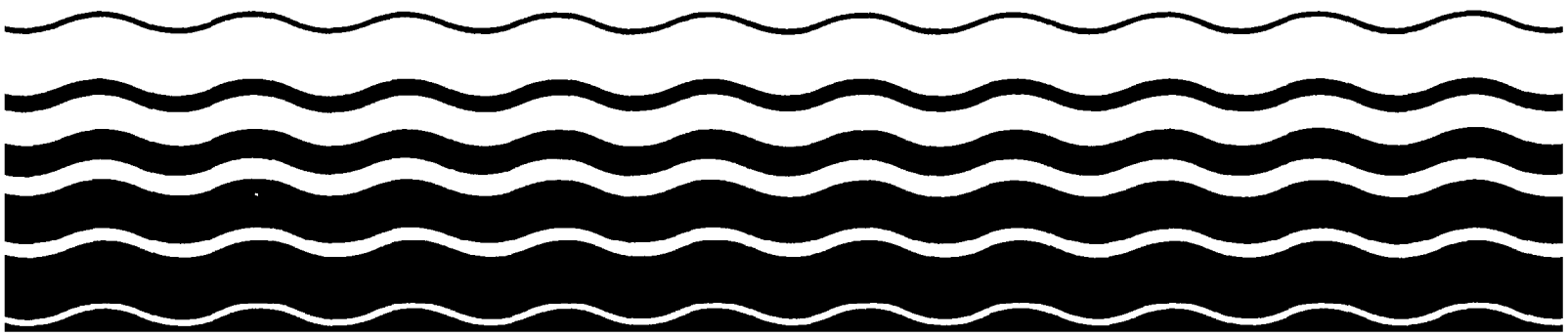
United States
Environmental Protection
Agency

Office of Water
Regulations and Standards
Criteria and Standards Division
Washington DC 20460

EPA 440/5-80-053
October 1980

EPA

Ambient Water Quality Criteria for Hexachlorobutadiene



AMBIENT WATER QUALITY CRITERIA FOR

HEXACHLOROBUTADIENE (HCBD)

Prepared By
U.S. ENVIRONMENTAL PROTECTION AGENCY

Office of Water Regulations and Standards
Criteria and Standards Division
Washington, D.C.

Office of Research and Development
Environmental Criteria and Assessment Office
Cincinnati, Ohio

Carcinogen Assessment Group
Washington, D.C.

Environmental Research Laboratories
Corvalis, Oregon
Duluth, Minnesota
Gulf Breeze, Florida
Narragansett, Rhode Island

DISCLAIMER

This report has been reviewed by the Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

AVAILABILITY NOTICE

This document is available to the public through the National Technical Information Service, (NTIS), Springfield, Virginia 22161.

FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

STEVEN SCHATZOW
Deputy Assistant Administrator
Office of Water Regulations and Standards

ACKNOWLEDGEMENTS

Aquatic Life Toxicology:

William A. Brungs, ERL-Narragansett
U.S. Environmental Protection Agency

David J. Hansen, ERL-Gulf Breeze
U.S. Environmental Protection Agency

Mammalian Toxicology and Human Health Effects:

Robert D. Lingg (author) HERL-Cin
U.S. Environmental Protection Agency

Michael L. Dourson (doc. mgr.) ECAO-Cin
U.S. Environmental Protection Agency

Bonnie Smith (doc. mgr.) ECAO-Cin
U.S. Environmental Proteciton Agency

Si Duk Lee, ECAO-Cin
U.S. Environmental Protection Agency

Joseph Santodonato
Syracuse Research Corporation

Roy E. Albert, CAG*
U.S. Environmental Protection Agency

Nancy Dwyer
Mobil Oil Corporation

John L. Laseter
University of New Orleans

Shane Que Hee
University of Cincinnati

Technical Support Services Staff: D.J. Reisman, M.A. Garlough, B.L. Zwayer, P.A. Daunt, K.S. Edwards, T.A. Scandura, A.T. Pressley, C.A. Cooper, M.M. Denessen.

Clerical Staff: C.A. Haynes, S.J. Faehr, L.A. Wade, D. Jones, B.J. Bordicks, B.J. Quesnell, C. Russom, R. Rubinstein.

*CAG Participating Members: Elizabeth L. Anderson, Larry Anderson, Ralph Arnicar, Steven Bayard, David L. Bayliss, Chao W. Chen, John R. Fowle III, Bernard Haberman, Charalingayya Hiremath, Chang S. Lao, Robert McGaughy, Jeffrey Rosenblatt, Dharm V. Singh, and Todd W. Thorslund.

TABLE OF CONTENTS

CRITERIA DOCUMENT

HEXACHLOROBUTADIENE

CRITERIA

Aquatic Life

The available data for hexachlorobutadiene indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 90 and 9.3 µg/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for hexachlorobutadiene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 32 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of hexachlorobutadiene to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of hexachlorobutadiene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 4.47 µg/l, 0.45 µg/l, and 0.045 µg/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 500 µg/l, 50 µg/l, and 5.0 µg/l, respectively.

INTRODUCTION

Hexachlorobutadiene (HCBD; C_4Cl_6) is produced deliberately in the United States as a by-product of the manufacture of chlorinated hydrocarbons such as tetrachloroethylene, trichloroethylene, and carbon tetrachloride. Secondary production estimates range from 7.3 to 14.5 million pounds (~ 5,000 MT) per year (U.S. EPA, 1975a). In 1974, approximately 0.5 million pounds (~ 230 MT) were imported into the U.S. (U.S. EPA, 1975a).

HCBD is used as a solvent for many organic substances; its relatively low vapor pressure gives it a distinct advantage over some other chlorohydrocarbons for this purpose. The largest domestic users of HCBD are chlorine producers, who use it to recover chlorine from "snift" gas which is cleaned by passage through HCBD. Other applications of HCBD include its use as an intermediate in the manufacture of rubber compounds and lubricants and as a fluid for gyroscopes (U.S. EPA, 1975a).

HCBD, a colorless liquid with a faint turpentine-like odor, has a water solubility of 5 µg/ml at 20°C. It has a melting point of about -21°C, a vapor pressure of 22 mm Hg at 100°C, and a specific gravity of 1.675 (Hawley, 1977).

Unlike most short chain halogenated aliphatics, hexachlorobutadiene has a low vapor pressure and, thus, may not volatilize rapidly from the aqueous environment to the atmosphere. Hexachlorobutadiene has been reported to be present in domestic drinking water supplies in low concentrations (U.S. EPA, 1975b) and has been detected at concentrations of 1.9 and 4.7 µg/l in water at two areas near Geismar, Louisiana. These concentrations indicate that HCBD may be quite persistent in natural waters. However, hexachloroethane, which is structurally somewhat similar to HCBD and exhibits a vapor pressure

of 0.4 torr at 20°C (Verschueren, 1977) in comparison to 0.15 torr for HCBD (Pearson and McConnell, 1975), appears to be volatilized rather rapidly from water. Dilling (1977) determined a volatilization half-life in water of 40.7 minutes for hexachloroethane initially present at 0.72 mg/l in an open system stirred constantly at 200 rpm. Although no specific rate data were found for HCBD, volatilization may be an important transport process for this compound in aqueous systems.

Sorption may also be an important process for HCBD. The currently reviewed literature contains an appreciable amount of information pertaining specifically to the adsorption of HCBD onto sediments. In a study of the Mississippi Delta region it was found that the level of hexachlorobutadiene in water was less than 2 µg/l while the concentration of hexachlorobutadiene in mud or soil samples exceeded 200 µg/l (U.S. EPA, 1976b). In this same study, water samples from the waste of an industrial company in Geismar, Louisiana, contained from <0.1 µg/l to 4.5 µg/l HCBD. Levels of HCBD in the mud, however, reached a maximum of 2,370 µg/l, indicating selective concentration of several orders of magnitude. Leeuwangh, et al. (1975) found that the concentration of HCBD in uncontaminated sediment after equilibration with water that contained HCBD was 100 times that found in the water.

Samples taken from Liverpool Bay (England) showed the presence of HCBD, but rarely at levels greater than 1 µg/l (Pearson and McConnell, 1975). McConnell, et al. (1975) noted that coarse gravels have little adsorptive capacity for chlorinated aliphatics, whereas sediments rich in organic detritus have a much higher adsorptive capacity. The calculated log P (octanol/water partition coefficient) of 3.74 implies that hexachlorobutadiene should be strongly adsorbed by humus material (U.S. EPA, 1979).

Environmental contamination by HCBD results primarily during the disposal of wastes containing HCBD from chlorinated hydrocarbon industries (U.S. EPA, 1976a). Disposal methods include landfill, high temperature incineration, deep-well injection, and lagoon storage (U.S. EPA, 1975a).

REFERENCES

Dilling, W.L. 1977. Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes, and propylenes from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11: 405.

Hawley, G.G. (ed.) 1977. The Condensed Chemical Dictionary. Van Nostrand Reinhold Co., New York.

Leeuwangh, P., et al. 1975. Toxicity of Hexachlorobutadiene in Aquatic Organisms. Sublethal Effects of Toxic Chemicals on Aquatic Animals. In: Proc. of Swedish-Netherlands Symp., Sept. 2-5. Elsevier Scientific Publishing Co., Inc., New York. (Abst.)

McConnell, G., et al. 1975. Chlorinated hydrocarbons and the environment. Endeavor. 34: 13.

Pearson, C.R. and G. McConnell. 1975. Chlorinated C_1 and C_2 hydrocarbons in the marine environment. Proc. Royal Soc. Lond. B. 189: 305.

U.S. EPA. 1975a. Survey of industrial processing data. Task I - Hexachlorobenzene and hexachlorobutadiene pollution from chlorocarbon processing. Midwest Res. Inst., Kansas City, Missouri. EPA 560/3-75-003. Off. Toxic Subst., U.S. Environ. Prot. Agency, Washington, D.C.

U.S. EPA. 1975b. Preliminary assessment of suspected carcinogens in drinking water. EPA 560/4-75-003. Off. Toxic Subst., U.S. Environ. Prot. Agency, Washington, D.C.

U.S. EPA. 1976a. Sampling and analysis of selected toxic substances. Task IB - Hexachlorobutadiene. EPA 560/6-76-015. Off. Toxic Subst., U.S. Environ. Prot. Agency, Washington, D.C.

U.S. EPA. 1976b. An ecological study of hexachlorobutadiene. EPA 560/6-76-010. Off. Toxic Subst., U.S. Environ. Prot. Agency, Washington, D.C.

U.S. EPA. 1979. Water-related environmental fate of 129 priority pollutants. EPA 68-01-3852. U.S. Environ. Prot. Agency, Washington, D.C.

Verschueren, K. 1977. Handbook of Environmental Data on Organic Chemicals. Van Nostrand Reinhold, New York.

Aquatic Life Toxicology*

INTRODUCTION

Available data for hexachlorobutadiene indicate that it is acutely toxic to freshwater aquatic life in the range of 90 to 326 µg/l. A physiological change in fish blood occurred at much lower concentrations and a chronic effect was observed on fathead minnows at 13.3 µg/l.

Static tests have been conducted with four saltwater species and the range of 96-hour LC_{50} values of 59 to 557 µg/l is similar to that for freshwater species.

EFFECTS

Acute Toxicity

A freshwater snail has been exposed to hexachlorobutadiene and the 96-hour LC_{50} value is 210 µg/l (Table 1).

Goldfish have been tested (Leeuwangh, et al. 1975) and the 96-hour LC_{50} is 90 µg/l (Table 1). The LC_{50} values for the fathead minnow, rainbow trout, and bluegill are 102, 320, and 326 µg/l, respectively. All the tested species, both fish and invertebrate, demonstrated a relatively narrow range of sensitivity.

Static tests have been conducted with the saltwater mysid shrimp, grass shrimp, pinfish, and sheepshead minnow; the 96-hour LC_{50} values are 59, 32, 399, and 557 µg/l, respectively (Table 1). The fish are approximately 10 times more resistant than the invertebrate species. Both fish species were affected, but not killed, by hexachlorobutadiene at concentrations below the LC_{50}: Cyprinodon variegatus at 240 µg/l and above, and Lagodon

*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

rhomboides at 180 µg/l and above. Within 24 hours of exposure, some swam in spirals at the surface of the water, while others swam on their sides or lay motionless, except for opercular movement, on the bottom.

Chronic Toxicity

An embryo-larval test with the fathead minnow (U.S. EPA, 1980) resulted in a chronic value of 9.3 µg/l (Table 2). After division of this into the 96-hour LC_{50} of 102 µg/l, an acute-chronic ratio of 11 is derived.

Plant Effects

No freshwater algal or vascular species have been tested.

Residues

Bioconcentration factors have been determined for goldfish (Leeuwangh, et al. 1975) and range from 920 to 2,300 (Table 3). Laseter, et al. (1976) obtained bioconcentration factors for an algal species, a crayfish species, and largemouth bass of 160, 60, and 29, respectively.

Miscellaneous

Laska, et al. (1978) exposed largemouth bass to concentrations of hexachlorobutadiene between 3.43 and 31.95 µg/l for 10 days and observed elevated blood corticosteroid levels (Table 4).

Summary

There is a narrow range of LC_{50} values, for freshwater fish and invertebrate species and hexachlorobutadiene, from 90 to 326 µg/l. The chronic value for the fathead minnow is 9.3 µg/l with an acute-chronic ratio of 11. There was a wide range of bioconcentration factors of 29 to 2,300 for a variety of organisms.

As with the freshwater organisms, there is a narrow range of LC_{50} values for saltwater fish and invertebrate species of 59 to 5578 µg/l with the invertebrate species being more sensitive. No data are available to estimate chronic toxicity.

CRITERIA

The available data for hexachlorobutadiene indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 90 and 9.3 µg/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for hexachlorobutadiene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 32 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of hexachlorobutadiene to sensitive saltwater aquatic life.

Table 1. Acute values for hexachlorobutadiene

Species	Method*	LC50/EC50 (µg/l)	Species Acute Value (µg/l)	Reference
		FRESHWATER SPECIES		
Snail, *Lymnaea stagnalis*	R, M	210	210	Leeuwangh, et al. 1975
Rainbow trout, *Salmo gairdneri*	FT, M	320	320	U.S. EPA, 1980a
Goldfish, *Carassius auratus*	R, M	90	90	Leeuwangh, et al. 1975
Fathead minnow, *Pimephales promelas*	FT, M	102	102	U.S. EPA, 1980a
Bluegill, *Lepomis macrochirus*	FT, M	326	326	U.S. EPA, 1980a
		SALTWATER SPECIES		
Mysid shrimp, *Mysidopsis bahia*	S, U	59	59	U.S. EPA, 1980b
Grass shrimp, *Palaemonetes pugio*	S, U	32	32	U.S. EPA, 1980b
Pinfish, *Lagodon rhomboides*	S, U	399	399	U.S. EPA, 1980b
Sheepshead minnow, *Cyprinodon variegatus*	S, U	557	557	U.S. EPA, 1980b

* S = static, FT = flow-through, R = renewal, U = unmeasured, M = measured

No Final Acute Values are calculable since the minimum data base requirements are not met.

Table 2. Chronic values for hexachlorobutadiene (U.S. EPA, 1980a)

Species	Test*	Limits (µg/l)	Chronic Value (µg/l)
FRESHWATER SPECIES			
Fathead minnow, *Pimephales promelas*	ELS	6.5-13.3	9.3

* ELS = Early life state

Acute-Chronic Ratio

Species	Acute Value (µg/l)	Chronic Value (µg/l)	Ratio
Fathead minnow, *Pimephales promelas*	102	9.3	11

Table 3. Residues for hexachlorobutadiene

Species	Tissue	Bioconcentration Factor	Duration (days)	Reference
		FRESHWATER SPECIES		
Alga, *Oedogonium cardiacum*	whole body	160	7	Laseter, et al. 1976
Crayfish, *Procambarus clarki*	whole body	60	10	Laseter, et al. 1976
Goldfish, *Carassius auratus*	-	920-2,300	49	Leeuwangh, et al. 1975
Largemouth bass, *Micropterus salmoides*	whole body	29	10	Laseter, et al. 1976

Table 4. Other data for hexachlorobutadiene (Laska, et al. 1978)

Species	Duration	Effect	Result (µg/l)
	FRESHWATER SPECIES		
Largemouth bass, Micropterus salmoides	10 days	Elevated blood corticosteroid levels	3.43-31.95

REFERENCES

Laseter, J.L., et al. 1976. An ecological study of hexachlorobutadiene (HCBD). U.S. Environ. Prot. Agency, EPA-560/6-76-010.

Laska, A.L., et al. 1978. Acute and chronic effects of hexachlorobenzene and hexachlorobutadiene in Red Swamp Crayfish (Procambarus clarki) and selected fish species. Toxicol. Appl. Pharmacol. 43: 1.

Leeuwangh, P., et al. 1975. Toxicity of hexachlorobutadiene in aquatic organisms. In: Sublethal effects of toxic chemicals on aquatic animals. Proc. Swedih-Netherlands Symp., Sept. 2-5. Elsevier Scientific Publ. Co., Inc., New York.

U.S. EPA. 1980a. Unpublished laboratory data. Environmental Research Laboratory - Duluth, Minnesota.

U.S. EPA. 1980b. Unpublished laboratory data. Environmental Research Laboratory - Gulf Breeze, Florida.

Mammalian Toxicology and Human Health Effects

EXPOSURE

Ingestion from Water

In 1974, the U.S. Environmental Protection Agency (EPA) conducted a survey of finished water from three New Orleans area water plants (Keith, et al. 1976). Immediately upstream on the Mississippi River from New Orleans are numerous petrochemical and chemical plants including some producing chlorohydrocarbon compounds. The concentrations of HCBD found ranged from 0.07 to 0.7 µg/l HCBD for the three test sites.

In 1975, a follow-up study conducted by EPA included 10 cities (U.S. EPA, 1975). HCBD was identified in one of the drinking water supplies surveyed in this group and the concentration was less than 0.01 µg/l. HCBD has been found in water samples taken from inland sites bordering the lower Mississippi River (Laseter, et al. 1976). In this study, a landfill pond near an industrial source was found to contain 4.49 µg/l and the corresponding value for the surrounding mud was 920 µg/kg (corrected to dry weight). Effluents from industrial plants suspected of being sources of HCBD contained concentrations ranging from 0.04 to 240 µg/l (Li, 1976).

The conclusions regarding HCBD contamination of drinking water supplies are: (1) HCBD contamination of U.S. finished drinking water supplies does not appear to be a widespread problem; (2) the problem is localized in areas with raw water sources near industrial plants producing HCBD; (3) the physical and chemical characteristics of HCBD favor rapid extraction of HCBD from

contaminated water into the surrounding sediment and mud (Laseter, et al. 1976).

Ingestion from Food

Since the water, soil, and air surrounding certain chlorohydrocarbon plants have been shown to be contaminated with HCBD (Li, et al. 1976), food produced in the vicinity of these plants is most likely to contain residual levels of HCBD. A survey of foodstuffs within a 25-mile radius of tetrachloroethylene and trichloroethylene plants was made. Milk, eggs, and vegetable samples did not contain measurable levels of HCBD. Freshwater fish caught in the lower Mississippi river contained HCBD residues in the range of 0.01 to 4.65 mg/kg (Yip, 1976; Yurawecz, et al. 1976).

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of

the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

Some bioconcentration factors are available for hexachlorobutadiene (Laseter, et al. 1976; Leeuwangh, et al. 1975), but the necessary data concerning percent lipids are not. The equation "Log BCF = (0.85 Log P) - 0.70" can be used (Veith, et al. 1979) to estimate the BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). Based on a measured log P value of 1.82 (Dec, et al. Manuscript), the steady-state bioconcentration factor for hexachlorobutadiene is estimated to be 7.03. An adjustment factor of 3.0/7.6 = 0.395 can be used to adjust the estimated BCF from the 7.6 percent lipids, on which the equation is based, to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for hexachlorobutadiene and the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 7.03 x 0.395 = 2.78.

Studies on HCBD contamination of food supplies have been reported from several foreign countries. In England, McConnell, et al. (1975) detected HCBD in fresh milk (0.08 µg/kg), imported grapes (3.7 µg/kg), and in tomatoes (0.8 µg/kg) grown on a reclaimed lagoon which was once used as a disposal area for a chemical plant. In Germany, Kotzias, et al. (1975) found 4 µg/kg HCBD in evaporated milk, 42 µg/kg in egg yolk, 33 µg/kg in vegetable oil

margarine, and 39 and 2 ug/kg in chicken grain feed and laying rations, respectively.

Inhalation

The air in the vicinity of plants producing tetrachloroethylene, trichloroethylene, and carbon tetrachloride has been shown to be contaminated with HCBD (Li, et al. 1976). A value as high as 460 ug/m^3 has been detected in one instance, but generally, the levels of HCBD detected in air surrounding these chlorohydrocarbon plants were less than 5 ug/m^3. There are uncertainties concerning the atmospheric fate of HCBD, but McConnell, et al. (1975) reported efficient tropospheric destruction of aliphatic organochlorine compounds via photo-oxidation. Unless someone lives or works in the vicinity of a chemical plant producing HCBD as a major byproduct, exposure to HCBD through inhalation would not seem to pose a problem.

PHARMACOKINETICS

Jacobs, et al. (1974) gave oral doses of a mixture of seven chlorinated hydrocarbons (2 mg/kg/component and 4 mg/kg/component) to rats daily for up to 12 weeks. Rats sacrificed at 4, 8, and 12 weeks had roughly 7 mg/kg or less HCBD accumulated in fatty tissue taken from the inner genital and kidney regions. Concentrations of HCBD on tissues were apparently the same at both dose levels. The quantity found in the liver, heart, kidney, and blood was less than that found in the fatty tissue. These results showed that HCBD did not have a strong tendency to accumulate in fatty tissue of selected organs if administered in a mixture with other chlorinated

hydrocarbons, some of which were aromatic and accumulated significantly in the fat.

EFFECTS

In 1971, Gehring and MacDougall (1971) completed a review of the toxic properties of HCBD. These authors concluded that the data available at that time did not allow for a critical evaluation of any potential hazard associated with long-term, repeated low level exposure to HCBD. Since then, several excellent studies have been published on the effects of repeated low level exposure to HCBD in rats. Kociba, et al. (1977) conducted a two-year study during which rats were given doses of 0.2, 2.0, and 20 mg/kg/day HCBD in their diet, and found HCBD induced renal neoplasms at the highest dose level. Schwetz, et al. (1977), working in the same laboratory, found that these same dosage levels had virtually no effect on the reproduction and offspring of rats. Only a slight decrease in body weight of weanlings at 21 days of age was observed at the highest dose level.

Acute, Subacute, and Chronic Toxicity

A table summarizing the acute toxicity data on HCBD which was published prior to 1971 was prepared by Gehring and MacDougall (1971) in their review and is reproduced in this document as Table 1. Pre-1971 acute toxicity data not included in their table and acute data published since are summarized in Table 2. Oral LD_{50} values range from 64 mg/kg in the female weanling rat to 580 mg/kg in the adult male rat, indicating that HCBD has a relatively high acute toxicity by the oral route. Toxicity varied with sex and age differentiation exists. Data on acute dermal and inhalation

Table 1

Acute Toxicity of Hexachlorobutadiene*

Reference	Route	Species	No. of Animals	Solvent	Dose	Exposure Time	LD_{50} mg/kg	Observations
1	Oral	Rat					270	
2							350	
3			6	Corn oil, 3%			200	
2		G. Pig					90	
2		Mice					87	
1							116	
4	Dermal	G. Pig			500 mg			Some Deaths
3		Rabbits	4	Propylene glycol	126 mg/kg	24 hr		All died
3			2		126 mg/kg	7 hr		50% mortality
3			2		126 mg/kg	4 hr		No deaths
3			2		63 mg/kg	24 hr		No deaths
3	Inhalation	Rats	3		500 ppm	4 hr		All died
3			3		200 ppm	4 hr		Two death
3			3		200 ppm	2 hr		One death
5			6		34 ppm	3.5 hr		No deaths
5			5		161 ppm	0.83 hr		No deaths
5			6		274 ppm	3.5 hr		All died
5			6		314 ppm	0.88 hr		One death
3			6		133 ppm	7 hr		All died
5		G.Pig	2		34 ppm	3.5 hr		One death
5			2		161 ppm	0.88 hr		One death
3			4		27.5 ppm	7 hr		All died
3		Cats	2		34 ppm	3.5 hr		All died
5			2		161 ppm	0.88 hr		All died

*Source: Gehring & MacDougall, 1971
Blank means no data available.

1 Gulko, et al. 1964.
2 Murzakaev, 1963.
3 Unpublished data, The Dow Chemical Company.
4 Murzakaev, 1966.
5 Treon & Edwards, 1948.

Table 2

Additional Acute Toxicity Data on Hexachlorobutadiene

Reference	Route	Species	HCBD Purity	No. of Animals	Dose	Exposure Time	LD_{50} mg/kg	Observations
1	Oral	Rat(F)	99%				200-400	
1		Rat(M)	99%				580	
1		Rat(F)	99%				65	21 days old
1		Rat(M)	99%				64	21 days old
2		Rat(M)					250	Caused hepatic and renal disorders, and appeared to affect central nervous of mice
2		Mice(M)					80	
3		Rat(M)					298*	
3		Rat(M)		5	529 mg/kg*	Single		All died in 5 days
3		Rat(M)		5	16 mg/kg	Single		No deaths
2	IP	Rat(M)					216	
2	IP	Mice(M)					105	
4		Mice(M)					76	
3	Inhalation	Rat			6800 ppm[a]			Lt50 275 min.
		Mice			6800 ppm[a]			Lt50 310 min.
		Guinea Pig			6800 ppm[a]			Lt50 200 min.
3	Dermal	Rabbit		5	5290 mg/kg*	Single		All died
3		Rabbit					2981	
5		Rabbit			2000 mg/kg	Single		All in three died
5		Rat			5000 mg/kg	Single		Absolute lethal dose
5		Rat					4330	

* Converted from ul/kg using density of 1.675 mg/ul
Blank means no data available
1 Schwetz, et al. 1977.
2 Gradiski, et al. 1975.
3 Hazelton Labs., 1978.

[a]Aerosol dose; estimated concentration
Lt = Lethal time
4 Gradiski, et al. 1974.
5 Chernokan, 1970.

exposure to HCBD show that HCBD is hazardous when encountered by these routes.

The subacute and chronic toxicity data are summarized in Table 3. A column has been included in Table 2 and 3 giving the purity of the HCBD reagent used in each study. This piece of information is essential in making a final judgment on the usefulness of published data for assessing the toxicity of a particular substance. Unfortunately, no indication of the purity of the reagent has been given in many of the publications on HCBD toxicity.

A considerable portion of the literature on HCBD toxicity is reported in the Russian language. For the majority of these publications, only an abstract was available. All publications on HCBD toxicity reported in the literature are given in the tables. Those publications for which translations were available are so labeled in the references. Because details of the experimental procedures and results were not included in many of the abstracts of the Russian articles (and often not in the full translations), only a limited reference to Russian literature is made in the main body of this document. None of the Russian articles attributed carcinogenic properties to HCBD.

Renal damage, as evidenced by renal tubular epithelial degeneration, necrosis, and an increase in the kidney weight:body weight ratio occurred in female rats receiving 30, 65, or 100 mg/kg/day HCBD for 30 days in their diet (Kociba, et al. 1971). No histopathologic changes were observed at the 3 mg/kg/day HCBD dosage level. Other observed effects were: decreased food consumption and body weight gains for female rats consuming 10, 30, 65, and 100

Table 3

Subacute and Chronic Toxicity of Hexachlorobutadiene

Reference	Route	Species	No. of Animals	% HCBD Purity	Dose	Duration	Observations
1	Oral (diet)	Rat(M)	39	99	20 mg/kg/day	22 mo	Renal tubular neoplasms; metastasis to the lung
		Rat(F)	40	99	20 mg/kg/day	24 mo	
		Rat(M)	40	99	2 mg/kg/day	22 mo	Increased urinary coproporphyrin, increase in renal tubular epithelial hyperplasia
		Rat(F)	40	99	2 mg/kg/day	24 mo	
		Rat(M)	40	99	0.2 mg/kg/day	22 mo	No effects
		Rat(F)	40	99	0.2 mg/kg/day	24 mo	
2	Oral (diet)	Rat(F)	4	99	1 mg/kg/day	30 days	No effects
		Rat(F)	4	99	3 mg/kg/day	30 days	Marginal change in kidney/body weight ratio; no pathologic alterations
		Rat(F)	4	99	10 mg/kg/day	30 days	Decreased body weight gain; no pathologic alterations
		Rat(F)	4	99	30 mg/kg/day	30 days	Renal tubular epithelial degeneration,
		Rat(F)	4	99	65 mg/kg/day	30 days	individual cell necrosis and regeneration,
		Rat(F)	4	99	100 mg/kg/day	30 days	decreased body weight gain; increase in mean kidney:body weight ratio; increase in hemoglobin concentration
3	Oral (diet)	Rat(M)	12	99	0.2 mg/kg/day	148 days	No effects among adults or neonates
		Rat(F)	24	99	0.2 mg/kg/day	148 days	No effects among adults or neonates
		Rat(M)	10	99	2.0 mg/kg/day	148 days	Kidney "roughened," mottled cortex; other kidney changes which normally occur appeared to be accentuated
		Rat(F)	20	99	2.0 mg/kg/day	148 days	Accentuation of normal kidney changes; one had renal lesions identical to those on 20 mg/kg/day; no effects on neonates
		Rat(M)	12	99	20 mg/kg/day	148 days	Change in kidney body weight ratio; kidney roughened with mottled cortex; renal tubular dilation and hypertrophy with foci of renal tubular epithelial degeneration and regeneration
		Rat(F)	24	99	20 mg/kg/day	148 days	Renal tubular dilation and hypertrophy with foci of renal tubular epithelial degeneration and regeneration; decreased value of body weight and heart, increased values for relative weight of brain and kidney; slight decrease in body weight of neonates at time of weaning.

Table 3 (Continued)

Reference	Route	Species	No. of Animals	% HCDD Purity	Dose	Duration	Observations
4	Oral	Rat			0.0005 mg/kg/day	6 mo	Was at threshold level with respect to toxicity
		Rat			0.004 mg/kg/day	4 mo	Weakly toxic
		Rat			0.02 mg/kg/day	2 mo	Highly toxic
5	Oral	Rat			8.4 mg/kg 100 mg/kg		Severe necrotic nephrosis, as well as abnormal changes in the brain, liver, and other internal organs
6	Oral	Guinea Pig			60 mg/kg 90 mg/kg	Single	Decompensatory acidosis most significant at 5 days; those surviving 15 days, the blood indicators of acid-base equilibrium were normalized
7,8		Dog			1 mg/kg/day	6 mo	Administered to puppies from birth to 6 mos. Increased secretion of total N-containing compounds. Increased vol. and total acidity of the gastric juice
9	Oral	Dog			0.05 mg/kg/day	45 days	Administered 1.5 to 3 months postnatal; increased total vol., acidity, and amount of HCl and chloride secreted by the stomach.
10	Oral	Guinea Pig			0.004-2 mg/kg/day	7 mo	2 mg dose caused a decrease in -SH group conc. in blood plasma without change in blood protein plasma spectrum
11	Parent-eral	Rat(F)	5		0.004 mg/kg	6 mos	No effect on rate of forming positive conditioned reflexes
		Rat(F)	5		0.04 mg/kg	6 mos	
		Rat(F)	5		7.0 mg/kg	6 mos	Acceleration of differentiation was noted. Decrease in the SH content of the cerebral cortex homogenate; disturbed ability of the animal to form conditioned reflex connections; Exhibited morphological changes in the liver, kidneys, and cardiac muscle in the form of parenchymatous dystrophy
12	IP	Mice	20		4 mg/kg	3 wk to total of 52 mg	No statistically significant increase in the production of adenomas
					8 mg/kg	3 wk to total of 96 mg	

Table 3 (Continued)

Reference	Route	Species	No. of Animals	% HCBD Purity	Dose	Duration	Observations
13	Subcutaneous	Rat(F)			20 mg/kg	Single	Administered to pregnant mice, caused disturbances of motor coordination; weight loss, lymphocytosis, neutropenia, myelcytosis, death occurred in 100% of the offspring within 3 mos. after injection
14	Inhalation	Rat			24 mg/m^3-air	7 mos	Caused no alterations
		Mice			24 mg/m^3-air	7 mos	
15	Inhalation	Rat			0.01 mg/m^3-air	6 mos (5 hr daily)	No effects observed
16	Inhalation	Rat(4 M)	4		25 ppm	15 daily	Caused respiratory difficulty; decreased weight gain and pathologic injury to the tubular epithelium of the kidneys
		Rat(4 F)	4		25 ppm	for 6 hrs	
		Rat(4 M)	4		100 ppm	12 daily	Severe toxicity including death
		Rat(4 F)	4		100 ppm	for 6 hrs	
		Rat(4 M)	4		10 or 5 ppm	15 daily	Caused no toxicity except for retarded weight gain in females at 10 ppm
		Rat(4 F)	4		10 or 5 ppm	for 6 hrs	
17	Cutaneous & ocular	Rat				Single	Irritant
18	Dermal	Rat			1,675 mg/kg*	24 hrs	Mild to moderate erythema
19	Inhalation oral, and topical	Rat, Mice, Guinea pig & cats					Acute and chronic toxicity experiments performed on large groups of rats, mice, guinea pigs, and cats. Hematological study showed leucocytosis, lymphocytosis and decreased erythrocytic permiability to water, immunological depression and decreased antibody formation were observed
20					8.4 mg/kg	Single	Largest concentration of HCBD found in proximal sections of the nephrons: dystropic changes in kidneys caused by 8.4 mg/kg; necrosis at 10 mg/kg
					10 mg/kg	Single	

* Converted from µl/kg using density of 1.675 mg/µl. Blank areas mean no data available.

Table 3 (Continued)

References for Table 3

1. Kociba, et al. 1977
2. Kociba, et al. 1971
3. Schwetz, et al. 1977
4. Poteryaeva, 1973
5. Dimitrienko and Vasilos, 1972
6. Popovich, 1975
7. Boranova, 1974a
8. Boranova, 1974b
9. Kravitskaya and Boranova, 1974
10. Murzakaev, 1965
11. Murzakaev, 1967
12. Theiss, et al. 1977
13. Poteryaeva, 1966
14. Gulko, et al. 1964
15. Poteryaeva, 1972
16. Gage, 1970
17. Duprat, et al. 1976
18. Mumma and Lawless, 1975
19. Poteryaeva, 1971
20. Shroit, et al. 1972

mg/kg/day HCBD in their diet; depletion of abdominal fat at 65 and 100 mg/kg/day; minimal hepatocellular swelling at 100 mg/kg/day; and hemoconcentration at 10, 30, 65, and 100 mg/kg.

Dietary ingestion of 20 but not 2.0 or 0.2 mg/kg/day HCBD for two years caused depression of the body weight gain of both male and female rats (Kociba, et al. 1977). Evaluation of the mean organ weights and organ:body weight ratios for rats killed at the termination of this study indicated the males had no alterations in weights of the brain, heart, and liver relative to control values. The relative and absolute weights of the kidneys of males ingesting the 20 mg/kg/day were found to be increased. For females, the body weight loss was accompanied by a significant decrease in the absolute weight of the heart and liver, and an increase in the relative weight of the kidney. A decreased survival rate was observed for males but not for females at 20 mg/kg/day HCBD.

Extensive gross and microscopic pathological examination of all rats necropsied during the course of this study was conducted. Significant abnormalities were observed in the urinary systems of rats receiving the 20 mg/kg/day dosage level. Among effects related to HCBD treatment was an increase over the controls in renal tubular neoplasms in both male and female rats. The effects at 2 mg/kg/day HCBD were slight, including possible renal tubular epithelial hyperplasia. No effects were observed in rats receiving 0.2 mg/kg/day HCBD in their diet for two years.

Blood, serum, and urine samples were analyzed for a wide variety of clinical indicators. A statistically significant increase in urinary coproporphyrin was observed in male rats ingesting 20

mg/kg/day HCBD for 12 months, in females ingesting 2.0 mg/kg/day for 14 months, and in females ingesting 20 mg/kg/day HCBD for 24 months.

A study of the effects of HCBD on reproduction in rats included gross and microscopic examination of internal organs, bone, and other tissues (Schwetz, et al. 1977). Pathological changes considered to be related to ingestion of HCBD were found in the kidneys of both male and female rats at dietary dose levels of 2 and 20 mg/kg/day HCBD for 148 days. Kidneys from male rats at these two dose levels were "roughened" and had a mottled cortex. There was renal tubular dilation and hypertrophy with foci of renal tubular epithelial degeneration and regeneration in kidneys from male and female rats on 20 mg/kg/day HCBD. No effects were observed at 0.2 mg/kg/day.

An effect on the central nervous system was observed at a 7 mg/kg (frequency of administration unknown) dose level as a reduction in the response capability of the rat to conditioned reflexes (Murzakaev, 1967). Results at 0.04 mg/kg were not statistically significant, while 0.004 mg/kg HCBD gave no indication of a neurotoxic response. Poteryaeva (1973) measured urinary acid-base equilibrium, serum peroxidase, residual nitrogen, and neuromuscular chronaxie in rats receiving daily oral doses of HCBD. HCBD toxicity was observed at 0.02 mg/kg/day for one month, at 0.004 mg/kg/day for four months, and threshold toxicity at 0.0005 mg/kg/day after six months.

Kidney damage is also induced by inhalation of HCBD. Fifteen daily six-hour exposures of rats to 25 ppm HCBD caused respiratory

difficulty, decreased weight gain, and pathologic injury to the tubular epithelium of the kidneys. Twelve daily six-hour exposures to 100 ppm HCBD caused more severe toxicity, including the death of some rats. Fifteen daily six-hour exposures to 5 or 10 ppm HCBD caused no toxicity except for retarded weight gain in females exposed to 10 ppm (Gage, 1970).

Chernokan (1970) determined the dermal LD_{50} of HCBD for the rat at 4,330 mg/kg. Animals receiving 1/5 dermal LD_{50} showed general weight loss, sluggishness, and paresis of the extremities within one day of the start of the experiment, and all had died by the fifth to sixth day. With repeated applications of 1/20 dermal LD_{50} HCBD, features of intoxication began showing up by the sixth day which were expressed in weight loss, flaccidity, and rigidity of muscles. The red blood cell was the most sensitive initial indicator of toxicity as manifested by a loss in hemoglobin level and a drop in erythrocyte count.

Boranova (1974b) observed that oral administration of 1 mg/day HCBD to puppies from birth to six months increased excretion of total nitrogen-containing compounds and of urea nitrogen starting on the 20th day of growth. Total acidity of gastric juices and content of free HCl also were increased. A single dose of 60 or 90 mg/kg to guinea pigs caused decompensatory acidosis which was most significant at five days (Popovich, 1975). Indicators of acid-base equilibrium in blood and urine tended toward normalization at 15 days.

The kidney appears to be the organ most sensitive to HCBD. Possible chronic effects are observed at doses as low as 2 to 3

mg/kg/day (Kociba, et al. 1971, 1977; Schwetz, et al. 1977). Renal tubular neoplasms were observed during a two-year study in which 20 mg/kg/day was administered to rats in their diet (Kociba, et al. 1977). Single oral doses as low as 8.4 mg/kg have been observed to have a deleterious effect on the kidney (Shroit, 1972). Neurotoxic effects have been reported to occur at dose levels as low as 4 ug/kg/day (Poteryaeva, 1973; Murzakaev, 1967). Acute HCBD intoxication affects acid-base equilibrium in blood and urine (Popovich, 1975; Poteryaeva, 1971). Some investigators report a cumulative effect for HCBD during chronic dosing by dermal (Chernokan, 1970) or oral (Poteryaeva, 1973) routes. An increase in urinary coporphyrin was observed in rats receiving 2 mg/kg/day and 20 mg/kg/day HCBD for up to 24 months (Kociba, et al. 1977).

In Russia, where HCBD is used as a soil fumigant for grape phylloxera, exposure of vineyard workers has occurred. Measurements showed that HCBD persisted in the air at levels from 0.0012 to 0.01 mg/l for up to 5 days depending upon weather, application method, and soil tillage (Krasniuk, et al. 1969). A medical examination was done of 205 workers, 153 with 4 years exposure to HCBD and polychlorobutane-80 (a mixture of partly chlorinated 1,3 butadienes) and 52 working under the same conditions but with no exposure to these compounds. Arterial hypotension, as a rule, was noted in workers exposed to HCBD and polychlorobutane-80. Heart changes, including myocardial dystrophy, were found more frequently in workers in contact with the fumigant than in workers with no contact. Dyspeptic signs and periodic epigastric pains associated with food intake were observed in 38 subjects. Nervous disorders

and upper respiratory tract changes occurred more frequently in exposed than in nonexposed workers. Details and tests for statistical significance were not presented.

McConnell, et al. (1975) reported finding HCBD in human liver samples at 12 µg/kg wet tissue and in body fat at around 4 µg/kg.

Synergism and/or Antagonism

Murzakaev (1967) found a decreased -SH group content in the cerebral cortex homogenate and blood serum of rats receiving a 7 mg/kg parenteral dose of HCBD over a six-month span (frequency of dose not given). Mizyukova, et al. (1973) determined the relationship between the structure and detoxifying power of thiols and amines against HCBD. Rats poisoned with 300 mg/kg or larger doses of HCBD were given thiols or amines 20 to 30 minutes before and immediately after poisoning. The antidotes were administered orally in twofold molar excess relative to the poison. As determined from the survival rates, mercaptide, cysteine, and especially unithiol were highly effective antidotes for HCBD. The ethanolammonium salts of 2,3-dimercaptopropane sulfonic acid were even more powerful antidotes than unithiol, while the ethanolamines themselves, either alone or combined with unithiol, were ineffective. The reduction in free -SH groups following HCBD injection and the effective antidote with mercapto (-SH) compounds suggests that HCBD reacts readily with compounds containing these groups.

Teratogenicity

Nonpregnant female rats were given single 20 mg/kg subcutaneous doses of HCBD and then bred (Poteryaeva, 1966). The course of the subsequent pregnancy and its outcome were followed. The

pregnancy rate for the treated rats was the same as in the control group. No stillbirths occurred, but all the offspring of the rats that had received HCBD died within 3 months of birth with 38 of 86 dying between days 1 and 28, and the rest of the offspring dying between days 29 and 90. In the control group, 13 of 61 newborns died within 14 days of birth with no deaths occurring thereafter for up to 90 days. The weights of the young rats from the dosed mothers were markedly lower than the controls. At 2½ months, when the rats showed marked toxic effects, gross pathologic changes were noted in the internal organs at autopsy. Damage to the kidney was observed in the form of glomerulonephritis. Blood smears showed degenerative changes in the red cells.

Schwetz, et al. (1977) studied the dietary effects of HCBD on reproduction in rats. Male and female rats were fed dose levels of 0.2, 2.0, or 20 mg/kg/day HCBD 90 days prior to mating, 15 days during mating, and subsequently throughout gestation and lactation. The toxic effects observed in these rats are given in Table 3. Signs of toxicity observed among the adult rats at the two higher dose levels were decreased weight gain, low food consumption, and alterations in the kidney structure in the form of a mottled cortex. The only effect on weanlings consisted of a slight decrease in body weight at 21 days of age at the 20 mg/kg/dose level. All parameters of the reproductive process including neonatal growth, survival, and development were normal at levels of 0.2 or 2.0 mg/kg/day.

The discrepancy between the survival rates and observations on young rats reported by Schwetz, et al. (1977) and Poteryaeva (1966)

suggests the need for more research on the teratogenic effects of HCBD. The difference in routes of administration and absorption may account for differences in survival rates and toxic effects. Schwetz, et al. (1977) reports a 94 percent survival rate for newborn controls to day 14, whereas Poteryaeva (1966) reports only a 79 percent survival rate for controls.

A study published by Schwetz, et al. (1974) dealt with the effects of HCBD on reproduction in Japanese quail (Coturnix japonica). Adult male and female Japanese quail were fed diets containing 0.2, 3, 10, or 30 mg/kg of HCBD for 90 days. The birds showed no evident signs of toxicity during the study. HCBD at all dose levels had no effect on body weight, demeanor, food consumption, egg production, the fertility and hatchability of eggs, the survival of hatched chicks, and eggshell thickness. In addition, at the termination of the study there were no gross or histopathologic changes evident in the organs or tissues of birds that could be related to treatment.

Mutagenicity

Taylor (1978) has tested the mutagenic potential of HCBD on S. typhimurium TA 100. Due to problems in solubilizing HCBD in the test system, Taylor concluded that HCBD mutagenicity was 'not proven.' A dose-dependent increase in reversion rate in the absence of activation was observed, but the usual criterion for mutagenicity (at least a twofold increase in reversion rate; U.S. EPA, 1977) was not reached. The final conclusion was that HCBD was nonmutagenic. HCBD was also found to be nonmutagenic in the presence of an activating system.

Carcinogenicity

Kociba, et al. (1977) completed a two year study of the chronic effects of HCBD in the rat. The results of the study are covered in Table 4. The most significant finding of these workers was the development of renal tubular neoplasms in the kidneys of rats receiving 20 mg/kg/day HCBD in their diet. Histological examination of the tumors revealed renal tubular adenomas and adenocarcinomas. Metastasis to the lung was observed in two cases.

The HCBD was 99 percent pure. Test diets to supply 0, 20, 2, or 0.2 mg/kg/day were prepared weekly for the first three months of the study and monthly thereafter with concentrations of test material being adjusted to maintain the designated dosages on a mg/kg/day basis. Seven week-old male and female Sprague-Dawley (Spartan substrain) specific-pathogen-free-derived rats were used. The rats were randomized into test groups of 39 to 40/sex/dose level plus 90/sex for controls.

In a 30-day toxicity study by Kociba, et al. (1971), female rats received HCBD in doses ranging from 1 to 100 mg/kg/day; no effects were observed in rats receiving 3 mg/kg/day, while effects were only marginal in rats receiving 10 mg/kg/day. The kidney was identified as the target organ. The no-effect-observed levels are roughly in the same range as those reported by other investigators, and the kidney has been identified repeatedly as the target organ.

Feeding of the male rats continued for 22 months while the females were treated for 24 months. During the course of the study, gross and microscopic examinations were conducted on: (1) representative portions of all major organs; and (2) any tissue having a

Table 4

Response to HCBD Feeding in Male and Female Rats*

Dose mg/kg/day	Numbers Male rat	% Response Male rat	Numbers Female rat	% Response Female rat	Observations
20.0	9/39	23	3/40	7.5	Renal tubular adenocarcinomas; Undifferentiated carcinoma; metastasis to the lung
2.0	0/40	0	0/40	0	
0.2	0/40	0	0/40	0	
Control	1/90	1.1	1/90	1.1	Nephroblastoma

*Source: Kociba, et al. 1977

gross lesion suggestive of a significant pathological process taken from rats killed in a moribund condition. Terminal necropsy examinations were conducted on rats surviving the full term on the test diets. There was a significant increase in the mortality of the male rats ingesting 20 mg/kg/day HCBD during the last two months of the study. This was not the case with the males on the lower dose levels or in females at any dose level. Fifty percent of the high dose males had died by 18 months, while the survival rate for females was 21 to 22 months before a 50 percent death count was observed.

No significant increase in incidence of neoplastic lesions related to ingestion of HCBD was observed in any organ, tissue, or skeletal samples other than in the kidney. In the males receiving 20 mg/kg/day of HCBD, 18 percent (9/39) had renal tubular neoplasms which were classified as adenomas or carcinomas; 7.5 percent (3/40) of the females on the high dose developed renal carcinomas. Metastasis to the lung was observed in one case for both male and female rats. A nephroblastoma developed in one of the male control rats (1/90 or 1.1 percent) and in one of the female controls (1/90 of 1.1 percent). No carcinomas were observed in the kidneys of the control rats, and no nephroblastomas were observed in the kidneys of the rats maintained on diets containing HCBD. Table 4 summarizes the data from Kociba, et al. (1977).

The production of lung adenomas in strain A mice following multiple intraperitoneal (i.p.) injections of HCBD was investigated (Theiss, et al. 1977). Doses of 4 mg/kg and 8 mg/kg were administered i.p. (tricaprylin vehicle) three times a week to groups of

20 male mice six to eight weeks old. Treatment continued until totals of 52 mg (4 mg/kg) and 96 mg (8 mg/kg) were administered. Twenty-four weeks after the first injection, the mice were killed. There was no statistically significant increase in the mean number of lung tumors per test mouse as compared to vehicle-treated controls, nor was a dose-response relation obtained.

CRITERION FORMULATION

Existing Guidelines and Standards

There are no guidelines or standards of record other than a standard of 10 $\mu g/m^3$ for inhalation referenced by Poteryaeva (1972) of the Soviet Union.

Current Levels of Exposure

The analytical data available on the distribution of HCBD in the environment suggest that exposure is a localized problem, potentially affecting those living in areas with nearby chemical plants producing parent compounds for which HCBD is a by-product. However, due to the limited number of air and water samples taken, it is difficult to estimate the level of exposure even to those populations living in areas where the heaviest exposure might be encountered.

Special Groups at Risk

A special group at risk would be those workers in an industrial environment where concentrations of HCBD in the air might be present.

Basis and Derivation of Criteria

HCBD exhibits acute, subacute, and chronic toxicity in animal test systems; the overall data can be reviewed in Tables 1-4.

The kidney appears to be the organ most sensitive to HCBD. Chronic effects are observed at doses as low as 2 to 3 mg/kg/day in rats (Kociba, et al. 1971, 1977; Schwetz, et al. 1977). Renal tubular neoplasms were observed during a two-year study in which 20 mg/kg/day was administered to rats in their diet. Single oral doses as low as 8.4 mg/kg have been observed to have a deleterious

effect on the kidney (Shroit, 1972). The carcinogenic effects of renal tubular adenomas and adenocarcinomas were strongly demonstrated at the 20 mg/kg/day dosage. Even though HCBD was not determined to be mutagenic in S. typhimurium (Taylor, 1978), since the mutation rate was not double the background rate, the compound is a carcinogen, as strongly indicated by the study of Kociba, et al. (1977).

The evidence of carcinogenicity is sufficient to conclude that HCBD is a suspect human carcinogen. As carcinogens can conservatively be assumed to have a nonthreshold dose/response characteristic, the carcinogenic effect is the most significant exposure effect from which to estimate an ambient water quality criterion value.

Under the Consent Decree in NRDC v. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." Hexachlorobutadiene is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of HCBD in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of HCBD corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be

expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register (44 FR 15926) notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6}, or 10^{-7} as shown in the table below.

Exposure Assumptions	Risk Levels and Corresponding Criteria(1)		
	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 6.5 grams of fish, shellfish (2).	0.045 µg/l	0.45 µg/l	4.47 µg/l
Consumption of fish and shellfish only.	5.00 µg/l	50.0 µg/l	500 µg/l

(1) Calculated by applying a linearized multistage model as discussed in the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document. Appropriate bioassay data used in the calculation are presented in Appendix I. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.

(2) Approximately 1 percent of the HCBD exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 2.78-fold. The remaining 99 percent of HCBD exposure results from drinking water.

Concentration levels were derived by assuming a lifetime exposure to various amounts of HCBD, (1) occurring from the consumption of both drinking water and aquatic life grown in water containing the corresponding HCBD concentrations, and (2) occurring solely from the consumption of aquatic life grown in the waters containing the corresponding HCBD concentrations. Because data indicating other sources of HCBD exposure and their contributions to total body burden are inadequate for quantitative use, the figures reflect the incremental risks associated with the indicated routes only.

REFERENCES

Boranova, T.I. 1974a. Nitrogen releasing function of the stomach in ontogenesis under the effect of hexachlorobutadiene. Eksp. Issled. Khim. Biol. (no vol.): 198.

Boranova, T.I. 1974b. Effect of hexachlorobutadiene on the secretory function of the stomach of puppies of various ages. Protsessy Obmena Veshchestv S-kh. Zhivotn. Usloviyakh Normy Vozdeistv. Vysokochast. Elektromagn. Pol. (no vol.): 83.

Chernokan, V.F. 1970. Some data of the toxicology of hexachlorobutadiene when ingested into the organism through the skin. Vop. Gig. Toksikol. Pestits. Tr. Nauch. Tr. Sess. Akad. Med. Nauk. SSSR. (no vol.): 169. (Translation).

Dec, G., et al. Water Solubility and Octanol/Water Partition Coefficients of Organics: Limitations of the Solubility-Partition Coefficient Correlation. (Manuscript).

Dimitrienko, V.D. and A.F. Vasilos. 1972. Kidney damage during experimental acute poisoning with hexachlorobutadiene. Zdravookhranenie 15-11.

Duprat, O.P., et al. 1976. Irritant power of the principal chlorinated aliphatic solvents on the skin and ocular mucosa of the rabbit. Eur. Jour. Toxicol. Environ. Hyg. 9: 171.

Gage, J.C. 1970. The subacute inhalation toxicity of 109 industrial chemicals. Br. Jour. Ind. Med. 27: 1.

Gehring, P.J. and D. MacDougall. 1971. Review of the toxicity of hexachlorobenzene and hexachlorobutadiene. Dow Chemical Co., Midland, Michigan.

Gradiski, D. et al. 1974. Aliphatic chlorinated solvents: their compared toxicities. Eur. Jour. Toxicol. Environ. Hyg. 7: 247.

Gradiski, D., et al. 1975. Experimental toxicological study of hexachlorobutadiene. Eur. Jour. Toxicol. Environ. Hyg. 8: 180.

Gulko, A.G., et al. 1964. Toxicological study of the insecticide hexachlorobutadiene. Vopr. Gigieny i. Sanit. Ozderovl. Uneshn. Sredy, Kishinev, Sb. (no vol.): 128.

Hazelton Laboratories. 1978. Personal communication with W. Coates. Washington, D.C.

Jacobs, A., et al. 1974. Accumulation of noxious chlorinated substances from Rhine River water in the fatty tissue of rats. Vom. Wasser. 43:259.

Keith, L.H., et al. 1976. Identification of Organic Compounds in Drinking Water from Thirteen U.S. Cities. In: L.H. Keith (ed.) Identification and Analysis of Organic Pollutants in Water. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Kociba, R.J., et al. 1971. Toxicologic study of female rats administered hexachlorobutadiene or hexachlorobenzene for 30 days. Dow Chemical Co., Midland, Michigan.

Kociba, R.J., et al. 1977. Results of a two-year chronic toxicity study with hexachlorobutadiene in rats. Am. Ind. Hyg. Assoc. 38: 589.

Kotzias, D., et al. 1975. Ecological chemistry. CIV. Residue analysis of hexachlorobutadiene in food and poultry feed. Chemosphere. 4: 247.

Krasniuk, E.P., et al. 1969. The state of health of viticulturists having contact with the fumigants, hexachlorobutadiene and polychlorobutane-80. Vrachebnoe Delo. (no vol.): 111.

Kratvitskaya, P.S. and T.I. Boroanova. 1974. Secretory function of the stomach in pups from 1.5 to 3 months of age under the effect of hexachlorobutadiene. Eksp. Issled. Khim. Biol. (no vol.): 207.

Laseter, J.L., et al. 1976. An ecological study of hexachlorobuta diene. EPA 560/6-76-010. U.S. Environ. Prot. Agency, Washington, D.C.

Leeuwangh, P., et al. 1975. Toxicity of Hexachlorobutadiene in Aquatic Organisms. In: Sublethal Effects of Toxic Chemicals on Aquatic Animals. Proc. Swedish-Netherlands Symp., Sept. 2-5. Elsevier Scientific Publ. Co., Ind., New York.

Li, R.T., et al. 1976. Sampling and analysis of selected toxic substances. Task I B - hexachlorobutadiene. EPA 560/6-76-015. U.S. Environ. Prot. Agency, Washington, D.C.

McConnell, G., et al. 1975. Chlorinated hydrocarbons and the environment. Endeavour. 34: 13.

Mizyukova, I.G., et al. 1973. Relation between the structure and detoxifying action of several thiols and amines during hexachlorobutadiene poisoning. Fiziol. Aktiv. Veshchestva. 5: 22.

Mumma, C.E. and E.W. Lawless. 1975. Survey of industrial processing data: Task I - Hexachlorobenzene and hexachlorobutadiene pollution from chlorocarbon processes. EPA 560/3-75-003. U.S. Environ. Prot. Agency, Washington, D.C.

Murzakaev, F.G. 1963. Toxicity data for hexachlorobutadiene and its intermediates. Farmakol. i Toksikol. 26: 750.

Murzakaev, F.G. 1965. Effect of small hexachlorobutadiene doses on some biochemical and immunobiological indexes. Gigiena Truda i Prof. Zabolevaniya. 9: 50.

Murzakaev, F.G. 1966. Toxicological and hygienic characteristics of hexachlorobutadiene and its intermediate products. Dokl. Neftekhim. Sekts. Bashkir. Respub. Provl. Vses. Khim. Obshchest. 2: 245.

Murzakaev, F.G. 1967. Effect of small doses of hexachlorobutadiene on activity of the central nervous system and morphological changes in the organism of animals intoxicated with it. Gig. Tr. Prog. Zabol. 11: 23. (Translation).

Popovich, M.I. 1975. Acid-base equilibrium and mineral metabolism following acute hexachlorobutadiene poisoning. Issled. Obl. Farm. Khim. (no vol.): 120.

Poteryaeva, G.E. 1966. Effect of hexachlorobutadiene on the offspring of albino rats. Gig. Sanit. 31: 33. (Translation).

Poteryaeva, G.E. 1971. Sanitary and toxicological characteristics of hexachlorobutadiene. Vrach. Delo. 4: 130.

Poteryaeva, G.E. 1972. Substantiating the maximum permissible concentration of hexachlorobutadiene in the air of industrial premises. Gig. Sanit. 37: 32.

Poteryaeva, G.E. 1973. Toxicity of hexachlorobutadiene during entry into the organism through the gastrointestinal tract. Gig. Tr. 9: 98.

Schwetz, B.A., et al. 1974. Reproduction study in Japanese quail fed hexachlorobutadiene for 90 days. Toxicol. Appl. Pharmacol. 30: 255.

Schwetz, B.A., et al. 1977. Results of a reproduction study in rats fed diets containing hexachlorobutadiene. Toxicol. Appl. Pharmacol. 42: 387.

Shroit, I.G., et al. 1972. Kidney lesions under experimental hexachlorobutadiene poisoning. Aktual. Vop. Gig. Epidemiol. (no vol.): 73.

Stephan, C.E. 1980. Memorandum to J.F. Stara. U.S. EPA. July 3, 1980.

Taylor, G. 1978. Personal communication. Natl. Inst. Occup. Safety Health.

Theiss, J.C., et al. 1977. Test for carcinogenicity of organic contaminants of United States drinking waters by pulmonary tumor response in strain A mice. Cancer Res. 37: 2717.

Treon, J.F. and A. Edwards. 1948. Report on the toxicity of hexachlorobutadiene vapor. Personal communication from Kettering Lab., University of Cincinnati, to Dow Chemical Co.

U.S. EPA. 1975. Preliminary assessment of suspected carcinogens in drinking water. Rep. to Congress.

U.S. EPA. 1977. IERL-RTP procedures manual: Level 1 environmental assessment biological tests for pilot studies.

U.S. EPA. 1978. Contract No. 6803-2624.

U.S. EPA. 1980. Seafood consumption data analysis. Stanford Research Institute International. Menlo Park, California. Final Report, Task 11. Contract No. 68-01-3887.

Veith, G.D., et al. 1979. Measuring and estimating the bioconcentration factors of chemicals in fish. Jour. Fish. Res. Board Can. 36: 1040.

Veith, G.D. 1980. Memorandum to C.E. Stephan. U.S. EPA. April 14, 1980.

Yip, G. 1976. Survey for hexachloro-1,3-butadiene in fish, eggs, milk, and vegetables. Jour. Assoc. Official. Anal. Chem. 59: 559.

Yurawecz, M.P., et al. 1976. Determination of hexachloro-1,3-butadiene in spinach, eggs, fish, and milk by election capture gas-liquid chromatography. Jour. Assoc. Off. Anal. Chem. 59: 552.

APPENDIX I

Derivation of Criterion for Hexachlorobutadiene

During the two year feeding study of Kociba (1977) in rats, renal tubular adenomas and carcinomas were observed in males with significantly higher incidence in animals fed 20 mg/kg/day than control animals. Using a fish bioaccumulation factor of 2.78, the parameters of the extrapolation model are:

Dose (mg/kg/day)	Incidence (no. responding/no. tested)
0.0	1/90
0.2	0/40
2.0	0/40
20.0	9/39

le = 669 days w = 0.610 kg

Le = 730 days R = 2.78

L = 730 days

With these parameters the carcinogenic potency for humans, q_1^*, is 0.07752 $(mg/kg/day)^{-1}$. The result is that the water concentration should be less than 4.47 micrograms per liter in order to keep the individual lifetime risk below 10^{-5}.