mg/L. The chronic-effect level for fathead minnows is 0.54 mg/L, based on observed reductions in survival, growth, and hatchability in the embryo-larval stages. Freshwater algae are less sensitive to hexachloroethane than other freshwater species, with 96-hr EC₅₀ values for Selenastrum capricornutum of 87 and 93 mg/L, based on chlorophyll *a* content and cell number, respectively. Based on one steady-state bioconcentration factor of 139 for bluegill sunfish, hexachloroethane would be expected to moderately bioconcentrate and to rapidly depurate (half-life < 1 day).

Based on acute data for 14 freshwater species, a freshwater Final Acute Value of 0.8497 mg/L is calculated, resulting in a tentative Criterion Maximum Concentration (CMC) of 0.42 mg/L for hexachloroethane. Available data are insufficient to establish a freshwater Criterion Continuous Concentration (CCC) for hexachloroethane.

In general, saltwater species appear to be more sensitive to hexachloroethane than freshwater species. In static bioassays, the 96-hr LC₅₀ values for sheepshead minnow and mysid shrimp are 2.4 and 0.94 mg/L, respectively. The 96-hr EC₅₀ values for the saltwater algae Skeletonema costatum are 8.57 and 7.75 mg/L, based on chlorophyll *a* content and cell number, respectively. Available data are insufficient to establish either a saltwater CMC or saltwater CCC for hexachloroethane.

Hexachloroethane has low acute toxic effects, with oral LD₅₀ values ranging from >1,000 mg/kg (rabbits) to 7,690 mg/kg (rats). It is rapidly absorbed from the gastrointestinal tract, and most is taken up by fat. Hexachloroethane is metabolized in the liver, and the major metabolite is tetrachloroethylene. Uptake of hexachloroethane by the kidney is sex dependent, with more taken up by the male. The sex difference in uptake is associated with a sex difference in severity of kidney damage, which is the primary nonneoplastic lesion induced by acute, subchronic, and chronic oral exposure. Hexachloroethane is not teratogenic.

Hexachloroethane is not mutagenic in bacteria or in yeast, but it is carcinogenic in B6C3F1 mice, inducing a significant increase in hepatocellular carcinomas in both males and females. Hexachloroethane; however, is not carcinogenic in Osborne-Mendel rats. The USEPA guidelines were used to derive a criterion based on the mouse carcinogenicity data. The concentrations of hexachloroethane in water (associated with consumption of water and fish) corresponding to lifetime risks of 10⁻⁵, 10⁻⁶, and 10⁻⁷ are 13, 1.3, and 0.13 µg/L, respectively, and the concentrations associated with the consumption of fish only (excludes the consumption of water) are 58, 5.8, and 0.58 µg/L, respectively.

**Water Quality Criteria for
Hexachloroethane**

FINAL REPORT

**Kowetha A. Davidson
Patricia S. Hovatter
Robert H. Ross**

**Chemical Effects Information Task Group
Information Research and Analysis Section
Health and Safety Research Division**

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EXECUTIVE SUMMARY

Hexachloroethane is a saturated chlorinated ethane used by the military in white smoke pyrotechnics produced at the Pine Bluff Arsenal (PBA), Arkansas. It is considered a hazardous chemical and is listed by the U.S. Environmental Protection Agency (USEPA) as one of its 126 Priority Pollutants. Evidence of the environmental release of hexachloroethane is substantial; it has been detected in fish tissues and in all environmental media. However, since the installation of the Central Waste Treatment Facilities at the PBA in 1980, hexachloroethane has not been detected in wastewater samples from the arsenal. Using USEPA guidelines, water quality criteria are derived for the protection of aquatic life and its uses and of human health.

Hexachloroethane is thermodynamically stable when released into the atmosphere, with the main sinks being the stratosphere and the oceans. Experimental evidence indicates that hexachloroethane does not persist in surface water and groundwater; however, the residence time in groundwater (330 days) is substantially longer than in surface water. The transport and transformation processes affecting its persistence in surface water, groundwater, and soil are volatilization (half-life of 45 min at 25°C; half-life of 70 hr in a 180 cm-deep body of water), biological degradation, photooxidation (half-life of 93.7 hr at 90 to 95°C in water under aerobic conditions), and adsorption to organic matter and mineral surfaces. Hexachloroethane in water is microbially reduced to tetrachloroethylene under both aerobic (100 percent within 7 days) and anaerobic (92 percent within 14 days) conditions. In soil, hexachloroethane is more rapidly degraded under anaerobic conditions (99 percent in 7 days) than under aerobic conditions (99 percent in 4 weeks). Fifty percent of the total sorption of hexachloroethane in aquifer materials occurs rapidly (within 2 hr), followed by a gradual decline, with equilibrium reached in 3 days.

In fish, hexachloroethane acts as a narcotic, producing lethargy and anesthesia. Ninety-six-hr LC₅₀ values from acute dynamic bioassays with freshwater fishes range from 0.86 to 1.18 mg/L for the most sensitive species, bluegill sunfish and rainbow trout, to 1.52 to 2.36 mg/L for the least sensitive species, channel catfish. Hexachloroethane appears to be slightly less toxic in freshwater invertebrates than in fishes. In static bioassays, the most sensitive species tested is Tanytarsus dissimilis, with 48-hr LC₅₀ values ranging from 1.23 to 1.70 mg/L, and the least sensitive species is Daphnia pulex, with a 48-hr EC₅₀ of 13.0 mg/L. Acute data are available for 14 freshwater species, fulfilling 7 of the required 8 families as described in the USEPA guidelines. Therefore, a Final Acute Value of 0.8497 mg/L is calculated, resulting in a tentative freshwater Criterion Maximum Concentration (CMC) of 0.42 mg/L for hexachloroethane.

In static bioassays with saltwater species, the 96-hr LC₅₀ for sheepshead minnow is 2.4 mg/L, and the 96-hr LC₅₀ for mysid shrimp is 0.94 mg/L. Acute data are available for only two of the required eight families of saltwater species; therefore, a saltwater CMC cannot be calculated.

The survival, growth, and hatchability of embryo-larval fathead minnows are adversely affected at concentrations of hexachloroethane as low as 0.70 mg/L, with no effect observed at 0.41 mg/L. Therefore, the chronic-effect level (maximum acceptable toxicant concentration) for this species is 0.54 mg/L. According to the USEPA guidelines, chronic data are required for three species in order to calculate a Final Chronic Value (FCV).

Freshwater algae are significantly less sensitive to hexachloroethane than freshwater vertebrate or invertebrate species. The 96-hr EC₅₀ values for Selenastrum capricornutum are 87 and 93 mg/L, based on chlorophyll *a* content and cell number, respectively. Therefore, the freshwater Final Plant Value (FPV) is established as 87 mg/L, the lowest test result with a freshwater algal species. Hexachloroethane is significantly more toxic to saltwater algae than to freshwater algae. The 96-hr EC₅₀ values for Skeletonema costatum are 8.57 and 7.75 mg/L, based on chlorophyll *a* content and cell number, respectively. Therefore, the saltwater FPV is established as 7.75 mg/L, the lowest test result with a saltwater algal species.

Based on one acceptable steady-state bioconcentration factor of 139 for bluegill sunfish, hexachloroethane would be expected to moderately bioconcentrate and rapidly depurate (half-life < 1 day). Data from two other studies indicate that bioconcentration may be greater in other species and possibly correlated with total lipid content. Additional studies are needed to clearly define the bioconcentration potential of hexachloroethane and to establish a maximum permissible tissue concentration. Therefore, a Final Residue Value (FRV) cannot be calculated.

According to the USEPA guidelines, the Criterion Continuous Concentration (CCC) is equal to the lowest of the FCV, FPV, or FRV; consequently, available data are insufficient to establish either a freshwater or saltwater CCC for hexachloroethane.

In mammalian systems, hexachloroethane is rapidly absorbed from the gastrointestinal tract. The majority is taken up by fat, but the uptake by the kidney is sex dependent, with more taken up by the male. The sex difference is reflected in a sex difference in severity of kidney damage induced by acute, subchronic, and chronic oral exposure. Hexachloroethane is metabolized by reductive dechlorination reaction involving cytochrome P-450, the transfer of two electrons, and the loss of a second chlorine ion by β -elimination to form tetrachloroethylene as the major metabolite.

Hexachloroethane has low acute toxic effects; the oral LD₅₀ values are >1,000 mg/kg for rabbits, 4,970 mg/kg for guinea pigs, and 4,460 to 7,690 mg/kg for rats. Acute clinical signs of toxicity include loss of appetite, mild diarrhea, weakness, reeling, drowsiness, tremors, ataxia, convulsions, narcotization, prostration, and possibly death. The primary nonneoplastic target of hexachloroethane after acute, subchronic, and chronic oral exposure is the kidney. Kidney damage is more severe in males than in females, and has been observed in several animal species including cattle, rabbits, rats, and mice. The lesions are characterized

by degeneration and necrosis of the renal tubules. Hexachloroethane is not teratogenic.

Hexachloroethane is not mutagenic in bacteria or in yeast, but it is carcinogenic in B6C3F1 mice at doses of 590 and 1,179 mg/kg/day [time weighted average (TWA) doses] administered by gavage 5 days per week for 78 weeks. The incidence of hepatocellular carcinomas is significantly increased in both male and female mice. Hexachloroethane; however, is not carcinogenic in Osborne-Mendel rats tested under similar conditions at doses of 212 and 423 mg/kg/day (TWA doses).

The USEPA guidelines were used to derive a criterion based on the mouse carcinogenicity data. The animal carcinogenic potency is $1.6404 \times 10^{-3} \text{ (mg/kg/day)}^{-1}$, and the human carcinogenic potency is $2.1289 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$. The concentrations of hexachloroethane in water (associated with consumption of water and fish) corresponding with lifetime risks of 10^{-5} , 10^{-6} , and 10^{-7} are 13, 1.3, and 0.13 $\mu\text{g/L}$, respectively, and the concentrations associated with consumption of fish only (excludes the consumption of water) are 58, 5.8, and 0.58 $\mu\text{g/L}$. These values are different from those derived by the USEPA, (19, 1.9, and 0.19 $\mu\text{g/L}$ associated with consumption of water and fish and 87.4, 8.74, and 0.874 $\mu\text{g/L}$ associated with consumption of fish only) because, to transform the experimental doses to lifetime doses, the USEPA equated the value for the duration of the study with lifespan of the animals. The transformed doses were larger than those calculated for this report, and; consequently, the USEPA criterion was higher.

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1. INTRODUCTION

Hexachloroethane is a saturated chlorinated aliphatic hydrocarbon (chlorinated ethane). It is used by the military in various pyrotechnics to generate screening smoke. Upon combustion, hexachloroethane mixed with zinc oxide and aluminum produces a dense, white smoke composed primarily of zinc chloride (Kaye 1978, Katz et al. 1980). Zinc chloride is toxic in both aquatic and mammalian organisms (Hill and Wasti 1978); however, its toxicity will not be considered in this document. According to the National Institute for Occupational Safety and Health (NIOSH) (1981), the military use of hexachloroethane is the only confirmed domestic use for this compound. Although hexachloroethane is produced during the manufacture of other chlorinated ethanes, it is usually not commercially distributed from the plant site, but is recycled as feedstock (Santodonato et al. 1985). Hexachloroethane is considered a hazardous chemical and is listed by the U.S. Environmental Protection Agency (USEPA) as one of its 126 Priority Pollutants (USEPA 1987).

The production of pyrotechnics containing hexachloroethane could result in environmental release and exposure of humans. The use of these pyrotechnics in the field could result in limited exposure of humans to hexachloroethane, in environmental contamination, and in production and environmental dispersion of zinc chloride. The objectives of this report are (1) to review the available data regarding the environmental fate, aquatic toxicity, and mammalian toxicity of hexachloroethane and (2) to generate water quality criteria for the protection of aquatic life and its uses and of human health. These criteria are derived using current USEPA guidelines summarized in the appendixes.

1.1 PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of hexachloroethane are listed as follows:

CAS Registry No.:	67-72-1
Chemical name:	Hexachloroethane
Synonyms, trade names:	1,1,1,2,2,2-hexachloroethane, carbon hexachloride, perchloroethane, ethane hexachloride, ethylene hexachloride, hexachloroethylene, Avlothane, Distokal, Distopin, Egitol, Falkitol, Fasciolin, NA 9037 (DOT) (CHEMLINE 1987).
Structural formula:	$\begin{array}{c} \text{Cl} \quad \text{Cl} \\ \quad \\ \text{Cl}-\text{C} \cdots \text{C}-\text{Cl} \\ \quad \\ \text{Cl} \quad \text{Cl} \end{array}$
Molecular formula:	C_2Cl_6
Molecular weight:	236.74 (Weast et al. 1985)
Physical state:	Colorless rhombic (up to 46°C) triclinic (46-71°C) or cubic (above 71°C) crystals (Archer 1979), with camphorlike odor (Hawley 1977, as cited in IARC 1979)
Melting point:	186-187°C (sealed tube) (Weast et al. 1985)
Boiling point:	184.4°C (Dean 1979); 186.0°C (Archer 1979); 186.6°C (triple point) (Windholz et al. 1983)
Density:	2.091 at 20°C (Weast et al. 1985)
Vapor Density:	6.3 g/L at 186.8°C (Sax 1986)
Solubility:	50 mg/L H_2O at 22°C (Dean 1979); 14 mg/L H_2O at 25°C (Spanggord et al. 1985); very soluble in alcohol and ether (Dean 1979); soluble in benzene, chloroform, and oils (Windholz et al. 1983)
Heat of sublimation (kcal/mol):	12.2 (Windholz et al. 1983)
Specific heat, liquid at 25°C (J/(g·°C):	0.728 (Archer 1979)

Latent heat of vapor at boiling point (J/g):	194.1 (Archer 1979)
Heat of combustion (kJ/g):	110.0 at 760 mm Hg and 20°C (Weast et al. 1985)
Vapor pressure:	
25.0°C	0.34 mm H ₂ (Spanggard et al. 1985)
32.7°C (solid)	1 mm Hg
73.5°C (solid)	10 mm Hg
124.2°C (solid)	100 mm Hg
185.6°C (solid)	760 mm Hg (Weast et al. 1985)
Henry's Constant:	6100 L-torr/mole (Spanggard et al. 1985)
Octanol:water partition coefficient K_{ow} :	Log P = 4.62 (Veith et al. 1983a, calculated by fragment constant method of Hansch and Leo 1979) Log P = 3.93 (Veith et al. 1981a, measured using method of Fujita et al. 1964) 8.9×10^3 (Spanggard et al. 1985)
Soil sorption partition coefficient (K_{oc}):	600 (Spanggard et al. 1985)
Soil/air partition coefficient K_h :	18 (Spanggard et al. 1985)
Heat of vaporization (cal/g):	46.0 (Rhodia, Inc. 1978, as cited in Kitchens et al. 1978)
Volatility at 25°C:	770 ppm (Amoore and Hautala 1983)
Reactivity:	Reacts violently with zinc powder in alcoholic solutions; unreactive with aqueous alkalis and acids at moderate temperatures; decomposes to oxalic acid when heated with solid caustics above 200°C or alcoholic alkalis at 100°C (Archer 1979)

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

1.2.1 Manufacturing and Production

Hexachloroethane is produced by the chlorination of tetrachloroethylene in the presence of ferric chloride at 100 to 140°C in a lead-lined vessel (Archer 1979, NIOSH 1981). Dupont uses a method of photochemical chlorination of tetrachloroethylene under pressure and below 60°C [U.S. Pat 2,440,731 (May 4, 1948), J.S. Elliot and E.D. Edwards (to C.C Wakefield and Co. Ltd.), as cited in Archer 1979]. Hexachloroethane is

also formed when tetrachloroethylene is produced by pyrolysis of carbon tetrachloride at 800 to 900°C (Noller 1965, as cited in Dacre et al. 1979).

Hexachloroethane is no longer produced as an end product in the United States and has only minor industrial uses; the domestic needs are met by importation. Hexachloroethane is a by-product in the manufacture of other chloroethanes; it is not sold or distributed from the plant site, but is recycled as feedstock or thermally oxidized (NIOSH 1981, Santodonato et al. 1985). When hexachloroethane is in the gaseous phase at 400 to 500°C, it is thermally cracked, producing tetrachloroethylene, carbon tetrachloride, and chlorine (Archer 1979). A list of producers and importers of hexachloroethane is presented in Table 1.

TABLE 1. MANUFACTURERS AND IMPORTERS OF HEXACHLOROETHANE IN 1977^a

Location		1977 Production
<u>Manufacturers</u>		
Dow Chemical	Pittsburg, CA	0.1 to 1.0 million lb, not distributed
	Plaquemin, LA	1.0 to 10.0 million lb, not distributed
PPG Industries	Lake Charles, LA	1.0 to 10.0 million lb, not distributed
	New Martinsville, WV	10 to 100 thousand lb
Dupont	Ingleside, TX	Confidential, not distributed
<u>Importers</u>		
Hummel Chemical	South Plainfield, NJ	10 to 100 thousand lb
Rhone-Poulenc	Freeport, TX	1 to 10 million lb
ICI Americas	Wilmington, DE	1 to 10 million lb

a. Computer printout of non-confidential production data from TSCA inventory from USEPA in 1980, as cited in Santodonato et al. 1985.

Hexachloroethane is used by the military as a chlorine carrier in screening or obscurant white smoke compositions. The compositions are used in white smoke grenades and markers, which are produced at the Pine Bluff Arsenal, Arkansas (Kitchens et al. 1978). From 1966 to 1977, the average annual use of hexachloroethane at the arsenal was 192,802 lb (Fortner 1978, as cited in Kitchens et al. 1978). The full mobilization use rate of hexachloroethane at the arsenal could be 755,063 lb/month (Kitchens et al. 1978).

The standard hexachloroethane smoke composition used in AN-M8 grenades and MK 3 Mod 0 smoke pots is as follows: 43.53 percent (by weight) hexachloroethane, 46.47 percent zinc oxide, and 9 percent (may vary from 3 to 10 percent) aluminum (McIntyre and Rindner 1980). Other formulations used in screening smokes are as follows: (1) 45.5 percent hexachloroethane, 47.5 percent zinc oxide, and 7.0 percent aluminum (Katz et al. 1980); (2) 46.5 percent hexachloroethane, 48.3 percent zinc oxide, and 5.2 percent aluminum (Katz et al. 1980); and (3) 46.5 percent hexachloroethane, 38.3 percent zinc dust, 6.1 percent ammonium perchlorate, 3.0 percent calcium chlorate, and 6.1 percent ammonium chloride (float smoke pot, MK 1) (NAVWEPS OP 2793, 1963). To prepare the formulation for loading, hexachloroethane and zinc oxide are roughly premixed, then mixed to homogeneity in a hammermill equipped with a discharge screen. The aluminum is added to the mixture and blended in a baffled tumbling mixer (Katz et al. 1980). Other pyrotechnic units containing hexachloroethane include the M4A2, M1, and ABC-M5 smoke pots, M84A1 cartridge, and the M116A1 projectile (Novak et al. 1985).

1.2.2 Analytical Methods

Methods for separating, analyzing, and identifying substances in environmental and other media were not designed specifically for hexachloroethane, but for classes of organic compounds, primarily those chemicals on the USEPA Priority Pollutant list (USEPA 1987). Organic chemicals are first isolated by a procedure that quantitatively extracts or absorbs them from their media. The extracts are usually passed over sodium sulfate to remove residual water and concentrated in a Kuderna-Danish apparatus. Sometimes a cleanup procedure is required to remove interfering substances that are extracted along with the chemicals to be quantitated. The chemical(s) are then separated, identified, and quantitated. Table 2 summarizes the various techniques that have been used to identify and quantitate hexachloroethane in environmental and other types of samples. USEPA methods (USEPA 1985a,b,c) are not included in this list, because these methods are based on classes of chemicals and not on specific sources of the samples.

Procedures for extracting hexachloroethane and other organics from various environmental matrices are modified depending on the characteristic of the matrix (liquids such as wastewater from different sources, semisolids such as municipal or industrial sludges and sediments, or solids such as sand or soil). A widely used extraction method for liquids is liquid-liquid solvent extraction. Otson and Williams (1981) evaluated several solvents that could be used in liquid-liquid extraction techniques. They found that pentane gave the highest extraction efficiency, followed by hexane, hexane saturated with methanol, isooctane, 15 percent acetone in hexane, and benzene. Hexane was regarded as the best solvent because pentane was too volatile. Nevertheless, methylene chloride is the solvent most often used for extraction with and without alkaline and acidic pH adjustments, as in USEPA Methods 612, 625, and 1625 (USEPA 1985a,b,c). Most organic compounds are quantitatively extracted with this solvent.

TABLE 2. ANALYTICAL METHODS FOR HEXACHLOROETHANE AND OTHER ORGANIC COMPOUNDS^a

Sample Source	Extraction Method	Identification/ Quantitation Method	Detection Limit	Recovery	Standard Deviation (%)	Reference
Air	Charcoal absorption, carbon disulfide desorption	GC-FID, PC (3% OV-17 on 100/120 Gas Chrom Q)	5-25 mg/m ³	-	-	NIOSH 1977
Spiked industrial wastes; all types (liquids, solids, semisolids) (50 and 250 µg/g)	<u>Dry-Neutral Extraction</u> Methylene chloride extraction at pH 7.0 with sonification and sodium sulfate added to remove water	GC-FID (screening) GC/MS, FSCC coated with SE-54 (analysis)	1 ppm	70% at low conc, 54% at high conc	16 15	Warner et al. 1983
14 Spiked water (1, 5, 20, 100 µg/L)	<u>Continuous Liquid-Liquid Extraction</u> Hexane extraction, 24 hr	GC, PC (OV-17 SCOT)				Otson and Williams 1981
		FID	10	84, 89%	0.8, 3.7	
		ECD HECD	<1 <1	90-103% -	0.4-38 -	
Spiked water (1, 5, 20, 100 µg/L)	<u>Continuous Liquid-Liquid Extraction</u> Hexane extraction, 24 hr	PC (0.1 % SP-1000)				Otson and Williams 1981
		FID	ND	-	-	
		ECD	<1			
Spiked water	<u>Headspace Analysis</u> Purge-trap, with absorption onto Tenax-GC resin	GC/MS, HECD	1	82%	12	Otson and Williams 1982
		PC (Tenax-GC)				
Spiked National Bureau of Standards sediment	Serial methylene chloride extraction, pH 11 and pH 2, silica gel cleanup	GC/MS,				Lopez-Avila et al. 1983
		FSCC (SE-54)	-	41% (low conc)	15	
		FID, ECD	-	62% (high conc)	5	

TABLE 2. cont'd

Sample Source	Extraction Method	Identification/ Quantitation Method	Detection Limit	Recovery	Standard Deviation ^c (%)	Reference
Spiked secondary POTW sludges (310 µg/L)	Serial methylene chloride extraction, pH 11 and pH 2, GPC cleanup	GC/MS, PC (1% SP-2250 on 100/120 mesh Supelcoport)	-	70% using 80 mL spl	-	Lopez-Avila et al. 1981
Spiked POTW sludge (300 µg/L)	<u>Continuous Liquid-Liquid Extraction</u> : methylene chloride extraction, 10-20 hr, pH 11	GC/MS, FSCC (SE-54), FID, ECD	-	98% using 100 mL spl, 46% using 300 mL spl	-	Lopez-Avila et al. 1981
Spiked water (3.8 µg/L)	Serial methylene chloride extraction, pH 11 and pH 2	GC/MS, PC (3% SP-2250 on 100/120 mesh Supelcoport)	1-10	76% using 1 L spl	23	Eichelberger et al. 1983
Spiked water (10 µg/L)	Serial methylene chloride extraction, pH 7 and pH 2	GC/MS, FSCC (SE-54)	-	55% using 1 L spl	6.2	Eichelberger et al. 1983
Blood	Methanol:hexane (1:6)	GC, PC (5% OV-210)	0.3	98% using 2 mL spl	<5	Reznicek 1983

a. Abbreviations: ECD = electron capture detector; FID = flame ionization detector; FSCC = fused silica capillary column; GC = gas chromatography; GC/MS = gas chromatography/mass spectrometry; GPC = gel permeation chromatography; HECD = Hall electrolytic conductivity detector; PC = packed column; POTW = publicly owned treatment works; SCOT = support-coated open tubular; spl = sample.

Organic compounds in aqueous matrices can also be isolated by passing water over resins that can adsorb the organic compounds. The organic compounds are then desorbed from the resins. Van Rossum and Webb (1978) tested XAD resins (XAD-2, -4, -7, -8, and -4/8) for their efficiency in adsorbing organic compounds from spiked distilled water and found that the mixture XAD-4/8 was the most efficient. They reported that acetone followed by methylene chloride was an efficient eluant that was preferred over solvent systems containing chloroform, because methylene chloride is less toxic. XAD-2 and XAD-4/8 resins were tested for their efficiencies in adsorbing organic compounds, including hexachloroethane, from tapwater. Although both resins adsorbed hexachloroethane, only 66 percent of the hexachloroethane was recovered from XAD-2 and 69 percent from XAD-4/8. Carbon adsorption and Soxhlet extraction were tested and judged to be less effective than XAD-2 or XAD-4/8 resin. Van Rossum and Webb (1978) concluded that, because the XAD resins did not adsorb all materials, the best system may be a combination of both carbon and XAD resins.

Organic compounds can also be isolated by passing water samples over Tenax-GC (35/60 mesh) columns at low temperatures. The compounds are then released from the resin by raising the temperature (thermal desorption) (Pankow et al. 1982). The recovery of hexachloroethane from a 1-L water sample ranged from 62 to 75 percent. A Tenax-GC column can also be used to trap organic compounds (purgeable or semivolatile) in aqueous matrices that are volatilized by bubbling gas through the sample contained in a purging apparatus (purge-trap method) (Otson and Williams 1982).

Warner et al. (1983) described a dry neutral extraction procedure whereby the sample is extracted with methylene chloride at pH 7.0 accompanied by sonication to increase the contact of the solvent with waste particles. This procedure can be used to analyze all types of industrial wastes (water, oil, solvents emulsions, aqueous sludges, oily sludges, tars, soils, and sediments). Lopez-Avila (1981, 1983) described a procedure by which samples, adjusted to a pH >11 then to a pH <2, are serially extracted with methylene chloride to obtain a base/neutral (B/N) and an acidic fraction, respectively, for analysis. This procedure was used to analyze various municipal and industrial waste materials. The extracts usually require a cleanup procedure involving Florisil or silica gel chromatography prior to analysis by SE-54 coated-fused silica capillary column (FSCG) gas chromatography/mass spectrometry (GC/MS).

Harrold and Young (1982) described and evaluated procedures for extracting aqueous environmental samples containing large amounts of solids. The first step in this procedure was to separate the sample into an aqueous and solid fraction by either centrifugation or filtration. The aqueous fraction was extracted by continuous liquid-liquid extraction for 24 hr using methylene chloride with pH adjustments of 11 and 2 to obtain fractions of B/N and acidic compounds, respectively. The efficiency of the continuous liquid-liquid extraction procedure, tested by extracting distilled water spiked with organic pollutants at concentrations of 0.01, 0.10, 1.00, 10.0, or 100.0 mg/L, was 76, 67, 28, 83, and 97 percent, respectively. The low efficiency of extracting the 1.00 mg/L of

hexachloroethane was attributed to unknown systematic error; other compounds showed the same effect.

The solid fraction was processed by Soxhlet extraction for 24 hr using a solvent mixture containing hexane and methylene chloride, followed by a separatory funnel extraction using hexane-methylene chloride at pH 11 and 2 to obtain B/N and acidic fractions, respectively. The effects of particle size, the solvent system, and the length of time of extraction on the efficiency of extraction of organic compounds from solid matrices was tested on organic pollutants (10 and 300 $\mu\text{g/g}$ of solid) absorbed by activated carbon. The efficiency of recovery of hexachloroethane from carbon particles 125 to 1,000 μm in diameter was 82 to 86 percent, whereas the efficiency of recovery from particles less than 100 μm was only 45 percent. The efficiency of recovery of other compounds from the smaller particles was also reduced. The four solvent systems tested for Soxhlet extraction of organic compounds from carbon particles were hexane, benzene, methylene chloride, and hexane-methylene chloride. Benzene was most efficient in extracting hexachloroethane, with an efficiency of 113 percent. The efficiencies of methylene chloride, hexane, and hexane-methylene chloride were 86, 71, and 74 percent, respectively. Methylene chloride, however, was chosen as the preferred solvent because benzene was considered to be too toxic and a fire hazard. The optimum time for extracting hexachloroethane from carbon particles by the Soxhlet procedure was 6 to 24 hr. The efficiency of recovery was reduced when extraction times exceeded 24 hr (Harrold and Young 1982).

The methods by which extracted or isolated organic compounds are identified and quantitated are not dependent on the environmental or biological origin of the sample. The instrumentation for analysis include gas chromatography (GC) and GC/MS. These systems vary depending on the type of the detection system (flame ionization detector, electron capture detector, electrolytic conductivity detector) and the type of chromatography column used in the instruments. GC/MS using FSCC has been evaluated and judged to be a superior procedure over GC/MS using packed columns for accurately identifying and quantitating EPA environmental pollutants (Sauter et al. 1981, Eichelberger et al. 1983).

The USEPA established test procedures for quantitative and qualitative determination of classes of organic compounds that include hexachloroethane (USEPA 1985d). These procedures include Method 612 for chlorinated ethanes (USEPA 1985a), Method 625 for B/N and acid organic compounds (USEPA 1985b), and Method 1625 for semivolatile organic compounds (USEPA 1985c). The extracting solvent, methylene chloride, is the same for all procedures, but Method 612 uses serial extraction without pH adjustments. Method 625 uses serial or continuous extraction with pH adjustments ≥ 11 and < 2 , and Method 1625 uses continuous extraction with adjustment of pH similar to those in Method 625. If cleanup of the extract is necessary for better separation, then Florisil column chromatography is used (Method 612). The extracts are concentrated by use of a Kuderna-Danish apparatus and dried with anhydrous sodium sulfate.

Using Method 612, the extracts are analyzed by packed or capillary column GC with electron capture detection, the identity of substances detected by GC is confirmed by retention times, and the quantity of substances is determined by measurement of peak area (USEPA 1985b). Using Method 625, extracts are analyzed by packed or capillary column GC/MS; organic compounds are identified by retention time and relative abundance of three characteristic masses (m/z) and quantitated by using internal standards with a single characteristic m/z (USEPA 1985b). Using Method 1625, extracts are analyzed by capillary column GC/MS; organic compounds are identified as reported for Method 625 and are quantitated by using isotopically labeled compounds (when available) as internal standards (USEPA 1985c).

The retention time for hexachloroethane using Method 612 is 4.9 min [1.8 m x 2 mm (i.d.) glass column packed with 100/120 mesh Supelcoport coated with 1 percent SP-1000] or 8.3 min [1.8 m x 2 mm (i.d.) glass column packed with 80/100 mesh Supelcoport coated with 1.5 percent OV-1/2.4 percent OV-225]. The method detection limit is 0.03 $\mu\text{g/L}$. For four test measurements, the standard deviation for recovery at a test concentration of 10 $\mu\text{g/L}$ was 3.3 $\mu\text{g/L}$; the range for the average recovery was 2.4 to 12.3 $\mu\text{g/L}$; the range for the percent recovery was 8 to 139 percent (USEPA 1985a).

The retention time for hexachloroethane using Method 625 is 8.4 min, and the method detection limit is 1.6 $\mu\text{g/L}$. For four test measurements at a spike concentration of 100, the standard deviation was 24.5 $\mu\text{g/L}$, the range for the average recovery was 55.2 to 100 $\mu\text{g/L}$, and the range for the percent recovery was 40 to 113 percent (USEPA 1985b). The retention for hexachloroethane using Method 1625 is 13.7 min, and the method detection limit is 10 $\mu\text{g/L}$ (USEPA 1985c).

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

No information was found in the literature concerning the abiotic effects of hexachloroethane.

2.2 ENVIRONMENTAL FATE

2.2.1 Sources and Occurrences

From military uses, hexachloroethane may be released into the environment during manufacture of the chemical, during formulation and loading of the white smoke grenades and markers, or during training and testing operations. Hexachloroethane is also released into the environment as an intermediate or by-product waste during the production of other halogenated hydrocarbons; such as trichloroethylene, perchloroethylene, carbon tetrachloride, and fluorocarbons (USEPA 1981, Kitchens et al. 1978). Diffuse sources of release are ocean dumping, volatilization, and incineration and combustion of polyvinyl chloride wastes (Class and Ballschmiter 1986).

Evidence of the release of hexachloroethane is substantial; it has been detected in fish tissues and in all environmental media: drinking water, industrial and municipal effluents, surface waters, groundwater, aquatic sediments, soils, and air.

Kitchens et al. (1978) reported that, during typical production of pyrotechnic items at the Pine Bluff Arsenal, Arkansas, approximately 1 to 2 percent of the smoke formulation, or an estimated 150 to 300 lb of hexachloroethane per month would be discharged into receiving waters within the area of the arsenal. At full mobilization, 7,550 to 15,000 lb of hexachloroethane would be released per month. Combustion products resulting from detonation of the pyrotechnics can enter the aquatic environment directly as fallout, by runoff, or by leaching from soils, but the impact is usually local in nature, within 10 to 15 km downwind of the site (Cichowicz and Wentzel 1983).

Four main aquatic systems within the arsenal grounds that could receive pyrotechnic discharges drain into the Arkansas River, which fronts the arsenal for approximately 6 miles. Three of the aquatic systems originate on the installation. They are Triplett Creek, Yellow Creek (with associated drainages), and McGregor Reach. The fourth, Eastwood Bayou, originates off the installation. There is also a water table aquifer within the arsenal grounds. The pyrotechnic complex is located just southwest of Yellow Lake (Kitchens et al. 1978).

Prior to 1980, contamination from pyrotechnic residues and smoke mixtures was reported in Yellow Lake and within a munitions test area on

the Arkansas River (Pinkham et al. 1977, as cited in Kitchens et al. 1978). At this time the estimated average wastestream concentration of hexachloroethane was 168.0 mg/L (USAEHA 1973, 1974, as cited in Kitchens et al. 1978).

The waste loading was reduced in 1980 with the installation of the Central Waste Treatment Facilities at the arsenal. Fortner et al. (1983) reported that hexachloroethane was not detected in wastewater samples from five areas of the pollution abatement facility (central waste, filter backwash sludge, incinerator clearwell sludge, chain grate ash, and fuse residue from the rotary kiln). The wastewater is monitored chemically and biologically by static 96-hr bioassays with bluegill sunfish and by continuous monitoring procedures. Modernization of the waste treatment facility is currently under way with the implementation of granular activated carbon column treatment, which will control color, total organic carbon, and aquatic toxicity of the effluent (Fortner et al. 1983).

Shackelford and Keith (1976) reported the occurrence of hexachloroethane in eight samples of finished drinking water and in the effluent from a chlorinated sewage treatment plant in the United States. Suffet and Radzuil (1976) identified hexachloroethane in the Torresdale drinking water for Philadelphia, which comes from the tidal portion of the Delaware River. Hexachloroethane has been detected in drinking water in 4 of 13 U.S. cities at concentrations of 0.03 to 4.3 $\mu\text{g/L}$ (Keith et al. 1976, as cited in IARC 1979). Eurocop-Cost (1976, as cited in IARC 1979) reported that hexachloroethane has been detected in river water and tap water at a concentration of 4.4 $\mu\text{g/L}$ and in the effluent from a U.S. chemical plant at 8.4 $\mu\text{g/L}$. Hexachloroethane was detected 16 times in 4,000 samples of wastewater from industrial and publicly owned treatment works at concentrations ranging from 0.89 to 1,405.62 $\mu\text{g/L}$ (USEPA, ongoing study). Ewing et al. (1977) detected the chemical in two samples of surface waters collected from 2 of 204 sites near 14 heavily industrialized river basins in the United States: Hudson River, New Jersey, at 1.0- $\mu\text{g/L}$ level from mid-channel at low slack tide and Fields Brook, Ohio, at 3.0 $\mu\text{g/L}$ level from midstream. Hexachloroethane has been detected at levels <2.0 $\mu\text{g/L}$ in the Potomac River (Hall et al. 1987). Keith (1976) detected hexachloroethane in effluents from unbleached, treated Kraft paper mills at <1.0 $\mu\text{g/L}$.

Oliver and Kaiser (1986) reported concentrations of hexachloroethane in large-volume water samples from the St. Clair River (United States and Canada) ranging from 0.02 to 1,700 ng/L. The sources of this contamination are effluents from Dow Chemical Company and from Sarnia's Township Ditch, which drains one of Dow's waste disposal sites. Elevated concentrations persist for at least 25 km downstream. Low-level contamination of the watershed is indicated by measurable concentrations in tributaries entering the river. In suspended sediment samples from the St. Clair River, hexachloroethane concentrations ranged from 1.4 to 530 ng/g (Oliver and Kaiser 1986).

Hexachloroethane has been detected in sediments from Liverpool Bay, England, at levels ≤ 1.0 $\mu\text{g/L}$ (Pearson and McConnell 1975) and from Fields

Brook, Ashtabula, Ohio. The Fields Brook site was added to the National Priorities List in September 1983. For approximately 3.5 miles, the main channel flows through an industrial area with one of the largest and most diversified concentrations of chemical plants in Ohio (USEPA 1986). Hexachloroethane was detected in composite fish samples (bluegill, brown bullhead, yellow bullhead, and northern pike) from the Ashtabula River, Ohio, at concentrations of 0.1 mg/kg (DeVault 1985).

Silkworth et al. (1984) found concentrations of 1.0 $\mu\text{g/g}$ of hexachloroethane in soils from the Love Canal chemical dump site in Niagara Falls, New York. In air samples collected from a cage containing soil from Love Canal, the concentration of hexachloroethane ranged from 0.3 to 49.0 $\mu\text{g/m}^3$. The mean concentration was 8.0 $\mu\text{g/m}^3$ in fresh soil and 1.2 $\mu\text{g/m}^3$ in week-old soil.

Hexachloroethane has been detected in the troposphere with the primary source of release occurring from the production of chlorinated C-2 hydrocarbons in the northern hemisphere (NH) (Singh et al. 1980, Class and Ballschmiter 1986). The estimated global emission rate is <1 kton/yr. Class and Ballschmiter (1987) conducted studies to determine the tropospheric burden of some halocarbons, including hexachloroethane. Air samples were collected from sites in the North and South Atlantic Ocean and the Indian Ocean, far from anthropogenic sources. The average concentration of hexachloroethane in the NH was 0.17 ± 0.05 pptv (parts per trillion by volume), and the mean concentration in the southern hemisphere (SH) was 0.25 ± 0.05 pptv.

2.2.2 Transport Processes

2.2.2.1 Atmospheric transport

Pearson and McConnell (1975) theorized that the presence of organochlorine compounds in upland waters resulted from atmospheric transport and the subsequent precipitation from the atmosphere by rainfall and dewlike condensation. Callahan et al. (1979) stated that hexachloroethane released into the atmosphere would be expected to behave as its analogue, tetrachloromethane. Tetrachloromethane is stable in the troposphere because there are no functional groups present on the compound to react strongly with hydroxyl radicals.

Hexachloroethane is thermodynamically stable; therefore, the atmospheric half-life is long enough to allow interhemispheric exchange (Class and Ballschmiter 1986). Class and Ballschmiter (1987) reported an atmospheric burden of 4.7 kton in the NH and 4.2 kton in the SH, with a net flow from NH to SH of 0.45 kton/yr. No degradation by hydroxyl radicals is expected; therefore, a very long tropospheric lifetime is expected with the main sinks being the stratosphere and the oceans. At a transfer rate of 0.03/yr, transfer from the northern troposphere to the stratosphere would be approximately 0.14 kton/yr. Based on the water/air partition coefficient, the maximum expected flux into northern oceans would be

0.4 kton/yr. Therefore, the sum of the transport processes within the atmosphere (0.45 kton/yr and 0.14 kton/yr) and between the atmosphere and the oceans (0.4 kton/yr) is approximately 1 kton/yr (Class and Ball-schmitter 1987).

2.2.2.2 Surface water and groundwater transport

Dilling et al. (1975) conducted studies to determine the evaporation rates for several organic compounds, including hexachloroethane. The experimental half-life of 1.0 mg/L of hexachloroethane in water was determined using the hollow fiber-mass spectroscopic method. The experimental conditions were as follows: 200 mL of test solution at 25°C in a sealed 250 mL Erlenmeyer flask, 200 rpm stirring, still air <0.2 mph. Mass spectra were scanned after 1 min and periodically thereafter. The evaporative half-life of hexachloroethane was 45 min, with removal of 90 percent requiring >120 min. Using the same method, Dilling (1977) determined an average evaporative half-life of 40.7 min for 0.72 mg/L of hexachloroethane in stirred water. The evaporation rate followed first-order kinetics for the first two to five half-lives. This value is in close agreement with the 38-min half-life predicted by the theoretical model of Mackay and Leinonen (1975, as cited in Dilling 1977); however, it is quite different from the 4-min half-life determined by the model of Mackay and Wolkoff (1973, as cited in Dilling 1977). This variation could possibly be explained by differences in temperature or other experimental variables, such as stirring rate or air currents.

Using the equation given below, Spanggord et al. (1985) determined a volatilization rate constant (k_v) for hexachloroethane based on the liquid film mass-transfer coefficient (k_l) and depth in cm (L), which equals the liquid volume (F) divided by the interfacial area (A).

$$k_v = \frac{k_l}{L}$$

Spanggord et al. (1985) state that the k_l for lakes and ponds is of the order of 2 cm/hr; therefore, for a water body that is 180 cm deep, the first-order volatilization rate constant for hexachloroethane would be 0.01/hr with an estimated half-life of 70 hr.

Several investigators from Stanford University, California, and the University of Waterloo, Canada, conducted a large-scale field experiment to study the groundwater transport of five halogenated organic chemicals, including hexachloroethane, at a site in Borden, Ontario, Canada (Mackay et al. 1986). The site was a relatively uncontaminated portion of an unconfined glaciofluvial sand aquifer located above an existing landfill leachate plume. The groundwater was aerobic in some areas, but the dissolved oxygen (DO) content varied throughout from 0.0 to 8.5 mg/L. The organic carbon content (0.02 percent) of the groundwater was very low. An

equal and uniform flow of 12 m^3 of solution was injected into nine wells. The solution comprised two inorganic solutes, 892.0 mg/L of chloride ion and 324.0 mg/L of bromide ion, which served as tracers, and five organic solutes: 0.032 mg/L of bromoform, 0.031 mg/L of carbon tetrachloride, 0.030 mg/L of tetrachloroethylene, 0.332 mg/L of 1,2-dichlorobenzene, and 0.020 mg/L of hexachloroethane. The solutes migrated under the influence of the natural hydraulic gradient, with an average linear groundwater velocity of approximately 0.09 m/day. Hexachloroethane was the least water soluble compound and was expected to exhibit the lowest mobility. The solute plume was traced for approximately two years, and the solutes were measured by ion and gas chromatographic techniques. A dense, three-dimensional network of multilevel sampling wells was established to obtain "snapshots" of the spatial distribution of concentrations of the chemicals at particular points in time (synoptic sampling). Time-series data were also collected to determine solute concentration at a high sampling frequency for a few sampling points (breakthrough sampling). A total of 19,900 samples have been collected from 20 synoptic monitoring sessions and time-series sampling from 12 sampling points (Mackay et al. 1986).

In order to understand the long-term behavior of the organic solutes during transport, Roberts et al. (1986) determined mass balances and retardation estimates for the chemicals. Moment calculations were used to estimate the mass in solution and the position of the center of the mass. Concentrations of hexachloroethane decreased below the detection limit by 330 days, the eighth sampling time. The center of mass of hexachloroethane moved a total of 1.35 m from the center of the injection zone. The position of the center of mass could be quantified only up to 85 days due to the disappearance of hexachloroethane from solution. The disappearance was approximated as a first-order rate process with a rate constant of 0.02/day. This temporal decline in total mass was interpreted as evidence of the transformation of hexachloroethane. The retardation factor (the average travel time relative to that of chloride), which ranged from 1.2 to 4.4 for hexachloroethane, indicated that hexachloroethane was the most strongly retarded compound during the comparable time period $16 < t < 85$ days. In this study, the greatest retardation factors were observed for the two most hydrophobic compounds, 1,2-dichlorobenzene and hexachloroethane. The authors suggested that the observed retardation was caused by sorption. The transformation and sorption of hexachloroethane in Borden aquifer material were further investigated by Criddle et al. (1986) and Curtis et al. (1986), respectively (see Sects. 2.2.3 and 2.2.5).

2.2.3 Biological Degradation

Tabak et al. (1981) conducted studies to determine the biodegradability of USEPA-designated organic priority pollutants, including hexachloroethane. The static-culture, flask-screening method was used, in which BOD water containing 5 and 10 mg/L concentrations of hexachloroethane was inoculated with 5 mg/L of yeast extract and 10 mL of settled domestic wastewater. The cultures were incubated in the dark at 25°C for a 7-day static period, followed by three weekly subcultures, giving a total of 28 days of incubation. The samples were analyzed for degradation

products by GC using a flame ionization detector; total organic carbon was analyzed to determine loss of substrate from volatilization. At both concentrations, 100 percent of the hexachloroethane was degraded within 7 days in the original culture and in each of the three subcultures, with 0 percent loss from volatilization in 10 days under refrigeration at 25°C. Therefore, hexachloroethane exhibited significant degradative activity with rapid adaptation of the microbiota.

Criddle et al. (1986) conducted two laboratory experiments to evaluate the biotransformation of hexachloroethane in groundwater at the Borden site, Ontario, Canada. In the first experiment, a standard biological oxygen demand (BOD) microcosm study, BOD dilution water was inoculated with settled municipal wastewater seed, aerated to a DO of 8.3 mg/L, and spiked with 6.5 µg/L of hexachloroethane in methanol. Triplicate samples were analyzed on days 0, 10, and 20, and duplicate samples were analyzed on days 30, 40, and 60 by GC using an electron capture detector. In the second experiment, Borden aquifer material (1.5 to 3 m depth) was used instead of wastewater for the microbial source, and Borden site groundwater was used instead of BOD water. The groundwater was spiked with approximately 50 µg/L of hexachloroethane in methanol. Aerobic conditions were not guaranteed. Samples were analyzed on days 10, 17, 24, 38, 52, and 66 by GC using an electron capture detector. In both experiments, bottles were stored upside down in the dark at 20°C. Results of the BOD microcosm study indicated that tetrachloroethylene was formed as hexachloroethane disappeared; therefore, microbial reduction under aerobic conditions was occurring. The authors stated that the reaction was a classic example of co-metabolism where the product is structurally similar to the reactant and is brought about by enzymes of broad specificity.

In the Borden microcosm study (Criddle et al. 1986), hexachloroethane was converted to tetrachloroethylene in some samples and not in others, which may be related to the heterogeneous distribution of the active agents in the aquifer material. In all of the samples, the sum of hexachloroethane and tetrachloroethylene decreased over time, probably due to the slow increase in sorption with time (consistent with Roberts et al. 1986). Although tetrachloroethylene was one of the five contaminants injected into the aquifer, Roberts et al. (1986) did observe a statistically significant excess mass of this chemical that would be consistent with the transformation of hexachloroethane to tetrachloroethylene. The authors concluded that hexachloroethane was transformed (enzymatically mediated) under aerobic conditions by microorganisms. Hexachloroethane in the presence of Borden aquifer materials was transformed by microbes and/or abiotic agents. Both aerobic and anoxic conditions existed in the materials, indicating the possible occurrence of anaerobic transformation.

Spanggard et al. (1985) conducted studies to investigate the biodegradation of hexachloroethane in natural surface waters under aerobic and anaerobic conditions. The natural waters used in these studies were collected from Searsville Pond, Woodside, California and Coyote Creek, San Jose, California. In the aerobic study, hexachloroethane (50 mg/mL) was added to bottles containing the natural water with either bottom sediment

or yeast extract plus glucose (nonsterile) and incubated in the dark at 25°C. The bottles containing pond water were not sealed or shaken; whereas, the bottles containing creek water were sealed and shaken. For the anaerobic study, the solutions were placed in Teflon-lined, screw-capped bottles and incubated in an anaerobic chamber. Autoclaved pond or creek water was used as sterile controls.

In both sterile and nonsterile aerobic waters, Spanggord et al. (1985) determined that 80 to 95 percent of the hexachloroethane was lost in 6 days in the non-sealed bottles, indicating that volatilization was probably a competitive process. In the sealed bottles containing aerobic waters with yeast extract plus glucose, 60 percent of the hexachloroethane was lost after 14 days; whereas, 38 percent was lost in the sterile waters, indicating that volatilization was still a significant factor. In the anaerobic studies, hexachloroethane was not lost in the sterile waters, indicating that volatilization was not occurring. However, in the nonsterile waters with either sediment or yeast extract plus glucose, 90 percent of the hexachloroethane was lost in 18 days in the pond water and 92 percent was lost in 14 days in the creek water. The metabolites were tentatively identified by GC as pentachloroethane, tetrachloroethylene, and trichloroethylene.

To confirm the microbial transformation of hexachloroethane in natural waters, Spanggord et al. (1985) inoculated media containing 10 ppm of hexachloroethane, glucose, and yeast extract with pond microorganisms under both aerobic and anaerobic conditions. Under both conditions, metabolites were observed as hexachloroethane concentrations decreased from; to, thereby confirming the initial results. Spanggord et al. (1985) concluded that the aquatic biotransformation of hexachloroethane is nutrient-dependent, faster under anaerobic than aerobic conditions, and competitive with volatilization under aerobic conditions.

Spanggord et al. (1985) also studied the biodegradation of hexachloroethane in soil under aerobic and anaerobic conditions. For each test, hexachloroethane was added to 20 g of either autoclaved or non-sterile soil to obtain a final concentration of 5 ppm of hexachloroethane. The aerobic studies were performed in both nonsealed and sealed flasks. In the anaerobic study, the test tubes were incubated in an anaerobic chamber. In the nonsealed aerobic flasks, 80 percent of the hexachloroethane was lost from the nonsterile soil and 64 percent was lost from the sterile soil after 7 days. In the sealed aerobic flasks, 99 percent of the hexachloroethane in the nonsterile soil was lost after 4 weeks of incubation and 17 percent was lost in the sterile soil after 4 weeks of incubation. After 7 days under anaerobic conditions, 99 percent of the hexachloroethane was lost from the nonsterile soil and 34 percent was lost from the sterile soil. Consequently, Spanggord et al. (1985) concluded that hexachloroethane is biotransformed rapidly in soils under anaerobic conditions and moderately fast under aerobic conditions without requiring an additional nutrient source.

Jafvert and Wolfe (1987) also observed the degradation of hexachloroethane in anoxic sediment suspensions, fractionated (by particle size)

sediment suspensions, and reconstituted sediment suspensions (dried USEPA 16 sediment resuspended in deionized water, seeded with anoxic sediment and incubated under a nitrogen atmosphere for two weeks).

2.2.4 Chemical and Physical Transformation

The chemical transformation of hexachloroethane is insignificant in determining the fate of the chemical in the environment (Spanggord et al. 1985). Hydrolysis by the hydroxyl radical is extremely slow with a rate constant of 6.3×10^{-6} /M/sec at pH 7 and 25°C (Taylor and Ward 1934, as cited in Spanggord et al. 1985).

Knoevenagel and Himmelreich (1976) studied the degradation of organic compounds by photooxidation (ultraviolet light) in the presence of water. The degradation was estimated by the quantitative determination of carbon dioxide formed. Water-insoluble chemicals, including hexachloroethane, were tested in a dispersed state. The half-life of 237.0 mg of hexachloroethane suspended in 900 mL of water at 90 to 95°C was 93.7 hr. Twenty-five percent was degraded in 25.2 hr, and 75 percent was degraded in 172.0 hr. The authors concluded that the two carbon atoms in hexachloroethane were completely transformed to carbon dioxide and that the degradation velocity depended on how finely the compound was dispersed in water, the temperature, and the wavelength of light. The degradation velocity was slightly delayed by the addition of 69.0 mL of 0.1 N hydrochloric acid, introduced as a catalyst. The half-life for hexachloroethane increased to 111.5 hr, 25 percent was degraded in 29.7 hr, and 75 percent was degraded in 190.3 hr.

Using the free radical spin trapping technique, Davies and Slater (1986) studied the photolysis of hexachloroethane in either toluene or water at room temperature under anaerobic conditions to determine if cleavage of the carbon-carbon bond would occur to give two $\cdot\text{CCl}_3$ radicals. Ultraviolet irradiation of hexachloroethane in toluene in the presence of *N*-tert-butyl- α -phenylnitrone (PBN) resulted in three radicals. Two of the signals indicated that substantial cleavage of the C-Cl bonds occurred; the third signal could have been assigned to either $\cdot\text{CCl}$ or $\cdot\text{CCl}_2\text{CCl}_3$ adducts. A further study was conducted to confirm the identity of the radicals by using 2-methyl-2-nitrosopropane in the spin trap. Results indicated that the PBN-photolysis product was most likely the $\cdot\text{CCl}_2\text{CCl}_3$ adduct, indicating that C-Cl rather than C-C bond cleavage was observed. Single radical species were not detected in aqueous hexachloroethane solutions in the presence of PBN. This is probably attributed to the lower concentration of the substrate and the shorter lifetime of the spin-adducts because of their limited aqueous solubility.

2.2.5 Sorption Processes

Based on a calculated soil/air partitioning coefficient (K_H) of 18 and a measured octanol/water partition coefficient (K_{OW}) of 8.9×10^3 , Spanggord et al. (1985) determined that hexachloroethane deposited on the

surface of soil would readily volatilize back to the atmosphere. However, if the chemical is allowed to penetrate significantly below the soil surface, approximately 95 percent would bind to soil, 2 percent would remain in the soil-water phase, and 3 percent would occur in the soil-air phase (Spangford et al. 1985).

Curtis et al. (1986) conducted sorption equilibrium, desorption, and mass balance laboratory experiments to determine if the observed field retardation of the organic solutes, including hexachloroethane, in the Borden aquifer site could be attributed to sorption. The uptake behavior for all five solutes was characterized by an initial rapid sorption step in <2 hr accounting for approximately 50 percent of the total sorption, followed by a gradually declining rate. After 5 days it became difficult to differentiate between sorption or losses from the system, which were attributed through mass balance studies to volatilization. An equilibration time of 3 days was determined for hexachloroethane. The sorption and desorption isotherms were linear and reversible for hexachloroethane for concentrations ranging from 1 to 50 $\mu\text{g/L}$, indicating that partitioning into the organic matter was the observed mechanism of sorption. This was tested by comparing the organic carbon partition coefficients (K_{oc}) obtained in this study with estimates based on octanol-water partition coefficients. The observed K_{oc} value for hexachloroethane exceeded the estimated value by a factor of 1.7. This difference can be attributed to adsorption to mineral surfaces, which would be expected due to the low organic carbon content (0.02 percent) of the Borden aquifer materials.

2.3 SUMMARY

Hexachloroethane may be released into the environment from its military use in white smoke grenades and markers, from the industrial production of other halogenated hydrocarbons, or from diffuse sources such as the incineration of polyvinyl chloride wastes. Evidence of the release of hexachloroethane is substantial; it has been detected in all environmental media and in fish tissues.

The Arkansas River and associated drainages are the primary aquatic system receiving wastewater discharges from the production of white smoke pyrotechnics at the Pine Bluff Arsenal, Arkansas. Prior to the installation of the Central Waste Treatment Facilities in 1980, the estimated average wastestream concentration of hexachloroethane was 168.0 mg/L . However, since treatment has begun, hexachloroethane has not been detected in wastewater samples from the arsenal.

Hexachloroethane has been detected in drinking water at concentrations ranging from 0.03 to 4.4 $\mu\text{g/L}$; in surface waters at concentrations ranging from 1.0 to 8.4 $\mu\text{g/L}$; in wastewater from industrial and publicly owned treatment works at concentrations ranging from 0.88 to 1,405.62 $\mu\text{g/L}$; in industrial effluents at concentrations ranging from <1.0 to 4.4 $\mu\text{g/L}$; in suspended sediments at concentrations as high as 0.53 $\mu\text{g/L}$; in aquatic sediments at concentrations <1.0 $\mu\text{g/L}$; in soils at concentrations

of 1.0 $\mu\text{g/g}$; in air samples at concentrations as high as 0.25 ± 0.05 pptv; and in fish tissues at concentrations of 0.1 mg/kg.

Hexachloroethane is thermodynamically stable when released into the atmosphere; no degradation by hydroxyl radicals is expected. Therefore, a very long tropospheric lifetime is expected, with the main sinks being the stratosphere and the oceans. The sum of the transport processes within the atmosphere and between the atmosphere and the oceans is approximately 1 kton/yr.

Experimental evidence indicates that hexachloroethane does not persist in surface water and groundwater; however, the residence time in groundwater is substantially longer than in surface water. Hexachloroethane (0.02 mg/L) injected into a glaciofluvial sand aquifer disappeared from groundwater by 330 days following a first-order rate process with a rate constant of 0.02/day. The center of the mass of hexachloroethane moved only 1.35 m from the center of the injection zone.

The transport and transformation processes affecting the persistence of hexachloroethane in surface water, groundwater, and soil are volatilization, biological degradation, photooxidation, and adsorption to organic matter and mineral surfaces.

The evaporative half-life of 1.0 mg/L of hexachloroethane in water is 45 min at 25°C, with removal of 90 percent requiring >120 min. The evaporation rate follows first-order kinetics for the first two to five half-lives. The first order volatilization rate constant for hexachloroethane in lakes and ponds is on the order of 2cm/hr; consequently, for a water body that is 180 cm deep, the rate constant would be 0.01/hr. with an estimated half life of 70 hr.

Hexachloroethane in surface water is microbially reduced to tetrachloroethylene under both aerobic and anaerobic conditions. The reaction is a classic example of cometabolism where the product is structurally similar to the reactant and is brought about by enzymes of broad specificity. In static culture BOD studies under aerobic conditions, 100 percent of hexachloroethane at concentrations of 5 and 10 mg/L was degraded in 7 days with rapid adaptation of the microbiota. Under anaerobic conditions in natural surface water, 90 percent of hexachloroethane at a concentration of 50 mg/mL was degraded in 18 days in pond water and 92 percent was degraded in 14 days in creek water.

BOD microcosm studies using aquifer materials indicate that hexachloroethane in groundwater is converted to tetrachloroethylene (under aerobic and possible anaerobic conditions) by microbes and/or abiotic agents which are nonuniformly distributed in the aquifer materials.

Hexachloroethane is more rapidly biodegraded in soil under anaerobic conditions than under aerobic conditions. Ninety-nine percent of hexachloroethane at a concentration of 5 ppm was degraded in 7 days under anaerobic conditions and in 4 weeks under aerobic conditions in sealed flasks.

Aerobic solutions of hexachloroethane in water (237.0 mg in 900 mL of water) are photooxidized with complete transformation of the two carbon atoms to carbon dioxide. The half-life at 90 to 95°C is 93.7 hr, with 25 percent degraded in 25.2 hr and 75 percent degraded in 172.0 hr. The degradation velocity depends on how finely the compound is dispersed in water, the temperature, and the wavelength of light. Anaerobic solutions of hexachloroethane are photolyzed, with cleavage occurring at the C-Cl bond.

Fifty percent of the total sorption of hexachloroethane in aquifer materials occurs in <2 hr in a rapid initial step, followed by a gradually declining rate with equilibrium reached in 3 days. The sorption and desorption isotherms for concentrations of hexachloroethane ranging from 1 to 50 µg/L are linear and reversible, indicating that hexachloroethane is partitioning into organic matter. However, because the observed K_{oc} value exceeds the estimated value by a factor of 1.7, adsorption to mineral surfaces is also occurring. Based on a calculated soil/air partitioning coefficient of 18 and a measured octanol/water partition coefficient of 8.9×10^3 , 95 percent of hexachloroethane which penetrates below the soil surface would bind to soil.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY IN AQUATIC ANIMALS

3.1.1 Aquatic Vertebrates

3.1.1.1 Freshwater fishes

Thurston et al. (1985) determined the acute toxicity of hexachloroethane in six species of freshwater fishes, including: Salmo gairdneri (rainbow trout), Lepomis macrochirus (bluegill sunfish), Pimephales promelas (fathead minnow), Gambusia affinis (mosquitofish), Ictalurus punctatus (channel catfish), and Carassius auratus (goldfish). Ninety-six-hour flow-through tests were conducted with six measured concentrations and a control. Dilution water was obtained from a groundwater spring. Chemical parameters remained relatively constant during testing with only slight seasonal variations in temperature, pH, alkalinity, and hardness. These parameters, along with DO and conductivity, were measured during testing. LC₅₀ (lethal concentration causing 50 percent mortality) values, determined by the trimmed Spearman-Kärber method, ranged from 0.86 mg/L for bluegill, the most sensitive species tested, to 2.36 mg/L for channel catfish (weighing 3.48 g), the least sensitive species tested (Table 3).

Phipps and Holcombe (1985) conducted multiple-species acute toxicity tests for hexachloroethane in which seven species were simultaneously tested in a single flow-through compartmentalized exposure tank. The five fish species tested were rainbow trout, bluegill, fathead minnow, channel catfish, and goldfish. The flow-through system comprised two 2-L proportional diluters (dilution factor of 0.6) with a flow rate of 130 mg/min and 90 percent replacement every 6 hr. Lake Superior water with a mean temperature of $17.3 \pm 0.6^{\circ}\text{C}$ was used in all tests. Ranges of water chemistry parameters were as follows: pH, 7.1 to 7.8; DO, 4.7 to 10.0 ppm; total hardness, 40.7 to 46.6 mg/L as CaCO₃, and alkalinity, 42.3 to 57.0 mg/L as CaCO₃. Because of its low water solubility, hexachloroethane was dissolved in dimethylformamide (DMF). Fish (20 per treatment) were exposed to the following five measured concentrations and a control (in duplicate): 0.290 ± 0.050 , 0.410 ± 0.060 , 0.730 ± 0.010 , 1.28 ± 0.17 , or 2.10 ± 0.39 mg/L. The nominal concentration of DMF used in the highest concentration test tank was 327 mg/L.

Phipps and Holcombe (1985) calculated the 96-hr LC₅₀ values, with 95 percent confidence intervals, using the trimmed Spearman-Kärber method. The results are presented in Table 3. The two most sensitive species tested were rainbow trout and bluegill, each with an LC₅₀ of 0.970 mg/L. The least sensitive species tested was the goldfish, with an LC₅₀ greater than the highest test concentration of 2.10 mg/L. The 96-hr LC₅₀ values (Table 3) for the species tested in this study are similar to those obtained by other investigators conducting single-species flow-through

TABLE 3. ACUTE TESTS FOR IMMOBILIZATION OR MORTALITY OF FRESHWATER
AQUATIC SPECIES FOLLOWING EXPOSURE TO HEXACHLOROETHANE

Species	Age/Size	Test Method ^a	Test Duration	LC ₅₀ /EC ₅₀ mg/L (95% CI)	Genus Mean Acute Value	Reference
ARTHROPODA						
Crustacea						
Cladocera						
Daphnidae						
<u>Ceriodaphnia reticulata</u>	<24 hr old	S,N	48 hr	6.8 (4.7-8.6)	4.7370	Elnabarawy et al. 1986
<u>Ceriodaphnia reticulata</u>	<4 hr old	S,N	48 hr	3.3 (2.3-4.7)		Mount and Norberg 1984
<u>Daphnia magna</u>	<24 hr old	S,MM	48 hr	1.36 (1.04-1.79)	3.6007 ^b	Thurston et al. 1985
<u>Daphnia magna</u>	<24 hr old	S,MM	48 hr	2.1 (1.8-2.5) ^c		Richter et al. 1983
<u>Daphnia magna</u>	<24 hr old	S,MM	48 hr	2.9 (2.5-3.3) ^d		Richter et al. 1983
<u>Daphnia magna</u>	<24 hr old	S,MM	48 hr	1.8 (1.6-2.1) ^e		Richter et al. 1983
<u>Daphnia magna</u>	<24 hr old	S,MM	48 hr	2.4 (2.0-2.9) ^f		Richter et al. 1983
<u>Daphnia magna</u>	<24 hr old	S,N	48 hr	8.1 (4.3-16.0)		LeBlanc 1980
<u>Daphnia magna</u>	<24 hr old	S,N	48 hr	10.0 (8.8-12.0)		Elnabarawy et al. 1986
<u>Daphnia magna</u>	<24 hr old	S,N	48 hr	2.7 (2.0-3.6)		Mount and Norberg 1984
<u>Daphnia pulex</u>	<24 hr old	S,N	48 hr	13.0 (12.0-15.0)		Elnabarawy et al. 1986
<u>Daphnia pulex</u>	<24 hr old	S,N	48 hr	>10.0		Mount and Norberg 1984
<u>Simocephalus vetulus</u>	<24 hr old	S,N	48 hr	5.8 (3.7-9.1)	5.8	Mount and Norberg 1984
Decapoda						
Astacidae						
<u>Orconectes immunis</u>	0.4 - 2 g	FT,M	96 hr	2.70 (2.13-3.41)	2.70	Thurston et al. 1985
<u>Orconectes immunis</u>	2.2 g	FT,M		>2.10		Phipps and Holcombe 1985

TABLE 3. (Cont'd.)

Species	Age/Size	Test Method ^a	Test Duration	LC ₅₀ /EC ₅₀ mg/L (95% CI)	Genus Mean Acute Value	Reference
Insecta						
Diptera						
Chironomidae						
<u>Tanytarsus dissimilis</u>	3rd-4th instar	S,MM	48 hr	1.23 (1.07-1.42)	1.4460	Thurston et al. 1985
<u>Tanytarsus dissimilis</u>	3rd-4th instar	S,M	48 hr	1.70		USEPA 1980a
MOLLUSCA						
Gastropoda						
Physidae						
<u>Aplexa hypnorum</u>	Adult	FT,M	96 hr	>2.10	>2.10	Phipps and Holcombe 1985
CHORDATA						
Osteichthyes						
Salmonidae						
<u>Salmo gairdneri</u>	0.6-8g	FT,M	96 hr	1.18 (0.727-1.92)	1.0390	Thurston et al. 1985
<u>Salmo gairdneri</u>	1.8g	FT,M	96 hr	0.97 (0.73-1.28)		Phipps and Holcombe 1985
<u>Salmo gairdneri</u>	-	FT,M	96 hr	0.98		USEPA 1980a
Cyprinidae						
<u>Carassius auratus</u>	1-4g	FT,M	96 hr	1.42 (1.03-1.95)	1.42	Thurston et al. 1985
<u>Carassius auratus</u>	8.4g	FT,M	96 hr	>2.10		Phipps and Holcombe 1985
<u>Pimephales promelas</u>	0.44g	FT,M	96 hr	1.10 (0.967-1.25)	1.3667	Thurston et al. 1985
<u>Pimephales promelas</u>	0.56g	FT,M	96 hr	1.39 (1.08-1.78)		Thurston et al. 1985
<u>Pimephales promelas</u>	0.3g	FT,M	96 hr	1.23 (1.08-1.40)		Phipps and Holcombe 1985
<u>Pimephales promelas</u>	-	FT,M	96 hr	1.53		USEPA 1980a
<u>Pimephales promelas</u>	30-35 days	FT,M	96 hr	1.51 (1.43-1.58)		Walbridge et al. 1983
<u>Pimephales promelas</u>	30 days	FT,M	96 hr	1.50		Veith et al. 1983b

TABLE 3. (Cont'd.)

Species	Age/Size	Test Method ^a	Test Duration	LC ₅₀ /EC ₅₀ mg/L (95% CI)	Genus Mean Acute Value	Reference
<u>Ictaluridae</u>						
<u>Ictalurus punctatus</u>	0.31g	FT,M	96 hr	1.77 (1.18-2.70)	1.8518	Thurston et al. 1985
<u>Ictalurus punctatus</u>	3.48g	FT,M	96 hr	2.36 (1.90-2.94)		Thurston et al. 1985
<u>Ictalurus punctatus</u>	5.6g	FT,M	96 hr	1.52 (1.39-1.65)		Phipps and Holcombe 1985
<u>Poeciliidae</u>						
<u>Gambusia affinis</u>	0.1-1.0g	FT,M	96 hr	1.38 (1.05-1.81)	1.38	Thurston et al. 1985
<u>Centrarchidae</u>						
<u>Lepomis macrochirus</u>	0.3-2g	FT,M	96 hr	0.86 (0.712-1.03)	0.9350	Thurston et al. 1985
<u>Lepomis macrochirus</u>	0.7g	FT,M	96 hr	0.970 (0.730-1.28)		Phipps and Holcombe 1985
<u>Lepomis macrochirus</u>	0.32-1.2g	S,N	96 hr	0.98 (0.85-1.10)		Buccafusco et al. 1981
<u>Amphibia</u>						
<u>Ranidae</u>						
<u>Rana catesbiana</u>	4.12g	FT,M	96 hr	3.18 (2.88-3.51)		Thurston et al. 1985
<u>Rana catesbiana</u>	4.21g	FT,M	96 hr	2.44 (1.47-4.06)	2.7856	Thurston et al. 1985

a. S = static; N = nominal concentrations; MM = mean measured concentrations; FT = flow-through; M = measured concentrations.

b. This is the GMAV for both Daphnia species; Species Mean Acute Values are 3.0658 for D. magna and 13.0 for D. pulex.

c. EC₅₀ value for acute test in which daphnids were unfed.

d. LC₅₀ value for acute test in which daphnids were unfed.

e. EC₅₀ value for acute test in which daphnids were fed.

f. LC₅₀ value for acute test in which daphnids were fed.

tests, with the possible exception of the discrepancy in values for the goldfish, which is probably attributed to differences in size (1 to 4 g compared with 8.4 g). The LC₅₀ values of 1.51 mg/L and 1.50 mg/L for fathead minnows (Table 3) obtained in single-species acute tests conducted by Walbridge et al. 1983 and Veith et al. 1983b, respectively, at the USEPA Environmental Research Laboratory in Duluth, Minnesota, are very similar to the value obtained in this study (1.23 mg/L) using the multiple-species testing approach. The authors concluded that conducting acute toxicity tests on several species in different chambers of the same exposure tanks allows easy determination of the most sensitive species and allows all species to be subjected to the same stresses (i.e., changes in chemical characteristics of test water).

Buccafusco et al. (1981) determined the toxicity of hexachloroethane in bluegill (weighing 0.32 to 1.2 g). Ninety-six-hour static acute toxicity tests (nominal concentrations) following USEPA (1975) procedures were conducted in 19.6-L widemouth glass jars, which were capped to control volatilization. Ten fish were randomly selected from the test population and added to the test chamber 30 min prior to the addition of the test solution. With the exception of DO, the chemical characteristics of the test solution remained relatively constant: temperature, 22 ± 1°C; pH, 6.5 to 7.9; total hardness, 32 to 48 mg/L as CaCO₃; total alkalinity, 28 to 34 mg/L as CaCO₃; and specific conductance, 93 to 190 µmhos/cm. The DO decreased from 9.7 mg/L at time 0 to 0.3 mg/L at 96 hr. Buccafusco et al. (1981) reported a 96-hr LC₅₀ of 0.98 mg/L (Table 3); however, they also reported that this value may be high, because a precipitate formed in the test solution, indicating that hexachloroethane was tested above the solubility limit. For the group of chloroethanes tested in this study, toxicity was positively correlated ($P \leq 0.01$) with the degree of chlorine substitution with 96-hr LC₅₀ values ranging from 550 mg/L for 1,2-dichloroethane to 0.98 mg/L for hexachloroethane.

Acute toxicity two orders of magnitude lower for 1,2-dichloroethane than for hexachloroethane was observed in tests conducted by the USEPA (1980a, as cited in USEPA 1980b) with fathead minnows. In flow-through tests using measured concentrations, the 96-hr LC₅₀ values ranged from 118 mg/L for 1,2-dichloroethane to 1.53 mg/L for hexachloroethane (Table 3). Flow-through tests with rainbow trout using measured concentrations were also conducted in this study; the 96-hr LC₅₀ for hexachloroethane was 0.98 mg/L (Table 3).

Walbridge et al. (1983) observed the same relationship in 96-hr flow-through toxicity tests with fathead minnows. Tests were conducted following USEPA (1975) methods using two 2-L/cycle proportional diluters to deliver the toxicant to randomly arranged exposure chambers (41-L all-glass aquaria). Fish (50 per treatment) were randomly assigned to the chambers and exposed to five measured concentrations and a control, in duplicate. Chemical characteristics of the dilution water (Lake Superior water supply) were measured during testing. The temperature was held at 25 ± 2°C, pH ranged from 6.7 to 7.6, DO ranged from 7.6 to 9.2 mg/L, hardness ranged from 45.0 to 45.5 mg/L as CaCO₃, and alkalinity ranged from 35.6 to 43.4 mg/L as CaCO₃. The 96-hr LC₅₀, calculated by the trimmed Spearman-Kärber method, was 1.51 mg/L (Table 3). The narcotic

effect of hexachloroethane produced lethargy and anesthesia in the exposed fathead minnows.

Using 96-hr flow-through toxicity tests, Veith et al. (1983b) determined LC₅₀ values for fathead minnows exposed to various industrial chemicals, including hexachloroethane, that are all known to produce narcosis. Tests were conducted following USEPA (1975) methods with five measured concentrations and a control, in duplicate (25 fish/exposure). Lake Superior water was used in all tests and maintained at 25 ± 1°C. Mean measurements of other chemical parameters were as follows: pH, 7.5; DO, >60% saturation; hardness, 56.3 mg/L as CaCO₃; and alkalinity, 42.2 mg/L as CaCO₃. The LC₅₀ for hexachloroethane, calculated by the trimmed Spearman-Kärber method, was 1.5 mg/L (Table 3).

3.1.1.2 Saltwater fishes

Heitmüller et al. (1981) conducted 96-hr static acute tests following USEPA (1975) methods to determine the toxicity of hexachloroethane in Cyprinodon variegatus (sheepshead minnow, juveniles). Nominal test concentrations (which were not specified) were determined from range-finding tests and were prepared in natural seawater of ambient salinity 10 to 31‰ (per thousand) at 25 to 31°C. DO and pH were monitored during testing. The 96-hr LC₅₀ was 2.4 ppm (Table 4) and the no-observable-effect-concentration (NOEC) was 1.0 ppm. In this study, tests with five chlorinated ethanes were conducted; the LC₅₀ values for this saltwater fish did not correlate as well with increased chlorination as did the acute values for bluegill (Buccafusco et al. 1981) and fathead minnows (USEPA 1980a, as cited in USEPA 1980b). The 96-hr LC₅₀ values for the other chloroethanes were as follows: >130 and <230 for 1,2-dichloroethane, 71 for 1,1,1-trichloroethane, 12 for 1,1,2,2-tetrachloroethane, and 116 for 1,1,1,2,2-pentachloroethane.

3.1.1.3 Other vertebrate species

Thurston et al. (1985) studied the toxicity of hexachloroethane in Rana catesbeiana (bullfrog) tadpoles (2 to 5 g) in 96-hr flow-through acute tests. Tests were conducted in the same manner as those tests described in Sect. 3.1.1.1 for fish with six concentrations and a control. This amphibian species was the least sensitive of any vertebrate or invertebrate species tested by Thurston et al. (1985) with 96-hr LC₅₀ values of 3.18 mg/L for tadpoles weighing 4.12 g and 2.44 mg/L for tadpoles weighing 4.21 g (Table 3).

TABLE 4. ACUTE TESTS FOR MORTALITY OF SALTWATER
AQUATIC SPECIES FOLLOWING EXPOSURE TO HEXACHLOROETHANE

Species	Age/Size	Test Method ^a	Test Duration	LC ₅₀ /EC ₅₀ mg/L (95% CI)	Genus Mean Acute Value	Reference
PROTOZOA						
<u>Photobacterium</u> <u>phosphoreum</u>	-	MT	5 min	0.14	-	Curtis et al. 1982
<u>Photobacterium</u> <u>phosphoreum</u>	-	MT	15 min	8.3 (7.1-9.7)		Nacci et al. 1986
ECHINODERMATA						
Echinoidea						
<u>Arbacia punctulata</u>	Embryo	LTI	3 hr	9.32 (8.29-10.60)	-	Jackim and Nacci 1984
<u>Arbacia punctulata</u>	Embryo	LTI	3 hr	8.51 (7.43-9.19)		Jackim and Nacci 1984
<u>Arbacia punctulata</u>	Egg	LTI	5 hr	6.05 (4.67-7.60)		Nacci and Jackim 1985
<u>Arbacia punctulata</u>	Egg/Sperm	LTI	4 hr	4.97 (4.04-5.91)		Nacci and Jackim 1985
<u>Arbacia punctulata</u>	Embryo	LTI	3 hr	8.31 (5.80-12.93)		Nacci and Jackim 1985
<u>Arbacia punctulata</u>	Sperm	LTI	80 min	29.1 (27.6-30.8)		Nacci et al. 1986
ARTHROPODA						
Crustacea						
Mysidae						
<u>Mysidopsis bahia</u>	-	S,N	96 hr	0.94	0.94	USEPA 1978
CHORDATA						
Osteichthyes						
Cyprinodontidae						
<u>Cyprinodon variegatus</u>	Juvenile	S,N	96 hr	2.4 (1.9-3.1)	2.4	Heitmuller et al. 1981

a. S = static test; N = nominal concentrations; MT = Microtox bioassay; LTI = labeled thymidine incorporation bioassay.

3.1.2 Aquatic Invertebrates

3.1.2.1 Freshwater invertebrates

Thurston et al. (1985) studied the toxicity of hexachloroethane in Daphnia magna (water flea, neonates <24 hr old) and in Tanytarsus dissimilis (midge, 3rd to 4th instar) in 48-hr static acute tests, and in Orconectes immunis (crayfish) in a 96-hr flow-through acute test. The test containers for the static tests, soft-glass Ball canning jars, were sealed with aluminum foil between the covers and jar rims to reduce volatilization. Twenty individuals of each species were used for controls and for testing at each of five to seven concentrations. Test concentrations were measured at the beginning and end of each test. Conductivity, alkalinity, hardness, pH, and DO were measured on combined samples from each concentration at the conclusion of the tests; temperature was monitored during testing. The flow-through test for crayfish followed procedures as described for the acute tests for fish performed by Thurston et al. (Sect. 3.1.1.1), except that the test tanks were divided into 10 individual compartments to prevent cannibalism. LC₅₀ values for these invertebrates were 1.23 mg/L for T. dissimilis, 1.36 mg/L for D. magna, and 2.70 mg/L for O. immunis (Table 3). In a 48-hr static acute test using measured concentrations, USEPA (1980a, as cited in USEPA 1980b) obtained an LC₅₀ of 1.70 mg/L for T. dissimilis (Table 3).

Phipps and Holcombe (1985) also determined the acute toxicity of hexachloroethane in two invertebrate species, O. immunis and Aplexa hypnorum (snail), using the multiple-species test procedures described for the fish species tested in this study (Sect. 3.1.1.1). Crayfish were placed in an individual compartment that was covered with a stainless-steel screen to prevent their escape, and snails were placed in 10 x 3 cm diam stainless-steel screen cylinders, which were randomly distributed in an individual compartment. The invertebrate species (10 crayfish/treatment; 20 snails/treatment) were exposed to the same series of measured concentrations: 0.290 ± 0.050 , 0.410 ± 0.060 , 0.730 ± 0.010 , 1.28 ± 0.17 , or 2.10 ± 0.39 mg/L. Hexachloroethane was less toxic in both invertebrate species than in the fish species tested; the 96-hr LC₅₀ values, calculated using the trimmed Spearman-Kärber method, were higher than the highest test concentration; therefore, the LC₅₀ values were reported as >2.10 mg/L (Table 3). Welsh and Gordon (1947, as cited in Dacre et al. 1979) reported that 5 to 10 mg/L of hexachloroethane applied to the isolated chela of another crayfish species, Cambarus virilis, caused multiple discharge nerve axon response.

Elnabarawy et al. (1986) conducted 48-hr static acute tests with unfed D. magna, Daphnia pulex, and Ceriodaphnia reticulata to determine their relative sensitivities to hexachloroethane. The dilution water was unchlorinated, carbon-filtered well water with a total hardness of 240 ± 10 mg/L as CaCO₃, total alkalinity of 230 ± 10 mg/L as CaCO₃, and specific conductance of 360 ± 10 μ mhos/cm². Temperature ($23 \pm 1^\circ\text{C}$), pH (8.0 ± 0.3), and DO (maintained at >5.0 mg/L) remained relatively constant from the beginning to the end of testing. Ten 1st-instar daphnids (<24 hr old)

of each species were exposed to at least five nominal concentrations and a control, in duplicate. EC₅₀ (median effective concentration causing complete immobilization) values were calculated using the moving average angle method. C. reticulata appeared to be the most sensitive of the three species with a 48-hr EC₅₀ of 6.8 mg/L; D. magna was less sensitive with a value of 10 mg/L; and D. pulex was the least sensitive with a value of 13 mg/L (Table 3).

Mount and Norberg (1984) compared the sensitivities of four cladoceran species (D. magna, D. pulex, C. reticulata, and Simocephalus vetulus) to 13 substances, including hexachloroethane, in 48-hr static tests using nominal concentrations. Dilution water was Lake Superior water with the following chemical characteristics; pH, 7.2 to 7.4; hardness, 45 mg/L as CaCO₃; alkalinity, 43 to 45 mg/L as CaCO₃. The water contained 50,000 to 100,000 bacterial cells/mL; therefore, the test organisms were provided with a food source during testing. Two replicates of five animals each were exposed to the test concentrations; control mortality was at ≤20 percent in every test. D. pulex, with a 48-hr LC₅₀ of >10.0 mg/L, was less sensitive to hexachloroethane than the other three cladoceran species tested (Table 3). D. magna was the most sensitive to hexachloroethane with a 48-hr LC₅₀ of 2.7 mg/L, followed by C. reticulata with a 48-hr LC₅₀ of 3.3 mg/L, and S. vetulus with a 48-hr LC₅₀ of 5.8 mg/L (Table 3).

Richter et al. (1983) conducted static bioassays following American Society for Testing and Materials (ASTM) (1980) methods to determine the acute toxicity of hexachloroethane in fed (trout chow and yeast mixture, 20 mg/L dry wt) and unfed D. magna. Four replicates of five animals each were exposed to six toxicant concentrations (each concentration was ~60 percent of the next higher one) and a control. Lake Superior water, maintained at 20 ± 1°C, was used in all tests. Measured chemical characteristics were as follows: hardness, 43.5 to 47.5 mg/L as CaCO₃; alkalinity, 37.0 to 45.5 mg/L as CaCO₃; DO, 7.9 to 9.9 mg/L in unfed tests and 4.1 to 8.4 mg/L in fed tests; and pH, 7.1 to 7.7 in unfed tests and 7.0 to 7.5 in fed tests. Forty-eight-hour EC₅₀ values and 48-hr LC₅₀ values (based on cessation of heart beat and gut movement) were calculated using probit, moving average, or binomial formulas. The EC₅₀ values, 2.1 mg/L for unfed daphnids and 1.8 mg/L for fed daphnids, were lower than the LC₅₀ values of 2.9 mg/L for unfed daphnids and 2.4 mg/L for fed daphnids (Table 3). Fed and unfed values were not significantly different. The authors tested a series of chlorinated ethanes in this study and observed the same relationship as Buccafusco et al. (1981) observed in 96-hr static tests with bluegill and as USEPA (1980a, as cited in USEPA 1980b) observed in 96-hr flow-through tests with fathead minnows. The chlorinated ethanes increased in acute toxicity by about two orders of magnitude in relationship to the degree of chlorination; LC₅₀ values ranged from 268 mg/L for 1,2-dichloroethane to 2.9 mg/L for hexachloroethane.

LeBlanc (1980) did not observe a trend between toxicity and degree of chlorination for the chloroethanes tested in 48-hr static tests with D. magna (<24 hr old). Following USEPA (1975) methods, 15 individuals per treatment were exposed to at least five nominal concentrations of each

chemical. The diluent water used in the tests was deionized reconstituted well water maintained at a temperature of $22 \pm 1^\circ\text{C}$ and a mean hardness of 72 mg/L as CaCO_3 . Water quality characteristics were measured at the beginning and end of each test in the high-, middle-, and low-concentration test chambers and in the controls. DO ranged from 6.5 to 8.1 mg/L, and pH ranged from 6.7 to 8.1. The 48-hr LC_{50} value for hexachloroethane, calculated using the moving average angle method, was 8.1 mg/L (Table 3), and the no-discernible-effect-concentration was 0.28 mg/L. The 48-hr LC_{50} values for the other chloroethanes tested were as follows: 218.0 mg/L for 1,2-dichloroethane, 18.0 mg/L for 1,1,2-trichloroethane, 23.9 mg/L for 1,1,1,2-tetrachloroethane, 9.32 mg/L for 1,1,2,2-tetrachloroethane, and 62.9 mg/L for pentachloroethane.

3.1.2.2 Saltwater invertebrates

USEPA (1978) determined the toxicity of a series of chlorinated ethanes, including hexachloroethane, in the mysid shrimp Mysidopsis bahia in 96-hr static acute tests using nominal concentrations. An increase in toxicity was observed with an increase in the degree of chlorination, 96-hr LC_{50} values ranged from 113 mg/L for 1,2-dichloroethane to 0.94 mg/L for hexachloroethane (Table 4). This saltwater invertebrate species appears to be more sensitive to hexachloroethane than the freshwater invertebrate species tested in this study and by other investigators.

Jackim and Nacci (1984) developed a critical life-stage toxicity assay (post-fertilization exposure) using labeled thymidine incorporation in Arbacia punctulata (sea urchin) embryos as a rapid means of determining the potential toxicity of eight compounds, including hexachloroethane. The assay is based on the principle that inhibition of thymidine incorporation is proportional to inhibition of DNA synthesis and, consequently, retardation of cell division. The increasing incorporation of thymidine during the test period should reflect growth of the embryo from the 4- to 32-cell stage. The tests were conducted in autoclaved and filtered seawater at $30.0 \pm 1^\circ\text{C}$ and $20 \pm 1^\circ\text{C}$. One hour after fertilization, 1 mL of washed embryo suspension (10,000 eggs/mL) was added to 99 mL of seawater in 70- x 50-mm culture dishes containing the test solutions (final density was 100 embryos/mL). Two hours after fertilization, 10 mL of the embryo suspension (containing 1,000 embryos) from each of the test solutions was added to 20-mL glass vials containing 1.52 μCi [^3H]thymidine (20 Ci/mmol) in 1 mL seawater (final activity 0.138 $\mu\text{Ci/mL}$). Two hours after incorporation of the labeled thymidine, the embryo solution was filtered and analyzed by scintillation spectrometry. Morphological data were also obtained by determining the percentage of original eggs that developed into normal larvae (plutei) 48 hr after fertilization. The data were analyzed using linear regression, and EC_{50} values were calculated as the effective (nominal) concentration that induced 50 percent inhibition compared to control values. The biochemically derived EC_{50} , with 95 percent confidence intervals, for hexachloroethane in this study was 9.32 mg/L (8.29 to 10.60) and 8.51 mg/L (7.43 to 9.19) (Table 4) in duplicate experiments.

Nacci and Jackim (1985) continued this study using the thymidine incorporation assay to determine the toxic effects of the eight compounds, including hexachloroethane, to additional life stages of A. punctulata. Tests were conducted in duplicate with five nominal concentrations and a control using the same basic procedure as described by Jackim and Nacci (1984), with the exception of the additional exposure regimes. In the pre-fertilization test, eggs were added to the toxicant 1 hr before the sperm; in the at-fertilization test, eggs and sperm were added simultaneously to the toxicant; and in the post-fertilization test [as described in Jackim and Nacci (1984) above], eggs were fertilized in uncontaminated seawater and the toxicant was added to the embryo suspension 1 hr after fertilization. Test concentrations, determined in preliminary testing, were approximately equal to the EC₂₀ (effective concentration causing 20 percent inhibition of thymidine incorporation), EC₄₀ (effective concentration causing 40 percent inhibition of thymidine incorporation), EC₆₀ (effective concentration causing 60 percent inhibition of thymidine incorporation), EC₈₀ (effective concentration causing 80 percent inhibition of thymidine incorporation), or EC₁₀₀ (effective concentration causing 100 percent inhibition of thymidine incorporation) for each compound. The EC₅₀ values, with 95 percent confidence intervals, for each exposure regime for hexachloroethane, calculated using linear regression, were as follows: pre-fertilization, 6.05 mg/L (4.67 to 7.60); at-fertilization, 4.97 mg/L (4.04 to 5.91); and post-fertilization, 8.31 mg/L (5.80 to 12.93) (Table 4). The authors compared these values with LC₅₀ values obtained by Thurston et al. (1985) in standard aquatic bioassays with fathead minnows (96-hr flow-through tests) and D. magna (48-hr static-renewal tests). An analysis of covariance revealed that the results from the Arbacia and fathead minnow tests were statistically equivalent. The Daphnia test was slightly more sensitive than, but highly correlated with, the Arbacia test.

Nacci et al. (1986) conducted another rapid bioassay, the sperm cell toxicity test, to measure the toxicity of the eight compounds in A. punctulata. A sperm suspension (100 μ L containing 1×10^6 sperm/mL) was added to 10 mL of test solution in glass vials and exposed for 1 hr at 20°C. One milliliter of egg suspension (1,000 eggs/mL) was added to each vial so that the final sperm:egg ratios were 1,000:1. After 20 min, buffered formalin was added, and fertilization (presence of fertilization membranes) was determined by microscopic observation. The EC₅₀ and 95 percent confidence interval for hexachloroethane were 29.1 mg/L (27.6 to 30.8) (Table 4).

3.2 CHRONIC TOXICITY TO AQUATIC ANIMALS

USEPA (1978) conducted early life-stage toxicity tests with fathead minnows to determine the effects of hexachloroethane on survival, growth, and hatchability. The tests were conducted under flow-through conditions with the embryo-larval stages exposed for 31 to 33 days. The highest NOEC was 0.41 mg/L, and the lowest-observable-effect-concentration (LOEC) was 0.70 mg/L. Therefore, the maximum acceptable toxicant concentration

(MATC) for fathead minnows exposed to hexachloroethane, derived as the geometric mean of the NOEC and LOEC, is 0.54 mg/L ($P \leq 0.05$).

DeFoe (unpublished data, as cited in Walbridge et al. 1983) reported a MATC for hexachloroethane of 0.069 to 0.207 mg/L for embryo-larval fathead minnows.

3.3 TOXICITY TO MICROORGANISMS AND PLANTS

3.3.1 Freshwater Algae

USEPA (1978) conducted 96-hr acute tests to determine the toxicity of a series of chlorinated ethanes, including hexachloroethane, to the green alga Selenastrum capricornutum. EC₅₀ values, based on chlorophyll a content and cell number, were 87 mg/L and 93 mg/L, respectively (Table 5). Freshwater algae appear to be less sensitive to hexachloroethane than any freshwater fish or invertebrate species that has been tested. Only a slight increase in toxicity was observed with increased chlorination for this species. EC₅₀ values, based on chlorophyll a content, for other chloroethanes were as follows: >433 mg/L for 1,2-dichloroethane, >669 mg/L for 1,1,1-trichloroethane, 136 mg/L for 1,1,2,2-tetrachloroethane, and 121 mg/L for pentachloroethane (USEPA 1978).

TABLE 5. TOXICITY OF HEXACHLOROETHANE IN FRESHWATER AND SALTWATER ALGAL SPECIES

Species	Test Duration	EC ₅₀ (mg/L)	
		Chlorophyll <u>a</u>	Cell number
<u>Freshwater</u>			
CHLOROPHYTA			
Chlorococcaceae			
<u>Selenastrum capricornutum</u>	96 hr	87.0	93.0
<u>Saltwater</u>			
CHRYSOPHYTA			
<u>Skeletonema costatum</u>	96 hr	8.57	7.75

a. Data from USEPA 1978.

3.3.2 Saltwater Algae

The USEPA (1978) also tested the toxicity of hexachloroethane in the saltwater alga Skeletonema costatum in 96-hr acute toxicity tests. The 96-hr EC₅₀ values for growth, based on chlorophyll *a* content and cell count, were 8.57 mg/L and 7.75 mg/L, respectively (Table 5). Hexachloroethane appears to be more toxic to saltwater algae than to freshwater algae.

3.3.3 Marine Bacteria

Curtis et al. (1982) conducted bacterial bioassays using the Microtox toxicity analyzer, which measured the decrease in natural light emission the luminescent bacterium Photobacterium phosphoreum in response to exposure to hexachloroethane. The 5-min EC₅₀ (measured concentration that effects a 50 percent reduction in light output) for hexachloroethane was 0.14 mg/L (Table 4). The authors compared the bacterial EC₅₀ values with 96-hr LC₅₀ values for fathead minnows and determined that a statistically significant relationship exists between the two end points, such that this bioassay could be used to predict order of magnitudes of fish LC₅₀ values and rank organic chemicals as to their potential toxicity.

Nacci et al. (1986) conducted the Microtox bioassay with P. phosphoreum and exposed aliquots of the bacteria to small volumes of hexachloroethane for 15 min at 15°C. Luminescence was measured using a photometer; an EC₅₀ of 8.3 mg/L (7.1 to 9.7) (Table 4) was obtained.

3.4 BIOACCUMULATION

Barrows et al. (1980) studied the bioconcentration and elimination of several chemicals, including hexachloroethane, by bluegill sunfish exposed in a flow-through system to sublethal concentrations of the carbon 14-labeled compounds. The bluegill were maintained for 30 days prior to testing and had mean wet weights ranging from 0.37 to 0.94 g and mean standard lengths ranging from 25 to 32 mm. The total lipid content for bluegill usually ranges from 2 to 4 percent. Fish were fed a dry pelleted food ad libitum throughout the study. Water and fish (five individuals/tank) samples were collected from each control and test tank on days 0, 1, 2, 4, 7, 10, 14, 21, and 28 of the exposure period until a steady-state equilibrium between concentrations in whole-body fish tissue and exposure water was observed. At the end of the 28-day exposure period, the remaining fish were transferred to pollutant-free water in flow-through tanks for a 7-day depuration period, in order to determine the rate of elimination of chemical residues. Fish tissues were sampled on days 1, 2, 4, and 7 to determine the half-life of chemical residues in the tissues. Temperature, DO, and pH were monitored throughout the exposure and depuration periods. The mean temperature was $16 \pm 1^\circ\text{C}$, DO ranged from 5.9 to 8.6 mg/L, and pH ranged from 6.3 to 7.9. A steady-state bioconcentration factor (BCF) was calculated as the quotient of the mean concentration of hexachloroethane in whole-body fish tissues during equilibrium and the mean measured concentration in test water during the entire exposure

period. The mean test water concentration during the exposure period was $6.17 \pm 1.95 \mu\text{g/L}$. A relatively low BCF of 139 was calculated for hexachloroethane. A short half-life of <1 day for hexachloroethane indicated that when exposed fish were transferred to clean water, 50 percent of the chemical residues in tissues was eliminated within 24 hr. A series of chlorinated ethanes were tested in this study, and it appeared that the bioconcentration potential was unaffected by the degree of chlorination up to and including 1,1,2,2-tetrachloroethane (BCF of 8). However, with the addition of the fifth chlorine atom in pentachloroethane (BCF of 67), the BCF increased by 7 times from 1,1,1-trichloroethane (BCF of 9) and by 33 times from 1,2-dichloroethane (BCF of 2). The bioconcentration potential then doubles with the addition of the sixth chlorine atom in hexachloroethane (BCF of 139).

Veith (1981, as cited in USEPA 1981) reported a measured BCF of 703 for fathead minnows with a total lipid content of about 8 percent. A description of the test procedure was not given, and it is unknown whether this factor represents a steady-state value.

Oliver and Niimi (1983) conducted flow-through tests to determine the bioconcentration of 10 chlorinated benzenes, 1,1,2,3,4,4-hexachloro-1,3-butadiene, and hexachloroethane in rainbow trout. The fish were exposed to a mixture of the chemicals at two exposure levels, low and high, based on environmental concentrations in Lake Ontario. The low-exposure test concentration for hexachloroethane was $0.32 \pm 0.08 \text{ ng/L}$ and the high-exposure test concentration was $7.1 \pm 2.1 \text{ ng/L}$. The rainbow trout, with an average weight of 250 g, were maintained in Lake Ontario water at $15 \pm 1^\circ\text{C}$ for 30 days prior to testing. The total lipid content of rainbow trout can range from about 14 percent to as high as 50 percent. Fish samples (six fish per sample) were taken from the control tank on days 10, 34, 63, and 119; from the low-exposure tank on days 8, 39, 69, 99, and 119; and from the high-exposure tank on days 7, 22, 43, 63, and 105. Fish exposed to hexachloroethane at both levels attained equilibrium by the first sample interval. BCFs for whole fish were estimated after subtracting mean residue levels of the control fish ($0.03 \pm 0.01 \text{ ng/g}$ for hexachloroethane). The BCF for rainbow trout exposed to the low level of hexachloroethane was 510 ± 96 , and the BCF at the high exposure level was 1200 ± 450 . These data, however, are questionable due to the possible synergistic effects of exposure to a mixture of chemicals.

3.5 SUMMARY

Hexachloroethane acts as a narcotic, producing lethargy and anesthesia in fish. Results of acute dynamic bioassays with freshwater fishes indicate that bluegill sunfish and rainbow trout are the two most sensitive species, with 96-hr LC_{50} values ranging from 0.86 to 0.97 mg/L and 0.97 to 1.18 mg/L, respectively. The least sensitive fish species is channel catfish, with flow-through 96-hr LC_{50} values ranging from 1.52 to 2.36 mg/L. Results of static acute tests with the only saltwater fish species tested, sheepshead minnow, with a 96-hr LC_{50} of 2.4 mg/L, indicate that this species is not quite as sensitive to hexachloroethane as the

freshwater fish species. The NOEC for this species is 1.0 mg/L. Of all vertebrate species tested, the bullfrog (tadpoles) was the least sensitive, with 96-hr LC₅₀ values in dynamic assays ranging from 2.44 to 3.18 mg/L.

Hexachloroethane appears to be less toxic in general in freshwater invertebrates than in either freshwater or saltwater fishes. The most sensitive species is Tanytarsus dissimilis, with 48-hr LC₅₀ values in static tests ranging from 1.23 to 1.70 mg/L. The least sensitive species is D. pulex, with a 48-hr EC₅₀ in static tests of 13.0 mg/L. Of four cladoceran species tested, three can be clearly ranked according to their sensitivity to hexachloroethane: Daphnia pulex < Simocephalus vetulus < Ceriodaphnia reticulata. The data from static bioassays for Daphnia magna are more scattered, with 48-hr EC₅₀ values ranging from 1.8 to 10.0 mg/L and 48-hr LC₅₀ values ranging from 1.36 to 8.1 mg/L. The no-discernible-effect-concentration for D. magna is 0.28 mg/L. The saltwater invertebrate Mysidopsis bahia appears to be more sensitive to hexachloroethane than the freshwater invertebrates, with a 96-hr LC₅₀ of 0.94 mg/L in static tests.

In critical life-stage toxicity assays based on the inhibition of thymidine incorporation in sea urchins, the sensitivities of life stages to hexachloroethane can be ranked as follows: at-fertilization stage (egg and sperm) > pre-fertilization (egg) > post-fertilization (embryo). The biochemically derived EC₅₀ values are 4.97 mg/L for the egg and sperm, 6.05 mg/L for the egg, and 8.31 to 9.32 mg/L for the embryo. The sperm cell is the most tolerant, with an 80-min EC₅₀ of 29.1 mg/L.

The survival, growth, and hatchability of embryo-larval fathead minnows are adversely affected at concentrations as low as 0.70 mg/L of hexachloroethane. No effect was observed at 0.41 mg/L; therefore, the MATC was calculated as 0.54 mg/L.

Freshwater algae (Selenastrum capricornutum) are less sensitive to hexachloroethane than freshwater vertebrate and invertebrate species, with 96-hr EC₅₀ values of 87 mg/L and 93 mg/L, based on chlorophyll *a* content and cell number, respectively. However, hexachloroethane is more toxic to saltwater algae (Skeletonema costatum), with 96-hr EC₅₀ values of 8.57 mg/L and 7.75 mg/L, based on chlorophyll *a* content and cell number, respectively.

The steady-state BCF for bluegill sunfish is 139, which indicates that hexachloroethane moderately bioconcentrates in fish tissues. Depuration in bluegill is rapid, with a half-life of <1 day. Three other BCF values have been reported, 703 for fathead minnows, 510 ± 96 for rainbow trout exposed to 0.32 ± 0.08 ng/L, and 1200 ± 450 for rainbow trout exposed to 7.1 ± 2.1 ng/L. The elevated bioconcentration factors for these species could be related to higher lipid content.

For chloroethanes as a group, the severity of toxicity is positively correlated with the degree of chlorine substitution. Hexachloroethane is

two orders of magnitude more toxic than 1,2-dichloroethane in flow-through tests with fathead minnows and in static tests with D. magna. In static acute tests with bluegill, 96-hr LC₅₀ values range from 550 mg/L for 1,2-dichloroethane to 0.98 mg/L for hexachloroethane. This same relationship is observed for saltwater invertebrates, but not for saltwater fishes. Ninety-six-hour LC₅₀ values for Mysidopsis bahia (mysid shrimp) range from 113 mg/L for 1,2-dichloroethane to 0.94 mg/L for hexachloroethane. It appears that the bioconcentration potential of chloroethanes is unaffected by the degree of chlorination up to and including 1,1,2,2-tetrachlorethane; however, the BCF increases by a factor of 7 (1,1,1-trichloroethane) to 33 (1,2-dichloroethane) with the addition of the fifth chlorine atom and even doubles from that value for hexachloroethane.

4. MAMMALIAN TOXICOLOGY

4.1 PHARMACOKINETICS

4.1.1 Absorption, Distribution, and Excretion

The efficiency (percent) of absorption of hexachloroethane from the gastrointestinal tract has not been determined. Fowler (1969) showed that, in sheep given a dose of 0.5 g/kg, hexachloroethane appeared very rapidly in the venous blood, where it was first detected 27 min after dosing, but it was detected in bile 15 min after dosing. The peak blood level was reached within 24 hr; the level declined slowly up to 96 hr and rapidly after 96 hr. At the end of day 5, the blood level was almost nil. The average 24-hr blood level of hexachloroethane and metabolites was about 24 µg/mL.

Gorzinski et al. (1985) described the tissue distribution and clearance after subchronic exposure of Fisher 344 rats to dietary hexachloroethane. Table 6 shows the tissue concentrations of hexachloroethane after exposure of male and female rats to hexachloroethane at doses of 1.0, 15, or 62 mg/kg/day for 16 weeks. The data showed that, at all doses, the highest concentrations of hexachloroethane were found in the fat. A significant sex difference was found in the concentrations in kidney, which were 4, 35, and 47 times greater in males than in females at the low-, medium- and high-dose levels, respectively. Gorzinski et al. (1985) also showed that adverse effects observed in the kidney were more severe in male rats than in female rats (see Sect. 4.3). A study conducted by the National Cancer Institute (NCI) (1978) also showed that kidney lesions occurred with a higher frequency in male rats treated chronically with a low dose (212 mg/kg/day) of hexachloroethane, but not in rats treated with a higher dose (423 mg/kg/day). The sex difference in the uptake of hexachloroethane into the kidney could affect incidence and severity of kidney lesions.

The rate of clearance of hexachloroethane from tissues was determined by feeding an additional group of animals the highest dose of hexachloroethane (62 mg/kg/day) for 8 weeks. The animals were then placed on a control diet, and three to four rats (per time point) were killed after 3, 6, 13, 22, and 31 days on the control diet. Blood, liver, kidney, and fat were analyzed for hexachloroethane content. At the end of the 8-week treatment period, the concentration of hexachloroethane in each tissue was different, but the rates of clearance from the tissues were similar. Clearance from all tissues was first-order; the half-time of clearance was 2.7 days for fat and kidney, 2.3 days for liver, and 2.5 days for blood. Fowler (1969) also reported that the concentrations of hexachloroethane and metabolites were higher in fat than in liver, kidney, brain, and muscle (see Table 7, Sect. 4.1.2).

TABLE 6. TISSUE DISTRIBUTION OF HEXACHLOROETHANE IN RATS FED
HEXACHLOROETHANE IN THE DIET FOR 16 WEEKS^{a,b}

Sex	Dose ^c	$\mu\text{g}/\text{HCE}/\text{g Tissue}^{\text{d}}$			
		Blood	Liver	Kidney	Fat
M	1	0.079 $\pm$ 0.057	0.291 $\pm$ 0.213	1.356 $\pm$ 0.29	3.15 $\pm$ 0.37
	15	0.596 $\pm$ 0.653	1.736 $\pm$ 1.100	24.33 $\pm$ 5.73	37.90 $\pm$ 6.10
	62	0.742 $\pm$ 0.111	0.713 $\pm$ 0.343	95.12 $\pm$ 11.56	176.1 $\pm$ 14.50
F	1	0.067 $\pm$ 0.039 ^d	0.260 $\pm$ 0.035 ^d	0.369 $\pm$ 0.51	2.59 $\pm$ 0.72
	15	0.162 $\pm$ 0.049 ^d	0.472 $\pm$ 0.204	0.688 $\pm$ 0.17	45.27 $\pm$ 11.33
	62	0.613 $\pm$ 0.231	0.631 $\pm$ 0.262	2.10 $\pm$ 0.66	162.1 $\pm$ 7.10

a. Reprinted from Gorzinski et al. 1985, pp. 166, by courtesy of Marcel Dekker, Inc.

b. Values are mean $\pm$ S.D.; HCE = hexachloroethane.

c. Dose in mg/kg/day.

d. Blood samples from 3 animals, liver from 2, and all other from 4.

Hexachloroethane is excreted into feces, urine, and expired air. Fowler (1969) showed that sheep given hexachloroethane at a dose of 0.5 g/kg excreted almost all of the parent compound and metabolites into feces; the quantity excreted into urine was less than 10 percent of that excreted into feces. Excretion was almost completed by 24 hr. Jondorf et al. (1957) administered [¹⁴C]-hexachloroethane orally to rabbits at a dose of 0.5 g/kg and observed that only 5 percent of the radioactivity appeared in the urine within 3 days; 14 to 24 percent was exhaled. The fate of the remainder of the radioactivity was not reported.

Mitoma et al. (1985) gave Osborne-Mendel rats and B6C3F1 mice hexachloroethane (dissolved in corn oil) by gavage. The dose in rats was 500 mg/kg (2.11 mmol/kg) [the maximum tolerated dose (MTD)] or one-fourth the MTD, and the dose in mice was 1,000 mg/kg (4.22 mmol/kg) (MTD) or one-fourth the MTD. The animals were dosed 5 days/week for 4 weeks. The animals were then given a single dose of [1,2-¹⁴C]-hexachloroethane at 3.6 $\mu\text{Ci}/200$ g body weight (rats) or 3.5 $\mu\text{Ci}/20$ g body weight (mice) and placed in metabolism cages for 48 hr. In rats, 64.55 percent of the administered radioactivity was recovered in expired air, 2.37 percent was recovered as CO₂, 6.33 percent was in excreta (urine and feces), 20.02 percent was recovered in the carcass, and 6.73 percent was not recovered. In mice 71.51 percent of the administered radioactivity was recovered in expired air, 1.84 percent was recovered as CO₂, 16.21 percent was in excreta, 5.90 percent was recovered in the carcass, and 4.54 percent was not recovered. Mitoma et al. (1985) reported that the sum of radioactivity in expired CO₂, excreta, and the carcass was the total percentage of the dose metabolized. Because no data were presented on the identity of metabolites, only the radioactivity recovered in expired CO₂ should be represented as metabolized hexachloroethane.

4.1.2 Metabolism

4.1.2.1 Identity and distribution of metabolites

Hexachloroethane is a saturated chloroethane that can be metabolized by dechlorination or by other transformation reactions. Jondorf et al. (1957) used chromatographic and isotope-dilution methods to identify the metabolites in urine of rabbits given 0.5 g/kg of [¹⁴C]-hexachloroethane orally. The metabolites and the percentages of the administered dose were as follows: trichloroethanol, 1.3; trichloroacetic acid, 1.3; dichloroacetic acid, 0.8; monochloroacetic acid, 0.7; dichloroethanol, 0.4; and oxalic acid, 0.1. The metabolites expired in air (without percentages) were CO₂, tetrachloroethylene, and 1,1,2,2-tetrachloroethane. Hexachloroethane was also identified in expired air. Because of the limitation of techniques, volatile metabolites were not identified.

Fowler (1969) identified metabolites in several tissues (liver, kidney, brain, fat, and muscle), urine, feces, and bile taken from sheep administered hexachloroethane orally at 0.5 g/kg. Tetrachloroethylene was identified as the only major metabolite, and pentachloroethane was identified as a minor metabolite. The concentration of metabolites and parent compound in blood 24 hr after dosing ranged from 0.6 to 1.1 µg/mL for tetrachloroethylene, 0.06 to 0.50 µg/mL for pentachloroethane, and 10 to 28 µg/mL for hexachloroethane. The level of tetrachloroethylene increased rapidly and reached a maximum in about 3 hr; the levels remained constant for 4 days and declined rapidly during day 5. The blood levels of pentachloroethane increased rapidly and peaked at 24 hr; the concentration declined rapidly between 24 and 48 hr and declined more slowly thereafter. The concentration of hexachloroethane in blood 24 hr after dosing ranged from 10 to 28 µg/mL and declined over the next 4 days. The distribution of metabolites in tissues excised from two sheep killed 8.5 hr after administration of 0.5 g/kg of hexachloroethane orally is presented in Table 7. The concentration of tetrachloroethylene was almost always higher than the concentration of the parent compound. Tetrachloroethylene was also the major metabolite when fresh liver slices were incubated with hexachloroethane in vitro (Fowler 1969).

The metabolites were also found in higher concentrations in feces than in urine. During the first 24 hr, 854 µg of tetrachloroethylene was detected in feces of one sheep, and 1,300 µg in another, whereas only 25 and 29 µg, respectively, were detected in urine. Pentachloroethane was detected only in trace quantities in the feces of one sheep and 468 µg in the other, whereas 20 and 25 µg, respectively, were detected in urine. After 24 hr, the quantity of metabolites declined significantly in feces and urine, and the level of tetrachloroethylene approximated that of the parent compound. Tetrachloroethylene (0.3 µg/g) was also found in bile 4 hr after dosing (Fowler 1969).

TABLE 7. CONCENTRATIONS OF HEXACHLOROETHANE AND METABOLITES
IN TISSUES FROM TWO SHEEP ADMINISTERED
0.5 g HEXACHLOROETHANE ORALLY^{a, b}

Tissues	Tissue Concentration ($\mu\text{g/g}$) ^c		
	HCE	PCE	TCE
Fat	1.1/trace	0.02/nil	2.1/0.6
Kidney	0.1/trace	trace/trace	1.2/0.6
Liver	0.2/trace	0.01/trace	0.9/2.8
Muscle	0.04/trace	0.01/trace	0.01/trace
Brain	0.2/trace	0.02/trace	0.9/trace

a. Adapted from Fowler 1969.

b. HCE = hexachloroethane, PCE = pentachloroethane,
TCE = tetrachloroethylene.

c. Value for sheep No. 27/value for sheep No. 28, 8.5 hr
after dosing.

4.1.2.2. Mechanism of dechlorination

The means by which hexachloroethane is metabolized (dechlorinated) to tetrachloroethylene as the major metabolite rather than pentachloroethane or tetrachloroethane has received considerable attention. These studies were usually conducted using in vitro systems consisting of hepatic microsomes prepared from animals pretreated with phenobarbital.

According to Heppel and Porterfield (1948, as reported by Van Dyke and Wineman 1971), dechlorination (cleavage of the carbon-chlorine bond) in mammalian systems is an enzymatic process. Bray et al. (1952) measured the release of chloride ions released into the medium after incubating rabbit liver extracts with hexachloroethane and other chlorinated organic compounds and reported that the factor responsible for dechlorination was heat stable and for the most part nondialyzable. They suggested that dechlorination was not due to an enzyme, but to an interaction between the chlorinated compound and sulfhydryl compounds in the extract. An enzyme called alkyl-S-transferase was reported to catalyze the substitution of a halogen (chloride) on a halogenated aliphatic by glutathione through the sulfhydryl group, which causes release of the halide (chloride) and the formation of mercapturic acid (Barnsley 1966, as cited in Van Dyke and Wineman 1971).

Van Dyke and Wineman (1971) measured the release of inorganic chloride from hexachloroethane in a system containing the following components: rat liver microsomes (105,000-g pellet from a 9,000-g

supernatant), NADPH generating system (NADP^+ , glucose-6-phosphate, glucose-6-phosphate dehydrogenase), 105,000-g supernatant, and [^{36}Cl]-hexachloroethane. They reported that only 3.9 percent of the chloride was released enzymatically. Because a substantial amount of dechlorination took place in the absence of the NADPH generating system, they concluded that some dechlorination occurred by a nonenzymatic mechanism. A later report by Van Dyke (1977), however, stated that the 3.9 percent dechlorination thought to be enzymatic was in fact nonenzymatic and was due to the instability of hexachloroethane in aqueous systems.

Other studies have shown that dechlorination of hexachloroethane is indeed an enzymatic process initiated by the binding of hexachloroethane to cytochrome P-450 (Town and Leibman 1979, 1984, Nastainczyk et al. 1982a, Salmon et al. 1985). The binding of hexachloroethane with cytochrome P-450 is indicated by a shift in the spectra (Type I spectral change) of hepatic microsomes incubated with hexachloroethane (Pelkonen and Vainio 1975). A spectral shift, which is the difference between the wavelength at maximum and minimum absorbance, results when hexachloroethane or other chlorinated hydrocarbons interact specifically with cytochrome P-450. As the number of chlorine atoms on the carbon skeleton increases, the magnitude of the Type I spectral change also increases; consequently, the chloroethane causing the greatest spectral change is hexachloroethane. A double-reciprocal plot of the magnitude of Type I spectral changes against the concentration of microsomal cytochrome P-450 shows that, as the number of chlorine atoms increases, the value of the spectral dissociation constant decreases, indicating that compounds with the largest number of chlorine atoms have the highest affinity for cytochrome P-450. Therefore, of the chlorinated ethanes studied, hexachloroethane has the highest affinity for cytochrome P-450 (Pelkonen and Vainio 1975). Pelkonen and Vainio (1975) also showed that the double-reciprocal plots for hexachloroethane, pentachloroethane, and tetrachloroethylene are biphasic. This observation was interpreted as evidence for two binding sites with different affinities (high and low). The spectral dissociation constants for the high- and low-affinity binding sites for hexachloroethane were 8 μM and 48 μM , respectively.

Several investigators conducted in vitro studies to characterize the dechlorination reaction by hepatic microsomal enzymes. As in the in vivo studies, the major metabolite of hexachloroethane identified in the in vitro studies was also tetrachloroethylene; pentachloroethane and trichloroethylene were minor metabolites (Town and Leibman 1979, 1984; Nastainczyk et al. 1982a; Salmon et al. 1985). The reactions were quantitated by (1) identifying and measuring the volatile metabolites released into the headspace using gas chromatographic techniques (Town and Leibman 1979, 1984; Salmon et al. 1985), (2) identifying and measuring the metabolites in the extract of the reaction mixture using gas chromatographic techniques (Nastainczyk et al. 1982a,b), (3) measuring the oxidation of NADPH spectrophotometrically (Nastainczyk et al. 1982a,b; Salmon et al. 1985), or (4) measuring the amount of inorganic chloride released into the reaction mixture.

Town and Leibman (1979) used a 9,000-g supernatant from livers obtained from rats pretreated with phenobarbital to characterize the dechlorination of several chlorinated ethanes. The reaction, normally conducted under anaerobic conditions, was completely inhibited by pure O₂ and partially inhibited by air. Complete removal of O₂, however, was not required for maximum activity. The pH optimum ranged from 7.25 to 8.5. Using a 105,00-g microsomal pellet, parameters of the reaction kinetics were as follows: $K_m = 5.7 \mu M$ (K_m is the Michaelis-Menten constant, which is the substrate concentration at one-half the maximum velocity), $V_{max} = 29.7 \mu M/min/mg$ of microsomal protein (V_{max} is the maximum velocity when the substrate saturates all binding sites on the enzyme).

In a more detailed report, Town and Leibman (1984) used hepatic microsomes from male Holtzman rats, New Zealand white rabbits, and ICR mice. The rats and mice were pretreated with phenobarbital at 75 mg/kg/day (i.p.), and the rabbits with 15 mg/kg/day (i.p.) for 3 days. The incubation system was the same as that described by Town and Leibman (1979) and consisted of a 9,000-g supernatant or a 105,000-g microsomal pellet and a NADPH generating system. The metabolites (primarily tetrachloroethylene) released into the headspace were measured by GC. Inorganic chloride was also measured in the reaction mixture using a Buchler-Cotlove chloridometer.

Almost all of the enzymatic activity was localized in the microsomes; a small, but insignificant, amount was localized in the cytosol. Town and Leibman (1984) found that phenobarbital induced the maximum level of enzymatic activity in the microsomes relative to distilled water, cottonseed oil, or 3-methylcholanthrene, which did not induce the enzymatic activity. The formation of tetrachloroethylene was linear up to about 10 min, and the pH optimum ranged from 7.0 to 7.5. If any part of the NADPH generating system was omitted, the formation of tetrachloroethylene was decreased by at least 90 percent. In the presence of N₂ and glucose-glucose oxidase to remove residual O₂, 50.2 ± 0.45 nmol of tetrachloroethylene/min/mg of microsomal protein (mean $\pm$ SD) was formed; air inhibited the reaction by 97.5 percent, and pure O₂ by 100 percent.

Inhibitors of microsomal enzymes inhibited the formation of tetrachloroethylene--100 percent carbon monoxide by 100 percent, SKF 525-A by 18.3 percent (1 mM) or 52.1 percent (4 mM), and metyrapone (4 mM) by 58.4 percent. According to Town and Leibman (1984) the localization of the enzymatic activity in microsomes, inhibition of enzyme activity by carbon monoxide, SKF 525-A, and metyrapone, and the dependence of enzyme activity on the NADPH generating system suggest that the activity is mediated by cytochrome P-450. These data confirm the interaction of hexachloroethane with cytochrome P-450 indicated by Type I spectral changes as described by Pelkonen and Vainio (1975).

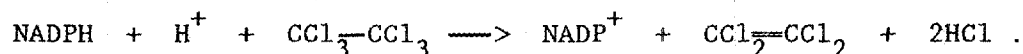
The kinetics for the formation of tetrachloroethylene using rat liver microsomes was 1.20 mM for the K_m and 52.0 nmol/min/mg of microsomal protein for the V_{max} . Using mouse liver microsomes, the kinetics for the formation of tetrachloroethylene was 3.34 mM for the K_m and 30.2 nmol/min/mg of microsomal protein for the V_{max} . The formation of

tetrachloroethylene using rabbit liver microsomes could not be measured (Town and Leibman (1984)). Salmon et al. (1981) reported a similar K_m of 2.37 mM, but a different V_{max} of 0.91 nmol/min/mg of rat microsomal protein (from phenobarbital-induced) for the release of radioactive chlorine from hexachloroethane. For uninduced rat liver microsomes the K_m was 6.0 μ M, and the V_{max} was 3.35 nmol (NADPH)/min/mg of microsomal protein (or 2.41 nmol (NADPH)/min/nmol of cytochrome P-450) (Salmon et al. 1985).

Salmon et al. (1985), using hepatic microsomes from rats and rabbits, also showed that tetrachloroethylene was the major metabolite under both aerobic and anaerobic conditions; trichloroethylene was formed under aerobic conditions in small quantities and in larger quantities under anaerobic conditions. Pentachloroethane was also formed under anaerobic conditions.

Salmon et al. (1985) proposed that the dechlorination of hexachloroethane, based on the effects of inhibitors and the nature of the products, is a reductive dechlorination reaction dependent on cytochrome P-450 and not an oxidative dechlorination reaction. Reductive dechlorination is less susceptible to the inhibitors than oxidative dechlorination (Town and Leibman 1984). Nastainczyk et al. (1982a) stated that hexachloroethane cannot be metabolized oxidatively due to its lack of hydroxylatable C-H bonds. Unlike the reductive dechlorination of carbon tetrachloride in which the direct release of a chloride ion leads to the formation of a carbon-centered radical that is released from the enzyme, the intermediate radical product of hexachloroethane is immediately subjected to a second reductive dechlorination, and another chloride ion is released (Nastainczyk et al. 1982a, Salmon et al. 1985).

A mechanism for the reductive metabolism of hexachloroethane (and other halogenated alkanes) by liver microsomal cytochrome P-450 was presented by Nastainczyk et al. (1982a). These authors showed that the rate of oxidation of NADPH correlated with the rate of formation of metabolites of hexachloroethane. The mean of three microsomal preparations from phenobarbital-induced rat livers was 1.07 ± 0.2 nmol of tetrachloroethylene per nmol of NADPH. According to Nastainczyk et al. (1982a) this observation indicated that two electrons are required for the formation of one olefin (tetrachloroethylene) molecule. The stoichiometric equation for the oxidation of NADPH and the formation of tetrachloroethylene is as follows:



The oxidation of NADPH is inhibited by carbon monoxide and high concentrations of metyrapone and α -naphthoflavone, which suggest that the reductive metabolism is dependent on cytochrome P-450. NADH, which could act synergistically with NADPH, also supported a small amount of

dechlorination. Nastainczyk et al. (1982a) suggested that the second electron could also be transferred by cytochrome b₅ to cytochrome P-450.

Nastainczyk et al. (1982a) further proposed that, as hexachloroethane accepts an electron from cytochrome P-450 under anaerobic conditions, a chloride ion leaves the molecule to form a radical ferric cytochrome complex ($\text{Fe}^{3+} \cdot \text{CCl}_2\text{-CCl}_3$), which is not released from the enzyme. The radical ferric cytochrome complex accepts a second electron from cytochrome P-450 or from cytochrome b₅ to form a carbanion complex ($\text{Fe}^{3+} \cdot \text{ICCl}_2\text{-CCl}_3$). Nastainczyk et al. (1982a) suggested several alternatives for the next step, depending on the products formed. Formation of pentachloroethane would suggest that the carbanion is protonated, and formation of a carbene complex would suggest that the second chloride is lost by α -elimination. Pentachloroethane was detected only in small amounts, and a carbene complex was not detected, but tetrachloroethylene was formed stoichiometrically. Therefore, the authors concluded that a second chloride ion is lost by β -elimination (Nastainczyk et al. 1982a,b).

4.2 ACUTE TOXICITY

4.2.1 Animal Data

Hexachloroethane was first tested as an anthelmintic in dogs by Hall and Cram (1925, as cited in Olsen 1947). It was first used as a fasciolicide in cattle by Thienel (1926, as cited in Olsen 1947), and Vianello (1937, as cited in Olsen 1947) noted that a dose of 1.42 g/kg was well tolerated by cattle. Another report stated that a single dose of 200 g of hexachloroethane suspended in bentonite and water did not cause adverse effects in cattle in average condition, but 100 g caused adverse effects in debilitated cattle (Olsen 1944). Bywater (1955) reported that debilitated animals are particularly susceptible to hexachloroethane and may die after only one-half the therapeutic dose. He also noted that deaths appeared to occur most often in susceptible herds, which were on a low nutrition diet and had been fasted 15 to 20 hr prior to dosing. Nervous symptoms and death occurred 1.5 to 20 hr after the second therapeutic dose. The death rate in these cattle was 12 per 1,000 cattle.

Clinical signs of toxicity associated with the administration of hexachloroethane to livestock include: temporary loss of appetite, mild diarrhea, weakness, reeling, drowsiness, prostration, narcotization, and death. Microscopic lesions include dilatation of the renal proximal convoluted tubules and degeneration and desquamation of the epithelial lining of the distal portions of the proximal convoluted tubules. Microscopic lesions were not found in the liver (Olsen 1947).

A summary of the lethality data for laboratory animals is presented in Table 8. Additional information is presented below.

Anesthetized dogs or rabbits were administered hexachloroethane (dissolved in olive oil) by either gavage, intravenous injection (i.v.),

TABLE 8. LETHALITY OF HEXACHLOROETHANE^a

Species	Route	Dose (mg/kg)	Remarks ^b
Rat	Oral	5,160	LD ₅₀ , male, corn oil diluent
		7,690	LD ₅₀ , male, methyl cellulose diluent
Rat	Oral	4,460	LD ₅₀ , female, corn oil diluent
		7,080	LD ₅₀ , female, methyl cellulose diluent
Guinea pig	Oral	4,970	LD ₅₀ , male, corn oil diluent
Rabbit	Oral	>1,000	ALD, male, methyl cellulose diluent
Rabbit	Dermal	32,000	LD ₅₀ , male, water paste
Rat	i.p.	2,900	ALD, male, corn oil diluent

a. From Weeks et al. 1979.

b. ALD = approximate lethal dose; LD₅₀ = lethal dose causing 50% mortality.

or subcutaneous injection (Barsoum and Saad 1934). The i.v. doses given to dogs ranged from 0.68 to 350 mg/kg. Doses $\leq$ 300 mg/kg did not cause death, whereas doses of 325 and 350 mg/kg caused death within 30 min. The minimum lethal dose (MLD) was 325 mg/kg. Another group of dogs were administered hexachloroethane by gavage at doses ranging from 1.0 to 6.0 g/kg; all animals were alive after 7 days. Because there were no deaths, an MLD could not be determined. A group of rabbits were subcutaneously injected with 1 to 4 g/kg of hexachloroethane. Although one animal died after 5 days, Barsoum and Saad (1934) concluded that an MLD could not be determined.

Male Sprague-Dawley rats (6 animals per dose) were administered hexachloroethane (dissolved in corn oil) by gavage (Weeks and Thomasino 1978). The animals were given doses of 2,510, 3,160, 3,980, 5,010, 6,310, 7,940, or 10,000 mg/kg and observed for 14 days. Toxic symptoms included tremors, ataxia, and convulsions at doses $\geq$ 5,010 mg/kg. A red exudate was

observed around the eyes throughout the observation period. All animals exposed to 2,510 or 3,980 mg/kg survived, two animals administered 3,160 and 5,010 mg/kg died, five animals administered 6,310 and 7,980 mg/kg died, and all animals administered 10,000 mg/kg died (Weeks and Thomasino 1978). The LD₅₀ was reported as 5,160 mg/kg (Table 8) (Weeks et al. 1979). Rats administered hexachloroethane by intraperitoneal injection exhibited the same symptoms as those administered the compound by gavage.

Male New Zealand rabbits (five per dose) were administered 100, 320, or 1,000 mg/kg (dissolved in methyl cellulose) of hexachloroethane by gavage (Weeks et al. 1979). The animals were treated daily for 12 days, weights were taken daily, and blood chemistry tests were performed on days 1, 4, 8, and 12 after initiation of treatment and day 4 after termination of treatment. The survivors were killed 4 days after termination of treatment, organs were weighed, and histopathological evaluations were performed.

Animals administered 1,000 mg/kg (high dose) showed a significant reduction in weight gain starting with day 7, whereas animals administered 320 mg/kg (medium dose) showed a significant reduction in weight gain starting with day 10, and animals administered 100 mg/kg (low dose) showed no gross toxic effects. The relative liver and kidney weights in the high-dose animals were increased significantly. Blood potassium and glucose levels were significantly reduced in medium- and high-dose animals; serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, and bilirubin levels were increased but not significantly (Weeks et al. 1979).

The histopathology evaluation showed hepatic and renal lesions in high- and medium-dose animals but not in low-dose animals or controls. The hepatic lesions were dose related as well as treatment related. Renal lesions, which did not increase in severity in high-dose animals, were treatment related but not dose related. The hepatic lesions consisted of degeneration and necrosis characterized by fatty degeneration, coagulation necrosis, hemorrhage, ballooning degeneration, eosinophilic changes, and hemosiderin-containing macrophages and giant cells. The renal lesions consisted of toxic tubular nephrosis involving the convoluted tubules in the corticomedullary region and minimal tubular nephrocalcinosis (Weeks et al. 1979).

In an acute inhalation study, six male rats per dose were exposed to 260 ppm (2.5 mg/L) of hexachloroethane for 8 hr, 5,900 ppm (57 mg/L) for 8 hr, or 1,000 ppm (17 mg/L) for 6 hr (Weeks and Thomasino 1978, Weeks et al. 1979). All animals were observed for 14 days for signs of toxicity and killed at the end of the observation period for gross necropsy and histopathological evaluation. Rats exposed to 260 ppm of hexachloroethane for 8 hr showed no effects related to exposure, and a staggered gait in two of six rats was the only effect observed in rats exposed to 1,000 ppm for 6 hr. In rats exposed to 5,900 ppm for 8 hr, one showed a staggered gait at 6 hr, two were dead by 8 hr, and body weight gain was reduced in the survivors. The histopathological examination revealed minimal to

moderate subacute interstitial pneumonitis with associated vascular congestion in two of the four survivors.

Studies to evaluate hexachloroethane for dermal and eye irritation in rabbits showed that 0.5 g of dry crystalline hexachloroethane was not irritating to either abraded or intact rabbit skin; 0.5 g of hexachloroethane in a water paste caused a slight transient redness; 0.1 g placed on the cornea caused transient irritation consisting of moderate corneal opacity, iritis, severe swelling, and severe discharge, which lasted for 72 hr in five of six rabbits. In dermal sensitization studies, guinea pigs treated with 0.1 percent hexachloroethane for induction and challenge showed no recognizable signs of sensitization (Weeks and Thomasino 1978, Weeks et al. 1979).

4.2.2 Human Data

Hexachloroethane causes neurologic effects manifested by the inability to close the eyelids. It also causes eye irritation, tearing, inflammation of the delicate membrane lining of the eye, and visual intolerance to light (photophobia) (NIOSH 1978).

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Animal Data

4.3.1.1 Oral exposure

In a subchronic toxicity study, Gorzinski et al. (1985) administered hexachloroethane (99.4 percent purity) in the diet of male and female Fischer 344 rats (10/sex/dose). The animals were kept on the diets for approximately 15 hr/day for 16 weeks (112 days). The doses estimated from measurements of food consumption and analysis of hexachloroethane in the food were 0, 1, 15, and 62 mg/kg/day. Body weights were measured weekly and food consumption was measured biweekly on nonconsecutive days. Urine and hematology analyses were performed during week 13, and serum chemistry, gross necropsy, and histopathology examination of major organs were performed when treatment was terminated.

The results showed no gross clinical signs of toxicity; body weight gain, food consumption, serum chemistry, and hematology and urine analyses were not altered due to treatment. Relative liver weights were increased by 4.5 percent and 4.9 percent in high-dose male and female rats, respectively; relative kidney weights were increased by 5.5 percent in high-dose males (Gorzinski et al. 1985). The increases were statistically significant but were probably not physiologically significant due to the small magnitude of the increase.

Gross and histopathological examination of the major organs showed significant damage only in the liver and kidneys. Lesions in other organs

were judged not to be treatment related. In male rats, nephrotoxic effects were manifested as slight hypertrophy and dilation of the renal proximal convoluted tubules; the incidence of this lesion was as follows: 0/0 (controls), 1/10 (low dose), 7/10 (medium dose), and 10/10 (high dose). Another renal lesion in male rats, described as slight atrophy and degeneration of the renal tubules with peritubular fibrosis, had the following incidence: 1/10 (controls), 2/10 (low dose), 7/10 (medium dose), and 10/10 (high dose). In female rats, nephrotoxic effects were less severe, and the only sign of toxicity was a very slight atrophy and degeneration of the renal tubules. The incidence of this lesion in female rats was as follows: 1/10 (controls), 1/10 (low dose), 2/10 (medium dose), and 6/10 (high dose) (Gorzinski et al. 1985). The more severe effects of hexachloroethane in male rats are consistent with the higher levels of hexachloroethane and metabolites found in the kidneys of male rats. The hepatotoxic effects of hexachloroethane were manifested as slight swelling of hepatocytes in male rats. The incidence was as follows: 4/10 (controls), 3/10 (low dose), 6/10 (medium dose), and 8/10 (high dose). No hepatotoxic effects were observed in female rats.

In a 6-week range-finding test to establish the MTD for a carcinogenicity study, Osborne-Mendel rats and B6C3F1 mice were administered hexachloroethane (dissolved in corn oil) by gavage. Five male and five female rats per dose group were given 178, 316, 562, 1,000, or 1,780 mg/kg/day, 5 days/week for 6 weeks. The same number of mice were given 316, 562, 1,000, 1,780, or 3,160 mg/kg/day for the same length of time. The survivors were observed for an additional 2 weeks (NCI 1978). Doses of 562 mg/kg/day or less were not lethal to any of the rats, a dose of 1,000 mg/kg/day killed some of the rats, and a dose 1,780 mg/kg/day killed all the rats. Mean body weights were reduced by 38 percent in male rats and 18 percent in female rats given 1,000 mg/kg/day. The estimated MTD in rats was established as 500 mg/kg/day (NCI 1978). Doses of 1,000 mg/kg/day or less were not lethal in male mice, and doses of 1,780 mg/kg/day were not lethal in female mice, but a dose of 3,160 mg/kg/day killed 4/5 male and 3/5 female mice. Mean body weight gain was substantially reduced in both male and female mice given 3,160 mg/kg/day. The MTD was established as 1,000 mg/kg/day in male and female mice (NCI 1978).

The MTD's from the 6-week range-finding test were used as the high-dose level in a long-term study in mice and rats (NCI 1978). This study evaluated both the chronic toxicity and carcinogenicity of hexachloroethane in both species. Mice were given 0, 500 (increased to 600), or 1,000 mg/kg/day (increased to 1,200), and rats were given 0, 250, or 500 mg/kg/day. After week 22, the rats were treated in cycles of 4 weeks treatment followed by 1 week without treatment. The animals were dosed by gavage 5 days/week for 66 weeks (rats) or for 78 weeks (mice). Detailed protocols along with time-weighted-average (TWA) doses are presented in Sect. 4.6. All animals were examined daily for mortality. Body weights, food consumption, signs of clinical toxicity, and location, size, and incidence of tissue masses were recorded weekly for 10 weeks and monthly thereafter. Gross necropsies were performed on all animals whether they died during the test, were killed when moribund, or were killed at the end

of the test. All major organs and tissues were subjected to histopathological examinations when possible.

The results showed a significant dose-related decrease in weight gain throughout the test in male rats. In female rats, weight gain was decreased up to 80 weeks, but at termination the weights of females were similar to the weights of control females. During the first year of treatment, the incidence of the following clinical symptoms was increased: hunched appearance; reddened, squinted, or lacrimating eyes; and abdominal urine stains. The effects were treatment related but not dose related. During the second year the incidence of these symptoms was decreased to the control levels.

The mortality rate and the incidence of significant nonneoplastic lesions in rats are presented in Table 9. The mortality rate includes the number of animals that died due to treatment as well as those that were killed in the moribund state, were killed on schedule, were accidentally killed, or were missing. In males, accelerated mortality was significantly associated with increased dosage ($P < 0.001$, Tarone test); only 8 of 50 low-dose males and 10 of 50 high-dose males survived to termination. Mortality in treated female rats was significantly higher than in controls; 27 of 50 low-dose females and 24 of 50 high-dose females survived to termination.

Histopathological evaluation revealed that toxic tubular nephropathy was the only treatment-related and dose-related lesion observed in rats (Table 9). This lesion appeared more frequently in low-dose male rats than in low-dose female rats, but in high-dose rats the incidence was similar, indicating a sex difference in the incidence only at the low dose. Toxic tubular nephropathy was characterized by degeneration, necrosis, and the appearance of large hyperchromatic regenerative epithelial cells in the kidney. A similar lesion was found in Fischer 344 rats administered hexachloroethane in their diets at doses of 1 to 62 mg/kg/day for 16 weeks (Gorzinski et al. 1985), in rabbits administered hexachloroethane by gavage at 320 or 1,000 mg/kg/day for 12 days (Weeks et al. 1979), and in cattle administered hexachloroethane therapeutically (Olsen 1947), but not in rats exposed by inhalation (Weeks et al. 1979). Therefore, kidney lesions appear to be common to animals exposed to hexachloroethane orally. The identity and incidence of neoplastic lesions will be discussed in Sect. 4.6.

In mice treated with hexachloroethane, mean body weight gain was not significantly affected in either males or females. Clinical toxicity was not significant, except that after week 38 a hunched or thin appearance was observed more frequently in treated mice than in controls. The mortality rate and incidence of nonneoplastic lesions in mice are presented in Table 9. Mortality in female mice was much lower than in male mice, and neither sex exhibited significant positive dose-related trends in mortality.

TABLE 9. MORTALITY AND INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS
AND MICE GIVEN HEXACHLOROETHANE BY GAVAGE^a

Parameters	Dose ^b					
	Controls		Low Dose		High Dose	
	M	F	M	F	M	F
<u>Rats</u>						
Mortality	12/20 (60%)	6/20 (30%)	42/50 (84%)	23/50 (46%)	40/50 (80%)	26/50 (52%)
Toxic nephropathy	0/20 (0%)	0/20 (0%)	22/49 (45%)	9/49 (18%)	33/50 (66%)	29/49 (59%)
<u>Mice</u>						
Mortality	15/20 (75%)	4/20 (20%)	43/50 (86%)	10/50 (20%)	21/50 (42%)	16/50 (32%)
Toxic nephropathy	0/20 (0%)	0/20 (0%)	49/50 (98%)	50/50 (100%)	47/49 (96%)	45/49 (92%)

a. Adapted from NCI 1978.

b. TWA doses in rats were 212 mg/kg/day (low dose) and 423 mg/kg/day (high dose) for 66 weeks; TWA doses in mice were 590 mg/kg/day (low dose) and 1,179 mg/kg/day for 78 weeks.

Toxic tubular nephropathy was found in more than 90 percent of treated male and female mice, but in 0 percent of the control mice (Table 9). This lesion was characterized by degeneration of the convoluted tubules in the corticomedullary region. The identity and incidence of neoplastic lesions in mice treated with hexachloroethane will be presented in Sect. 4.6.

4.3.1.2 Inhalation toxicity

Three mammalian species, rats (25 each sex per dose), guinea pigs (10 males), and dogs (4 males) were exposed to hexachloroethane at concentrations of 15 ± 4 (mean $\pm$ S.D.), 48 ± 15 , or 260 ± 49 ppm 6 hr/day, 5 days/week for 6 weeks (Weeks et al. 1979). Gross necropsy and histopathological evaluations were performed immediately after termination of exposure and after a 12-week recovery period. The animals were observed daily for clinical toxicity and were weighed weekly. Additional tests for behavioral abnormalities, pulmonary function, oxygen consumption, blood chemistry, and hematology were performed on selected animals.

Dogs exposed to the highest concentration showed signs of clinical toxicity consisting of tremors, ataxia, hypersalivation, severe head bobbing, and facial muscular fasciculation. These effects disappeared during the night and reappeared when the animals received the next exposure. Over the 6-week exposure period, body weights were normal, blood chemistry was unchanged, and resistance and compliance tests for pulmonary function were normal. One animal convulsed and died after the first exposure. In male guinea pigs, body weight was reduced from week 2 through termination, and relative liver weight was significantly increased. Two animals died, one each during weeks 4 and 5. Dermal sensitization tests performed on guinea pigs challenged with 0.1 percent hexachloroethane were negative (Weeks et al. 1979).

In young male and female rats exposed to hexachloroethane, one male and one female died during week 4. Symptoms of clinical toxicity observed in all animals included tremors, ruffled pelt, and the presence of a red exudate around the eyes. Body weight gain was reduced in males, but not in females. Relative kidney, spleen, and testes weights in males and relative liver weights in females were increased. Oxygen consumption, measured after termination of treatment as a general assessment of metabolism, was significantly decreased in rats exposed to the highest concentration. Histopathological findings included mucopurulent exudate from the nose, pneumonitis sometimes associated with bronchiectasis, and lymphoid hyperplasia in the lamina propria of the trachea in rats exposed only to 260 ppm. These lesions were not observed after the 12-week recovery period. Older male rats (12 to 14 weeks of age) exposed to hexachloroethane showed significantly lower absolute body weights starting at week 4 in animals exposed to 260 ppm than in control rats. Relative liver, lung, kidney, and testes weights were increased. Behavioral tests, which included avoidance performance and spontaneous motor activity, showed that hexachloroethane had no effect on behavior (Weeks et al. 1979).

Overall, the subchronic inhalation study by Weeks et al. (1979) showed that dogs and guinea pigs may be more susceptible to hexachloroethane exposure than rats. In addition, the results indicated that exposure to hexachloroethane up to 260 ppm has only mild toxic effects, with the exception of nervous system stimulation in dogs. These studies also showed that, unlike oral exposure, microscopic lesions are not induced in systemic organs after inhalation exposure to hexachloroethane.

4.3.2 Human Data

No data were available on chronic toxicity in humans.

4.4 GENOTOXICITY

4.4.1 Animal Data

Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and the yeast Saccharomyces cerevisiae strain D4 were used to test hexachloroethane for mutagenic activity (Weeks and Thomasino 1978, Weeks et al. 1979). Hexachloroethane was dissolved in DMSO (dimethylsulfoxide) and used at concentrations of 0.1, 1.0, 10, 100, and 500 $\mu\text{g}/\text{plate}$, with and without S9 metabolic activation. Hexachloroethane did not exhibit mutagenic activity in either Salmonella or Saccharomyces, with or without metabolic activation.

A study performed by Simmon and Kauhanen (1978) also showed that hexachloroethane is not mutagenic in the Salmonella reversion assay or in the yeast mitotic recombination assay. Mutagenicity tests were performed with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100. Hexachloroethane was dissolved in DMSO and tested at concentrations of 10, 50, 100, 500, 1,000, and 5,000 $\mu\text{g}/\text{plate}$, with and without S9 activation. Because hexachloroethane was not mutagenic or toxic at these concentrations, the compound was again tested at concentrations of 500, 1,000, 2,500, 5,000, 7,500, and 10,000 $\mu\text{g}/\text{plate}$. Hexachloroethane was slightly toxic at the highest concentration, but it was not mutagenic. Positive controls were tested with sodium azide, 9-aminoacridine, 2-nitrofluorene, or 2-anthramine. The mitotic recombination assay was performed with Saccharomyces cerevisiae strain D3. Hexachloroethane was tested at concentrations of 0.1, 0.5, 1.0, and 5.0 percent (w/v or v/v, dissolved in DMSO) with and without S9 activation. Hexachloroethane was not mutagenic in yeast. The positive control was tested with 1,2,3,4-diepoxybutane (Simmon and Kauhanen 1978). These tests showed that hexachloroethane is slightly toxic, but it is not mutagenic in Salmonella or Saccharomyces.

4.4.2 Human Data

No data were available on genotoxicity in humans.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

4.5.1 Animal Data

Hexachloroethane administered orally (gavage) or by inhalation was tested in rats for maternal and fetal toxicity (Weeks et al. 1979). Each dose group consisted of 22 animals. Oral doses were 50, 100, and 500 mg/kg/day, and inhalation doses were 15, 48, and 260 ppm, 6 hr/day. The animals were exposed from day 6 to day 16 and killed on day 20 of gestation. All animals were weighed and observed daily for signs of clinical toxicity. Maternal effects included reduced body weight gain starting on day 8 of gestation in animals exposed to the high dose by gavage or

inhalation and on day 14 of gestation in animals exposed to the medium dose by inhalation. Toxic effects included moderate tremors and respiratory irritation in 70 percent and subclinical pneumonitis in 20 percent of the rats exposed orally to the high dose. The incidence of respiratory irritation and subclinical pneumonitis was only 10 percent in control rats. Additional effects in rats exposed by inhalation included moderate tremors and an 85 percent incidence of mucopurulent nasal exudate in medium-dose animals and a 100 percent incidence in high-dose animals. No significant soft tissue or skeletal malformations were observed in fetuses from rats exposed either by gavage or by inhalation. The gestation index was lower, fewer live fetuses per female were produced, and the resorption rate was higher in rats exposed to the high dose by gavage (data not presented).

4.5.2 Human Data

No data were available on developmental and reproductive toxicity in humans.

4.6 ONCOGENICITY

4.6.1 Animal Data

Male and female Osborne-Mendel rats and male and female B6C3F1 mice were used to test hexachloroethane for carcinogenic activity (NCI 1978). Groups of 50 animals were given hexachloroethane (dissolved in corn oil) by gavage. A vehicle control was given corn oil only, and another control was untreated. Protocols for the tests in rats and mice are presented in Tables 10 and 11, respectively. Additional information on the selection of dose levels from a 6-week range-finding test and data on nonneoplastic effects were presented in Sect. 4.3. Mortality data were presented in Table 9.

Statistical analysis included the one-tailed Fisher exact test (Cox, 1970, as cited in NCI 1978), which compared the tumor incidence in control with the incidence in treated animals at each dose level. The Cochran-Armitage test for linear trend in proportions with continuity correction (Armitage 1971, as cited in NCI 1978) was used to determine if the slope of the dose-response curve was significantly different from zero. Early deaths that were not associated with neoplastic lesions were excluded by basing the tumor incidence on the number of animals that survived 52 weeks. Data were analyzed using both matched vehicle controls and pooled vehicle controls.

Data on the incidence of neoplastic lesions in rats are presented in Table 12; the tumor incidence is based on the total number of animals or the total number of specific sites examined. According to the NCI (1978), the survival rate of treated male rats was too low for the appearance of late-developing lesions. Therefore, in males the statistical analysis was

TABLE 10. PROTOCOL FOR HEXACHLOROETHANE CARCINOGENICITY
EXPERIMENT IN OSBORNE-MENDEL RATS^a

Treatment	Group Size	Dose ^b (mg/kg)	Observation Period		TWA
			Treated (weeks)	Untreated (weeks)	Dose ^c (mg/kg)
<u>Males</u>					
Untreated control	20	0	0	112	0
Vehicle control	20	0	78	33	0
Low dose	50	250	22	0	212
		250 ^d	44	12	
		0		34	
High dose	50	500	22	0	423
		500 ^d	44	12	
		0		34	
<u>Female</u>					
Untreated control	20	0	0	112	0
Vehicle control	20	0	78	33	0
Low dose	50	250	22	0	212
		250 ^d	44	12	
		0		34	
High dose	50	500	22	0	423
		500 ^d	44	12	
		0		34	

a. NCI 1978.

b. Hexachloroethane was dissolved in corn oil and administered by gavage, 5 days/week. Vehicle controls received corn oil only.

c. TWA = time-weighted average dosage = $\frac{\sum (\text{dosage} \times \text{weeks received})}{78 \text{ weeks}}$.

d. These doses were cyclically administered with a pattern of 1 dose-free week followed by 4 weeks of dosing at the indicated levels. The number of weeks treated does not include the dose-free weeks.

TABLE 11. PROTOCOL FOR HEXACHLOROETHANE CARCINOGENICITY
EXPERIMENT IN B6C3F1 MICE^a

Treatment	Group Size	Dose ^b (mg/kg)	Observation Period		TWA
			Treated (weeks)	Untreated (weeks)	Dose ^c (mg/kg)
<u>Males</u>					
Untreated control	20	0	0	90	0
Vehicle control	20	0	78	12	0
Low dose	50	500	8	0	590
		600	70	0	
		0		13	
High dose	50	1,000	8	0	1,179
		1,200	70	0	
		0		13	
<u>Female</u>					
Untreated control	20	0	0	90	0
Vehicle control	20	0	78	13	0
Low dose	50	500	8	0	590
		600	70	0	
		0		13	
High dose	50	1,000	8	0	1,179
		1,200	70	0	
		0		13	

a. NCI 1978.

b. Hexachloroethane was dissolved in corn oil and administered by gavage, 5 days/week. Vehicle controls received corn oil only.

c. TWA = time-weighted average dosage = $\frac{\sum (\text{dosage} \times \text{weeks received})}{\sum (\text{weeks receiving HCE})}$.

TABLE 12. INCIDENCE OF TUMORS IN RATS GIVEN HEXACHLOROETHANE BY GAVAGE ^{a,b}

Tumor	Males			Female		
	Control	Low	High	Control	Low	High
Pituitary adenoma/ carcinoma	2/19	4/42	0/44	7/20	15/50	6/46
Thyroid adenoma or carcinoma	2/20	3/48	5/48	2/20	3/47	3/47
Mammary fibroadenomas	0/20	0/49	0/50	6/20	13/50	9/50
Kidney tubular cell adenoma	0/20	4/49	0/50	0/20	0/50	3/49 ^c
Testis/ovarian	0/20	0/48	3/50	0/20	4/48	0/49

a. NCI 1978.

b. Incidence = number of animals with tumors per number of animals examined histologically at that site or where appropriate, the number of animals grossly examined.

c. Hamartoma - considered to be a benign form of malignant mixed tumor of the kidney consisting of proliferative lipocytes, tubular structures, fibroblasts, and vascular spaces in varying proportions.

based on the number of animals that survived 52 weeks: 18 vehicle control, 36 low-dose, and 29 high-dose male rats. The Cochran-Armitage test ($P < 0.048$) indicated that the incidence of interstitial cell tumors of the testis was significantly associated with dose, but the Fisher exact test indicated no significance. The incidence of kidney tumors and tumors at other sites was not significant in either sex. Therefore, according to the data presented by the NCI (1978), hexachloroethane was not carcinogenic in rats under these test conditions.

Data on the incidence of neoplastic lesions in mice given hexachloroethane by gavage are presented in Table 13. Although very few male mice in the untreated-control, treated-control, and low-dose groups survived until the end of the experiment, adequate numbers of animals survived long enough for late-developing lesions to appear and to make statistical analyses meaningful. The incidence of hepatocellular carcinomas was significantly increased in both male and female mice treated with hexachloroethane ($P \leq 0.008$, Fisher exact test; $P \leq 0.001$, Cochran-Armitage test). The latency was decreased in male mice (55 weeks for controls, 53 weeks for low-dose males, and 41 weeks for high-dose males), but not in females. The incidence of lung adenomas/carcinomas and malignant lymphomas was also increased, but not significantly. Therefore, under the

TABLE 13. INCIDENCE OF TUMORS IN MICE GIVEN HEXACHLOROETHANE BY GAVAGE ^{a,b}

Tumor	Males			Female		
	Control	Low	High	Control	Low	High
Hepatocellular carcinoma ^c	3/20 (6/60)	15/50	31/49	2/20 (2/60)	20/50	15/49
Lung adenoma/ carcinoma	0/20 (0/60)	2/50	3/49	1/20 (2/60)	1/50	4/49
Malignant lymphoma	0/20	0/50	0/49	4/20 (8/60)	12/50	9/49

a. NCI 1978.

b. Incidence = number of animals with a tumor per number of animals examined grossly or histologically at that site. Numbers in parenthesis are incidences in pooled vehicle controls.

c. Statistics: Cochran-Armitage test, $P < 0.001$ for males and females vs pooled controls, and males only vs matched controls; Fisher exact test, $P \leq 0.012$ for high-dose males and low-dose females vs matched controls; $P \leq 0.008$ for all groups vs pooled controls.

conditions of this test, hexachloroethane was carcinogenic in mice, inducing an increase in the incidence of hepatocellular carcinomas in both males and females.

The microscopic appearance of the hepatocellular carcinomas varied somewhat. These lesions were described as follows: "Some contained well-differentiated hepatic cells that had a relatively uniform arrangement of the cords and others had very anaplastic liver cells with large hyperchromatic nuclei, often with inclusion bodies and with vacuolated pale cytoplasm. Arrangement of the neoplastic liver cells varied from short, stubby cords to nests of hepatic cells and occasionally pseudo-acinar formation. Mitotic figures were often present." The lesions in control mice were not different from those in treated mice (NCI 1978).

4.6.2 Human Data

No data were available on oncogenicity in humans.

4.7 SUMMARY

The efficiency of absorption of hexachloroethane has not been determined, but it is absorbed very rapidly from the gastrointestinal tract. It appears in bile within 15 min and in blood within 27 min after oral dosing. The distribution of hexachloroethane shows the highest uptake in fat, but the uptake by kidney is sex-dependent. The concentration in male

kidneys is 4 to 47 times higher than in female kidneys, depending on the dose. The sex difference in the uptake of hexachloroethane by the kidney reflects the difference in the severity of toxic effects in this organ. The primary route of excretion of orally administered hexachloroethane in sheep is the feces with less excreted into urine, but more hexachloroethane is excreted in expired air than into urine. In mice and rats, the primary route of excretion may be expired air.

Many metabolites of hexachloroethane have been identified, but the best designed experiments have shown that tetrachloroethylene is the major metabolite. Depending on the conditions, pentachloroethane and trichloroethane are minor metabolites. Hexachloroethane is metabolized in the liver by enzymatic dechlorination involving microsomal cytochrome P-450. The microsomal activity is induced in liver by phenobarbital, but not by methylcholanthrene. Maximum activity requires a NADPH-generating system and anaerobic conditions; the activity can be inhibited by air or O₂. The activity is also inhibited by carbon monoxide and high concentrations of SKF 525-A and metyrapone. Metabolism of hexachloroethane involves a two-electron-reductive dechlorination reaction, rather than an oxidative reaction.

In humans, hexachloroethane causes neurologic effects and irritation and inflammation of the eyes. Clinical signs of toxicity in animals given hexachloroethane include temporary loss of appetite, mild diarrhea, weakness, reeling, drowsiness, prostration, narcotization, tremors, ataxia, convulsions, and possibly death. Microscopic lesions in livestock were found in the kidney, but not in the liver. The oral LD₅₀s range from 4,460 mg/kg to 7,690 mg/kg depending on species and vehicle used to administer the compound. The ALD in rats given hexachloroethane by i.p. injection is 2,900 mg/kg, and the MLD in dogs by i.v. injection is 325 mg/kg. The dermal LD₅₀ in rabbits is >32,000 mg/kg.

Oral exposures in rabbits at doses between 100 and 1,000 mg/kg/day for 12 days cause a significant decrease in weight gain, increase in relative liver and kidney weights, and degenerative changes in the liver and kidney at medium- and high-dose levels. Single inhalation exposures of rats from 260 ppm (2,500 mg/m³) to 5,900 ppm (57,000 mg/m³) for 6 to 8 hr cause no effect at the lower dose, central nervous system (CNS) effects at the medium doses, and CNS effects, respiratory irritation, and 33.3 percent mortality at the higher doses.

Hexachloroethane placed on the skin as a dry powder is not irritating to rabbits, but hexachloroethane placed on the skin as a paste is transiently irritating. Hexachloroethane introduced into the eyes of rabbits is transiently irritating, causing corneal opacity, iritis, severe swelling, and severe discharge. Hexachloroethane does not sensitize the skin of guinea pigs.

The primary nonneoplastic target of hexachloroethane administered orally in an acute, subchronic, or chronic protocol is the kidney. Nephrotoxic effects (toxic tubular nephropathy) were observed in almost all animal species tested. In rats exposed to hexachloroethane in their

diets at doses from 1 to 62 mg/kg/day for 16 week, renal lesions are characterized by hypertrophy and dilation of the proximal convoluted tubules and atrophy and degeneration of the renal tubules with peritubular fibrosis. This lesion is more severe in male than in female rats and may be due to the significantly higher quantities of hexachloroethane and metabolites distributed to the kidneys in male rats. In mice, renal lesions are found in almost 100 percent of both male and female mice treated with 590 or 1,179 mg/kg/day for 78 weeks. The lack of a sex difference in mice may have been due to the magnitude of the low dose. The lesion is characterized by degeneration of the proximal convoluted tubules in the corticomedullary region. The nonneoplastic hepatotoxic effects in animals exposed to hexachloroethane in their diets are mild and are characterized by slight swelling of hepatocytes.

Subchronic inhalation exposure of dogs, guinea pigs, and rats to 15, 48, or 260 mg/m³, 6 hr/day, 5 days/week for 6 weeks causes only mild toxic effects in laboratory animals. These effects include respiratory tract irritation, CNS effects, changes in body weight gain, and increases in relative organ weights, but no histopathological effects. Thus, nephrotoxic effects, so prevalent in animals exposed orally, are not found in animals exposed by inhalation.

Hexachloroethane is not mutagenic in bacteria up to 10,000 µg per plate or in yeast up to 0.5 percent (w/v or v/v) with or without S9 activation. Carcinogenicity tests in rats given hexachloroethane at doses of 212 or 423 mg/kg/day (TWA doses) and in mice at doses of 590 or 1,179 mg/kg/day (TWA doses) by gavage showed that the compound induces an increase in hepatocellular carcinomas in mice, but not in rats. Pituitary adenomas and carcinomas, thyroid adenomas or carcinomas, mammary fibroadenomas, kidney adenomas or hamartomas, and testicular (or ovarian) tumors were observed in rats; the incidence of these lesions was not significantly related to exposure. Lung adenomas, lung carcinomas, and malignant lymphomas were induced in mice, but the incidence was not significantly related to exposure.

Tests for reproductive and developmental effects showed that hexachloroethane given either orally or by inhalation causes respiratory tract irritation in the mothers, but no soft tissue or skeletal malformation in the fetuses. A lower gestation index, fewer live fetuses per female, and a higher resorption rate were observed.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

Table 14 summarizes the guidelines, standards, and regulations enacted to protect individuals from excessive exposure to hexachloroethane in the workplace and in the environment.

5.2 OCCUPATIONAL EXPOSURE

Standards and guidelines related to occupational exposure to hexachloroethane are listed in Table 14. The latest figures on the number of workers estimated to be exposed to hexachloroethane were obtained from a survey conducted by NIOSH during 1972-74, which showed that approximately 1,500 workers were at risk for exposure (NIOSH 1978). The principal routes of exposure to hexachloroethane would be inhalation and dermal and ocular contact. In an industrial setting, an excessive amount of hexachloroethane dust was reported to cause irritation. The dust was assigned a moderate hazard rating, involving both reversible and irreversible changes, but not severe enough to cause permanent injury or death. Fumes of hexachloroethane were reported to be moderately irritating to the skin and mucous membranes (RTECS 1977, as cited in Cichowicz et al. 1983).

Pitt (1982) determined a vapor hazard index, which is a measure of the amount of a substance in a saturated atmosphere relative to the threshold limit value (TLV). Large values imply greater danger. The vapor hazard index for hexachloroethane was 0.3 at 20°C. According to Pitt (1982), values between 0 and 1 would represent low danger requiring only "ordinary ventilation and good practice." Amore and Hautala (1983) estimated the concentration in a saturated atmosphere as 770 ppm at 25°C, and using a TLV of 10 ppm and the formula proposed by Pitt (1982), the vapor hazard index would be 0.77, low enough to still be classified as low danger via inhalation.

Workers most likely to be exposed to hexachloroethane would be those involved in formulating and loading pyrotechnic agents (smoke grenades) containing hexachloroethane. Military personnel who use smoke grenades in the field would experience only limited exposure because, upon combustion, most of the hexachloroethane is consumed when the zinc chloride cloud is produced, or it is converted to other substances (carbon monoxide, hydrogen chloride, the highly toxic phosgene gas, and other chlorinated hydrocarbons) (Katz et al. 1980).

TABLE 14. SUMMARY OF GUIDELINES, STANDARDS, AND REGULATIONS^a

Agency/Source	Guidelines/Standards/Regulations	Reference
USOSHA	1 ppm, 10 mg/m ³ (skin) (PEL, 8-hr TWA)	USOSHA 1986
NIOSH	Lowest feasible limit, treat as carcinogen; 300 ppm (IDLH)	USDHHS 1985
ACGIH-TLV	10 ppm, 100 mg/m ³ (TWA)	ACGIH 1986-87
ILO	3 ppm, 30 mg/m ³ (STEL, ACGIH)	ILO 1980
USSR	10 µg/L (RMCL)	Tugarinova et al. 1962
USEPA	Listed as toxic pollutant designated pursuant to section 307(a)(1) of the Clean Water Act	USEPA 1985e
USEPA	Listed as chemical substance regulated pursuant to section 3(2) of TSCA	USEPA 1985f
USEPA	Listed as acute hazardous waste to be managed pursuant to Section 3001 of RCRA	Clansky 1986
USEPA	Listed as hazardous substance to be managed pursuant to Section 102(a) of CERCLA, with final RQ of 1 lb (0.454 kg) subject to change when assessment of potential carcinogenicity and/or chronic toxicity is completed	USEPA 1985g

a. ACGIH = American Conference of Governmental Industrial Hygienists; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; IDLH = Immediately dangerous to life or health; ILO = International Labor Organization; NIOSH = National Institute for Occupational Safety and Health; PEL = Permissible exposure limit; RCRA = Resource Conservation and Recovery Act; RMCL = Recommended maximum contaminant level; RQ = Reportable quantity; STEL = Short term exposure limit (15 min); TLV = Threshold limit value; TSCA = Toxic Substance Control Act; TWA = Time weighted average; USEPA = U.S. Environmental Protection Agency; USOSHA = U.S. Occupational Safety and Health Administration; USSR = Soviet Union.

5.3 PREVIOUSLY CALCULATED CRITERIA

The USEPA (1979a) recommended freshwater and saltwater aquatic criteria for hexachloroethane using procedures other than the guidelines. The criteria were based on inferences from toxicity data for pentachloroethane and saltwater organisms. In a lifecycle test with mysid shrimp exposed to pentachloroethane, the Final Acute Value (FAV) was less than the Final Chronic Value (FCV) by a factor of 0.44; therefore, the criteria for the other chlorinated ethanes are estimated as 0.44 times the FAVs for each chemical. Consequently, the freshwater criterion for hexachloroethane was calculated as 0.44 times the final fish acute value of 140 $\mu\text{g/L}$, and the saltwater criterion was calculated as 0.44 times the final invertebrate acute value of 16 $\mu\text{g/L}$. The criteria were stated as follows: for freshwater aquatic life, the 24-hr average concentration is recommended as 62 $\mu\text{g/L}$, with the concentration not exceeding 140 $\mu\text{g/L}$ at any time; the criterion for saltwater aquatic life is 7.0 $\mu\text{g/L}$ as a 24-hr average, with the concentration not exceeding 16 $\mu\text{g/L}$ at any time.

The USEPA (1979a, 1980b) derived two human criteria for hexachloroethane based on the same carcinogenicity data (data from NCI 1978) used in this report. The methodology used to calculate the original criteria was based on a modified "one hit" extrapolation model used to estimate the concentration of hexachloroethane corresponding to lifetime incremental risks of 10^{-5} , 10^{-6} , and 10^{-7} (USEPA 1979b, as cited in USEPA 1979a). The concentrations calculated to keep lifetime risk below the targets were 5.9, 0.59, and 0.059 $\mu\text{g/L}$, respectively, assuming individuals consumed both contaminated drinking water and contaminated fish. The concentrations associated with the consumption of contaminated fish only were 7.9, 0.79, and 0.079 $\mu\text{g/L}$ (USEPA 1979a). In calculating this criterion, the authors assumed that the duration of the study was equal to the life span of the experimental animals. Because all surviving animals were killed at a specified time after initiating treatment, this assumption would lead to a life span for B6C3F1 mice of only 91 weeks (637 days) and transformed doses of 421 and 842 mg/kg/day . According to Goodman et al. (1985), 75 to 80 percent of B6C3F1 mice are expected to survive 2 yr.

The human health criterion was recalculated (USEPA 1980b) using the "linearized" multistage extrapolation model developed by Crump (1984, cited as Crump 1980 in USEPA 1980c) in which the extra risk was calculated using the computer program GLOBAL 79 developed by Crump and Watson (1979). The concentrations of hexachloroethane corresponding to lifetime risks of 10^{-5} , 10^{-6} , and 10^{-7} were estimated as 19, 1.9, and 0.19 $\mu\text{g/L}$ for consumption of both water and fish and 87.4, 8.74, and 0.874 $\mu\text{g/L}$ for consumption of fish only (USEPA 1980b,d). Again the authors assumed that the life span of the animals was equal to the duration of the study (91 weeks). Consequently, the higher transformed doses (421 and 842 mg/kg/day) resulted in a higher criterion than that reported in Sect. 5.5.

5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by USEPA for the estimation of water quality criteria for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the FAV, whereas the CCC is equal to the lowest of the FCV, the Final Plant Value (FPV), or the Final Residue Value (FRV).

Acute data are available for fourteen freshwater species, fulfilling seven of the required eight families as described in the guidelines. Even though acute data are currently unavailable for the eighth required family (another family in any order of insect or another phylum not already represented), a tentative freshwater FAV can be estimated. Using Genus Mean Acute Values (GMAV) for 13 genera ($N = 13$), the freshwater FAV is 0.8497 mg/L (Table 15). Acute data are available for only two of the required eight families of saltwater species; therefore, a saltwater CMC cannot be calculated at this time.

Only one acceptable chronic study, an early life-stage bioassay with embryo-larval fathead minnows, is available. The data indicate that survival, growth, and hatchability are adversely affected at concentrations as low as 0.70 mg/L, with no effects observed at 0.41 mg/L. Therefore, the chronic-effect level, MATC, is calculated as 0.54 mg/L. It should be noted that this value is higher than the CMC (given below), indicating that further research is necessary to clearly define a true chronic-effect level. Because chronic data are not available for the other two required species (an invertebrate and a sensitive freshwater species), a FCV cannot be calculated at this time.

An acceptable 96-hr toxicity test with the freshwater alga Selenastrum capricornutum is available, with EC_{50} values of 87 and 93 mg/L, based on chlorophyll *a* content and cell number, respectively. Therefore, the freshwater FPV is established as the lowest test result, or 87 mg/L. An acceptable 96-hr toxicity test with the saltwater alga Skeletonema costatum is available, with EC_{50} values of 8.57 and 7.75 mg/L, based on chlorophyll *a* content and cell number, respectively. Therefore, the saltwater FPV is established as the lowest test result, or 7.75 mg/L.

One adequate steady-state BCF is available, 139 for bluegill sunfish; however, data from two other studies indicate that bioconcentration may be greater in other species, possibly correlated to total lipid content. In addition, a maximum permissible tissue concentration has not been established for hexachloroethane; therefore, a FRV cannot be calculated at this time.

In conclusion, the tentative freshwater CMC of 0.42 mg/L for hexachloroethane is calculated from the available data; however, it should be noted that data for one of the required eight families are missing. Even

TABLE 15. CALCULATIONS OF FINAL ACUTE VALUE (FAV)
FOR HEXACHLOROETHANE

Rank (R)	GMAV	ln GMAV	(ln GMAV) ²	P (R/(N+1))	$\sqrt{P}$
4	1.38	0.3221	0.1038	0.2857	0.5345
3	1.37	0.3148	0.0991	0.2143	0.4629
2	1.04	0.0392	0.0015	0.1429	0.3780
1	0.94	<u>-0.0619</u>	<u>0.0038</u>	<u>0.0714</u>	<u>0.2672</u>
Sum		0.6142	0.2082	0.7143	1.6426

GMAV = Genus Mean Acute Value in mg/L.

ln GMAV = natural log of GMAV.

N = 13.

P = Probability for each GMAV.

R = Rank of four lowest GMAV's.

$$S^2 = \frac{0.2082 - (0.6142)^2/4}{0.7143 - (1.6426)^2/4} = 2.8618; S = 1.6917$$

$$L = (0.6142 - (1.6917)(1.6426))/4 = -0.5412$$

$$A = (1.6917)(\sqrt{0.05}) + (-0.5412) = -0.1629$$

$$FAV = e^{-0.1629} = 0.8497 \text{ mg/L}; \text{ CMC} = 0.42 \text{ mg/L}$$

$$S^2 = \frac{\sum((\ln \text{GMAV})^2) - ((\sum(\ln \text{GMAV}))^2/4)}{\sum(P) - ((\sum(\sqrt{P}))^2/4)}$$

$$L = (\sum(\ln \text{GMAV}) - S(\sum(\sqrt{P}))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

though a freshwater FPV is established, data are insufficient to calculate the FCV and FRV; therefore, a CCC cannot be determined for hexachloroethane at the present time.

5.5 HUMAN HEALTH CRITERIA

No data were retrieved on the chronic toxicity or carcinogenicity of hexachloroethane in humans; therefore, a criterion cannot be derived from human data.

Hexachloroethane was tested in Osborne-Mendel rats and B6C3F1 mice, but was carcinogenic only in mice (NCI 1978). The protocols and results for the tests were presented in detail in Sect. 4.6 and will be stated only briefly in this section.

The mice were administered hexachloroethane by gavage 5 days/week at TWA dose of 0, 590, and 1,179 mg/kg for 78 weeks. Although mortality in control and low-dose male mice was high, adequate numbers of animals did survive long enough for late-developing lesions to appear. Statistical analysis showed that the incidence of hepatocellular carcinomas was significantly increased in both dose groups in both sexes when compared with the pooled vehicle controls (Fisher exact test). When compared with the matched vehicle controls, both low- and high-dose males and only the low-dose females showed a significant increase in hepatocellular carcinomas. The data from the study in male mice were judged to be adequate for deriving a criterion; standard protocols were followed with respect to sex and species used, numbers of animals per group, route of administration, duration of treatment, and duration of study. The treatment protocol (the dose was increased during treatment period) was unorthodox, but it was judged to be adequate. Statistical analysis showed that the overall quality of the data was adequate.

There are currently no methods available for determining a threshold for carcinogenic effects; consequently, a "safe" level cannot be estimated. Therefore, the recommended concentration for maximum protection of human health is "zero" (USEPA 1980e). Because it is usually not feasible to attain this concentration, USEPA (1980c,e) presented a range of concentrations corresponding to incremental cancer risks of 10^{-5} , 10^{-6} , and 10^{-7} . For example, a risk of 10^{-5} indicates that one additional case of cancer may occur for every 100,000 people exposed. The USEPA (1980c) adopted the "linearized" multistage model of Crump (1984, cited as Crump 1980 in USEPA 1980c), which used the computer program GLOBAL 79 developed by Crump and Watson (1979) and revised (GLOBAL 82) by Howe and Crump (1982). The USEPA (1980c) methodology for deriving water quality criteria for the protection of human health is summarized in Appendix B.

The pertinent data used to estimate the lifetime risk are summarized in Table 16. Pertinent formulae used to calculate the lifetime risk are listed below:

$$q_1^*(A) = \frac{95\% \text{ upper confidence interval}}{\text{MLE}} \times \left(\frac{L}{L_e} \right)^3,$$

$$q_1^*(H) = q_1^*(A) \times \left(\frac{W_H}{W_A} \right)^{0.3333},$$

$$C_A = \frac{70 \times 10^{-5}}{q_1^*(H) (2 + 0.0065 \text{ BCF})},$$

$$C_O = \frac{70 \times 10^{-5}}{q_1^*(H) (0.0065 \text{ BCF})}.$$

Where $q_1^*(A)$ is the animal carcinogenic potency; $q_1^*(H)$ is the human carcinogenic potency; C_A is the concentration of hexachloroethane in water that will keep the average lifetime risk below 10^{-5} ; C_O is the concentration of hexachloroethane in fish only, calculated to keep the average lifetime risk below 10^{-5} .

The other definitions are listed in Table 16.

The concentrations of hexachloroethane, derived for the consumption of water and fish, corresponding to extra lifetime risks of 10^{-5} , 10^{-6} , and 10^{-7} are 13 $\mu\text{g/L}$, 1.3 $\mu\text{g/L}$, and 0.13 $\mu\text{g/L}$, respectively. The concentrations derived for the consumption of fish only are 58 $\mu\text{g/L}$, 5.8 $\mu\text{g/L}$, and 0.58 $\mu\text{g/L}$, respectively. These values are different from those calculated (USEPA 1980b) and published by USEPA (1980d) (see Sect. 5.3). Because Goodman et al. (1985) reported that 75 to 80 percent of B6C3F1 mice survive an average of 2 yr, we assumed that the life span of the animals was 730 days, whereas USEPA (1980b) assumed the life span of the animals was equal to the duration of the study (637 days or 91 weeks). Because all surviving animals were killed 91 weeks after the study was initiated and did not live out their entire life span, the life span should not have been equated to the duration of the study. The lower value for life span resulted in higher values for transformed doses, consequently, higher criteria values.

TABLE 16. SUMMARY OF PERTINENT DATA FOR CALCULATING A CRITERION
FROM HEXACHLOROETHANE CARCINOGENICITY DATA

Reference	NCI 1978		
Exposure route	Oral (gavage)		
Species	Mice		
Strain	B6C3F1		
Sex	Male		
Vehicle or physical state	Corn oil		
Average body weight of mouse (W_A)	0.032 kg		
Duration of treatment (t_e)	78 weeks		
Duration of study (L_e)	91 weeks		
Life span of animal (L)	104.3 weeks (730 days) ^a		
Target organ	Liver		
Tumor type	Hepatocellular carcinoma		
Experimental doses (mg/kg/day)	0	590	1,179
Transformed doses ^b (mg/kg/day)	0	361	722
Tumor incidence	3/20	15/50	31/49
Tumor percentage	15%	30%	63%
95% Upper confidence limit	0.27207×10^{-2}		
Maximum likelihood estimate (MLE) (mg/kg/day)	2.49619		
Bioconcentration factor (BCF)	86.9 ^c		
Animal potency [$q_1^*(A)$] (mg/kg/day) ⁻¹	1.6404×10^{-3}		
Average body weight of human (W_H)	70 kg		
Human potency [$q_1^*(H)$] (mg/kg/day) ⁻¹	2.1289×10^{-2}		
Lifetime risk of 10^{-5} (C_A) (consumption of water and fish)	13 $\mu\text{g/L}$		
Lifetime risk of 10^{-5} (C_O) (consumption of fish only)	58 $\mu\text{g/L}$		

a. Life span assumed for mice (Goodman et al. 1985).

b. Experimental doses given 5 days/week, 78 weeks adjusted for 7 days/week for 104.3 weeks.

c. From Barrows et al. (1980) and adjusted for the percent lipid in ingested fish (3%) and the percent lipid in bluegill (4.8%) (USEPA 1980c).

5.6 RESEARCH RECOMMENDATIONS

In order to meet the requirements established by the USEPA for deriving water quality criteria, the following research studies are recommended to fill gaps in the existing data.

1. Acute toxicity tests, which follow ASTM procedures, should be conducted for a family in any insect order (e.g., Ephemeroptera - Hexagenia sp.) or phylum not already represented (e.g., Annelida - Lumbriculus variegatus).
2. Chronic flow-through tests should be conducted using measured concentrations for an invertebrate species (e.g., 21-day life cycle test with Daphnia magna) and a sensitive freshwater species (e.g., life cycle test with Lepomis macrochirus or Salmo gairdneri).
3. As a part of the chronic study explained in (2) above, acute flow-through tests using measured concentrations should be conducted for the same two species in order to calculate acute-chronic ratios.
4. Because only one definitive steady-state or 28-day bioconcentration study with bluegill is available, it is suggested that additional studies be conducted with other aquatic species (e.g., Salmo gairdneri; Ictalurus punctatus; Homarus americanus, American lobster).
5. A chronic wildlife feeding study or a long-term wildlife field study should be conducted in order to determine a maximum permissible tissue concentration for the calculation of an FRV.
6. The carcinogenicity test in rats was repeated by the National Toxicology Program. A draft report of the results should be available in August 1988. Because carcinogenicity data take precedence over other types of data for deriving human health criteria, additional studies are not required and are not recommended at this time.

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7. GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
ALD	Approximate lethal dose
ASTM	American Society for Testing and Materials
B/N	Base/neutral
BCF	Bioconcentration factor
BOD	Biological oxygen demand
C _A	Concentration of pollutant in water that will to keep the average lifetime risk below 10 ⁻⁵
CCC	Criterion Continuous Concentration
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CMC	Criterion Maximum Concentration
CNS	Central nervous system
O	Concentration of pollutant in fish only calculated to keep the average lifetime risk below 10 ⁻⁵
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DO	Dissolved oxygen
EC ₂₀	Effective concentration causing 20 percent inhibition of thymidine incorporation
EC ₄₀	Effective concentration causing 40 percent inhibition of thymidine incorporation
EC ₅₀	Median effective concentration causing 50 percent death based on immobilization, or 50 percent inhibition of thymidine incorporation, or a 50 percent reduction in light output
EC ₆₀	Effective concentration causing 60 percent inhibition of thymidine incorporation
EC ₈₀	Effective concentration causing 80 percent inhibition of thymidine incorporation

EC ₁₀₀	Effective concentration causing 100 percent inhibition of thymidine incorporation
ECD	Electron capture detector
FAV	Final Acute Value
FCV	Final Chronic Value
FID	Flame ionization detector
FPV	Final Plant Value
FRV	Final Residue Value
FSCC	Fused silica capillary column
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GMAV	Genus Mean Acute Value
GPC	Gel permeation chromatography
HCE	Hexachloroethane
HECD	Hall electrolytic conductivity detector
IDLH	Immediately dangerous to life or health
ILO	International Labor Organization
K _m	Michaelis-Menten constant, which is the substrate concentration at one-half the maximum velocity
K _{oc}	Organic carbon partition coefficient
L	Life span of animal
LC ₅₀	Lethal concentration causing 50 percent mortality
LD ₅₀	Lethal dose causing 50% mortality
L _e	Duration of study
l _e	Duration of treatment
LOEC	Lowest observable effect concentration
MATC	Maximum acceptable toxicant concentration

MLD	Minimum lethal dose
MLE	Maximum likelihood estimate
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NH	Northern hemisphere
NIOSH	National Institute for Occupational Safety and Health
NOEC	No observable effect concentration
PBN	<u>N</u> -tert-butyl- α -phenylnitrone
PC	Packed column
PCE	Pentachloroethane
PEL	Permissible exposure limit
POTW	Publicly owned treatment works
$q_1^*(A)$	Animal carcinogenic potency
$q_1^*(H)$	Human carcinogenic potency
RCRA	Resource Conservation and Recovery Act
RMCL	Recommended maximum contaminant level
RQ	Reportable quantity
SCOT	Support-coated open tubular
SGOT	Serum glutamic oxaloacetic transaminase
SH	Southern hemisphere
STEL	Short term exposure limit (15 min)
TCE	Tetrachloroethylene
TLV	Threshold limit value
TSCA	Toxic Substance Control Act
TWA	Time weighted average
USAEHA	U.S. Army Environmental Hygiene Agency

USDHHS	U.S. Department of Health and Human Services
USEPA	U.S. Environmental Protection Agency
USOSHA	U.S. Occupational Safety and Health Administration.
$V_{\max}$	Maximum velocity when the substrate saturates all binding sites on the enzyme
W_A	Average body weight of mouse
W_H	Average body weight of human

APPENDIX A:

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect aquatic life and is slanted towards the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species all of the time was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species was very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data: it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived: however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative: much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.
2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different non-ionizable covalently bonded organometallic compound should usually be considered a separate material.
3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants: (b) FDA action levels (FDA Guidelines Manual): and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.
2. All data used should be available in typed, dated and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.
3. Questionable data, whether published or not, should not be used.
4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.
5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.
6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:
 - a. the family Salmonidae in the class Osteichthyes:
 - b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish):
 - c. a third family in the phylum Chordata (e.g., fish or amphibian):
 - d. a planktonic crustacean (e.g., cladoceran or copepod):

- e. a benthic crustacean (e.g., ostracod, isopod, or amphipod):
 - f. an insect (e.g., mayfly, midge, stonefly):
 - g. a family in a phylum other than Arthropoda or Chordata (e.g., Annelida or Mollusca): and
 - h. a family in any order of insect or any phylum not represented.
2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.
 3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.
 4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.
2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).
3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L) should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.
5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:
 - a. Tests with daphnids and other cladocerans should be started with organisms < 24 hr old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC₅₀ based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC₅₀ is not available from a test, the 48-hr LC₅₀ should be used in place of the desired 48-hr EC₅₀. An EC₅₀ or LC₅₀ of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.
 - b. The result of tests with all other aquatic animal species should be the 96-hr EC₅₀ value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC₅₀ value is not available from a test, the 96-hr LC₅₀ should be used in place of the desired EC₅₀.
 - c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$ 96 hr.
 - d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.
6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.
7. If the data indicate that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV because a species can only be considered protected from acute toxicity if all life stages are protected.
8. Consider the agreement of the data within and between species. Questionable results in comparison to other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentration of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

NOTE: Data reported by original investigators should not be rounded off and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.
11. Order the GMAVs from high to low and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.
12. Calculate the cumulative probability (P) for each GMAV as $R/(N+1)$.
13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).
14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln \text{GMAV})^2) - ((\Sigma(\ln \text{GMAV}))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln \text{GMAV}) - S(\Sigma(\sqrt{P}))) / 4$$

$$A = S(\sqrt{0.05}) + L$$

$$\text{FAV} = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.
16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.
2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.
3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.
4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.
5. Similarly normalize the values of the water quality characteristic for each species individually.
6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are

actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).
9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.
10. For each species calculate the SMAV using: $SMAV = e^Y$.
11. Obtain the FAV at Z by using the procedure described in Section 5. (No. 10-14).
12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.
13. The Final Acute Equation is written as:

$$FAV = e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])}$$

where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one: this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.
3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.
5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:
 - a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young < 48 hr old, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young < 24 hr old and last for not < 21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.
 - b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.
 - c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.
7. If chronic toxicity of material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.
8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (No. 10-14). Then go to Section 7 (No. 13).
9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used. If acute tests were not conducted as part of the same study, acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.
10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.
11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.
 - a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

- b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute--Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and salt-water species.
- c. If the most appropriate species mean acute-chronic ratios are <2.0 , and especially if they are <1.0 , acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a FCV probably cannot be calculated.

- 12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.
- 13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.
- 14. Go to Section 9.

8. FINAL CHRONIC EQUATION

- 1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.
 - a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.
 - b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z by the Final Acute-Chronic Ratio.
 - c. Use V = pooled acute slope as L = pooled chronic slope.
 - d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.
 - a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.
 - b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.
 - c. Decide whether data for each species is useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.
 - d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.
 - e. Similarly normalize the values of the water quality characteristic for each species individually.
 - f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
 - g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

- h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.
- i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.
- j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^Q$).
- k. Obtain the FCV at Z by using the procedure described in Section 5 (No. 10-14).
- l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.
- m. The Final Chronic Equation is written as:

$$FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$$

where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.
2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.
3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation

factors. A maximum permissible tissue concentration is either (a) a FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.
3. If a maximum permissible tissue concentration is available for a substance (e.g., parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.
 - a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.
 - b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.
 - c. A BCF obtained from an exposure that adversely effected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.
 - d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be

converted to a wet tissue weight basis. If a conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

- e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, then the BCF for the longest exposure should be used.
4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:
 - a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.
 - b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.
 5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.
 - a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.
 - b. Calculate the geometric mean normalized BCF.
 - c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.
 - For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.
 - For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.
6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.
2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.
3. The Criterion Continuous Concentration (CCC) is equal to the lower of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.
4. Round both the CCC and CMC to two significant figures.
5. The criterion is stated as: The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) $\mu\text{g/L}$ more than once every three years on the average and if the one-hour average concentration does not exceed (4) $\mu\text{g/L}$ more than once every three years on the average.

Where,

- (1) = insert freshwater or saltwater,
- (2) = name of material,
- (3) = insert the Criterion Continuous Concentration, and
- (4) = insert the Criterion Maximum Concentration.

13. REFERENCES

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APPENDIX B

SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

- (a) Carcinogenicity, (b) Toxicity, and (c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish. Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to

be insignificant: however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).
2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.
3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980). Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}},$$

where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to 10^{-5} (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065 BCF)}$$

where,

C = ambient water concentration;
 PR = the probable risk (e.g., 10^{-5} ; equivalent to one case in 100,000);
 BCF = the bioconcentration factor; and
 q_1^* = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C (2 + 0.0065 BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065 BCF \times C)}{70}$$

where,

2C is the daily exposure resulting from drinking 2 liters of water per day and $(0.0065 \times BCF \times C)$ is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* \times X,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X},$$

showing that the coefficient q_1^* is the ratio of risk to dose: an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/-response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5} , which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared to the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then $RR(X) = 5$). In such cases the "excess" relative cancer risk is expressed as $RR(X) - 1$, and the actual numeric, or proportional excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(\text{control}).$$

Using the standard risk/dose equation:

$$PR(X) = b \times X$$

And substituting for PR(X):

$$[RR(X) - 1] \times PR(\text{control}) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(\text{control})}{X}$$

where b is equal to the carcinogenic potency or q_1^* .

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.
2. The data set giving the highest index of cancer potency (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.
3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.
4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the $2/3$ power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3}.$$

3. The dose must also be normalized to a lifetime average exposure. For an carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e , and the total length of the experiment is L_e , then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}}$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)^3$ to adjust for an age-specific increase in the cancer rate.
5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = \text{ppm} \times F \times r,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man: 0.05 for a 0.35-kg rat: and 0.13 for a 0.03-kg mouse).

Substituting $(\text{ppm} \times F)$ for m and fW for F , the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{\text{ppm} \times F}{W^{2/3}} = \frac{\text{ppm} \times f \times W}{W^{2/3}} = \text{ppm} \times f \times W^{1/3}$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol, and is absorbed proportionally to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption: which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp\{-g(d)H(t)\} ,$$

where $P(t;d)$ is the probable response for dose d and time t ; $g(d)$ is the polynomial function defining the effect of dose level, and $H(t)$ the effect of time:

$$g(d) = \sum_{i=0}^a \alpha_i d^i$$

$$H(t) = \sum_{i=0}^b \beta_i t^i$$

(with α and $\beta \geq 0$, and $\sum \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each S as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp\left\{-\sum_{i=0}^a \alpha_i d^i\right\},$$

or as given in the EPA guidelines (USEPA 1980):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \dots q_k d^k)] ,$$

where $P(d)$ is the lifetime risk (probability) of cancer at dose d .

For a given dose the excess cancer risk $A(d)$ above the background rate $P(o)$ is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

where,

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \dots + q_k d^k],$$

Point estimates of the coefficients $q_1 \dots q_k$ and consequently the extra risk function $A(d)$ at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function $A(d)$ has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R . Thus $A(d)$ and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1 . This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^* , the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_o - L_1) = 2.70554,$$

where L_o is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^h \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where N_i is the number of animals in the i th dose group, X_i is the number of animals in the i th dose group with a tumor response, P_i is the probability of a response in the i th dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (χ^2) is larger than the cumulative 99 percent point of the chi-square

distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at, and below which, the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065 \text{ kg} \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criteria.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

- (1) NOEL = No-Observed-Effect-Level,
- (2) LOEL = Lowest-Observed-Effect-Level,
- (3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
- (4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.
2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.
3. If a LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to a NOAEL or an LOAEL.
4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.
5. If for reasonably closely spaced doses only a NOEL and a LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, NOAEL, LOAEL, and clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.
2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.
3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported,

but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criteria (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times AA}{(AO \times SF)}$$

where,

BR = daily air intake (assume 10 m³),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
AA = efficiency of absorption from air,
AO = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{CA \times DE \times d \times AA \times BR \times 70 \text{ kg}}{(BWA \times AO \times SF)}$$

where,

- CA = concentration in air (mg/m³),
- DE = duration of exposure (hr/day),
- d = number of days exposed/number of days observed,
- AA = efficiency of absorption from air,
- BR = volume of air breathed (m³/day),
- 70 kg = standard human body weight,
- BWA = body weight of experimental animals (kg),
- AO = efficiency of absorption from oral exposure, and
- SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant: however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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