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Health Effects Support Document for Hexachlorobutadiene

External Review Draft

Health Effects Support Document for Hexachlorobutadiene

EXTERNAL REVIEW DRAFT

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FOREWORD

The Safe Drinking Water Act (SDWA), as amended in 1996, requires the Administrator of the Environmental Protection Agency (EPA) to establish a list of contaminants to aid the agency in regulatory priority setting for the drinking water program. In addition, SDWA requires EPA to make regulatory determinations for no fewer than five contaminants by August 2001. The criteria used to determine whether or not to regulate a chemical on the CCL are the following:

The contaminant may have an adverse effect on the health of persons.

The contaminant is known to occur or there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern.

In the sole judgment of the administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

The Agency's findings for the three criteria are used in making a determination to regulate a contaminant. The Agency may determine that there is no need for regulation when a contaminant fails to meet one of the criteria.

This document provides the health effects basis for the preliminary regulatory determination for hexachlorobutadiene. In arriving at the preliminary regulatory determination, data on toxicokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology, and mechanisms of toxicity were evaluated. In order to avoid wasteful duplication of effort, information from the following risk assessments by the EPA and other government agencies were used in development of this document.

U.S. EPA. 1991a. Drinking Water Health Advisory: Hexachlorobutadiene. In: Volatile Organic Compounds. United States Environmental Protection Agency, Office of Drinking Water. Lewis Publishers. Ann Arbor, Michigan.

ATSDR. 1994. Toxicological Profile for Hexachlorobutadiene. Agency for Toxic Substances and Disease Registry, Department of Health and Human Services.

U.S. EPA, 1998a. Draft Ambient Water Quality Criteria for the Protection of Human Health. Office of Water. EPA 822-R-98-004.

Information from the published risk assessments was supplemented with information from recent studies of hexachlorobutadiene identified by literature searches conducted in 1999 and 2000 and the primary references for key studies.

Generally a Reference Dose (RfD) is provided as the assessment of long-term toxic effects other than carcinogenicity. RfD determination assumes that thresholds exist for certain toxic

effects such as cellular necrosis. It is expressed in terms of milligrams per kilogram per day (mg/kg-day). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The carcinogenicity assessment for hexachlorobutadiene includes a formal hazard identification as well as a quantitative dose-response assessment of the risk from oral exposure. Hazard identification is a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen via the oral route and the conditions under which the carcinogenic effects may be expressed.

Guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991b), *Proposed Guidelines for Carcinogen Risk Assessment* (1996a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), and *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998b); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998c); and Memorandum from EPA Administrator, Carol Browner, dated March 21, 1995.

The chapter on occurrence and exposure to hexachlorobutadiene through potable water was developed by the Office of Ground Water and Drinking Water. It is based primarily on unregulated contaminant monitoring (UCM) data collected under SDWA. The UCM data are supplemented with ambient water data as well as information on production, use, and discharge.

ACKNOWLEDGMENTS

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1.0 EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) has prepared this Health Effects Support Document for Hexachlorobutadiene (HCBD) to assist in determining whether to regulate HCBD with a National Primary Drinking Water Regulation (NPDWR). The available data on occurrence, exposure, and other risk considerations suggest that, because HCBD does not occur in public water systems at frequencies and levels of public health concern, regulating HCBD will not present a meaningful opportunity for health risk reduction for persons served by public water systems. EPA will present a determination and further analysis in the Federal Register Notice covering the Contaminant Candidate List (CCL) proposals.

HCBD (Chemical Abstracts Services Registry Number 87-68-3) is a colorless liquid at room temperature. It is poorly soluble in water, and has a high affinity for organic particulate. HCBD has never been specifically manufactured as a commercial product in the United States. However, significant quantities of hexachlorobutadiene are generated in the United States as waste by-product from the chlorination of hydrocarbons. The chemical is used as an intermediate product in rubber manufacturing and chlorofluorocarbon and lubricant production, as well as for transformer and hydraulic fluids, fluid for gyroscopes, heat transfer liquid, solvents, laboratory reagents, and as a wash liquor for removing C_4- and higher hydrocarbons. Hexachlorobutadiene has also been used as a fumigant in some overseas countries. Some of the chemical properties for hexachlorobutadiene (CAS# 87-68-3) include the following: solubility = 2-2.55 mg/L; vapor pressure = 0.15 mmHg; Log K_{ow} = 4.78; and Log K_{oc} = 3.67.

Emissions into air is the major pathway of release. For hexachlorobutadiene, air emissions constitute most of the on-site releases. Hexachlorobutadiene is listed as a toxic release inventory (TRI) chemical. It is included in the Agency for Toxic Substances and Disease Registry's (ATSDR) Hazardous Substance Release and Health Effects Database (HazDat) and has been detected in site samples in fourteen States: AL, AZ, CT, IA, LA, MI, MN, NJ, NY, OH, PA, RI, SC, WA (ATSDR, 2000).

Ambient air concentration data are available from Shah and Heyerdahl (1988). The mean and median of all ambient concentrations were 0.42 $\mu g/m^3$ and 0.04 $\mu g/m^3$, respectively. Air intake for adults is estimated to be 1.2×10^{-4} mg/kg-day using the mean air concentration, and is the main pathway of exposure (U.S. EPA, 1998a). Hexachlorobutadiene is not found in non-fish dietary foods for the majority of regions of the US. It was detected in fish at 3% of 362 sites sampled. The mean fish concentration at all sites was 0.6 ng/g (Kuehl et al., 1994). An estimate of adult exposure via fish consumption is 1.54×10^{-7} mg/kg-day (U.S. EPA, 1998a).

Cross-sectional monitoring data from two rounds of sampling conducted under EPA's Unregulated Contaminant Monitoring (UCM) program indicate that the frequency of detection of HCBD in public water systems (PWSs) is low. Round 1, conducted from 1987 to 1992 in 24 States, detected HCBD at levels above the minimum reporting level (MRL) for 0.35% of the PWSs, while Round 2, conducted from 1993 to 1997 in 20 States, detected HCBD at levels above the MRL for 0.18% of the PWSs. For a health reference level (HRL) of 0.9 µg/L HCBD, 0.114% of the PWSs in Round 1 (74 systems) exceeded the HRL, while 0.018% (11 systems) in

Round 2 exceeded the HRL. The United States Geological Survey's National Ambient Water Quality Assessment (NAWQA) program did not detect HCBD in the ground water or well water samples surveyed. When average daily drinking water intakes for HCBD are compared with intakes from food, air and soil, drinking water accounts for a relatively small proportion of total HCBD intake.

Hexachlorobutadiene (HCBD) is absorbed following oral administration. Few data are available on absorption via other routes. HCBD and its metabolites distribute preferentially to the kidney, liver, adipose tissue and the brain (Reichert et al., 1985). The primary pathway for HCBD metabolism is conjugation with glutathione, with subsequent conversion to a cysteine conjugate. Activation of the cysteine conjugate by β-lyase yields a highly reactive thioketene intermediate which has been implicated in renal toxicity. Evidence exists for a male specific metabolic pathway in rats (Birner et al., 1995). The primary routes for elimination of absorbed HCBD are urinary and fecal excretion; a small amount of absorbed HCBD is oxidized to carbon dioxide (U.S. EPA, 1991; U.S. EPA, 1999; ATSDR, 1994).

There are no reliable dose-response data for humans exposed to HCBD. Studies in animals show the selective effect of HCBD on the kidney, specifically the proximal tubule. Subchronic (NTP, 1991) and chronic (Kociba et al., 1977) studies in rodents present a clear picture of dose-related renal damage at 2 mg/kg-day and above. Progressive events over time include changes in kidney weight, increased excretion of coproporhyrin, renal tubular degeneration and regeneration, hyperplasia, focal adenomatous proliferation, and renal tumor formation. Developmental effects were also associated with hexachlorobutadiene exposure in animals (Harleman and Seinen, 1979). However, these effects were observed at higher doses than for renal toxicity. Pups with lower birth weights and reduced growth were reported at maternal dose of 8.1-15 mg/kg-day in rats (Badaeva, 1983; Harleman and Seinen, 1979). In the presence of metabolic activation, HCBD and its reactive metabolites are mutagenic in some (Simmon, 1977; Reichert et al., 1984; Reichert and Schutz, 1986; Wild et al., 1986) but not all studies. Only one study of lifetime oral exposure to hexachlorobutadiene was located (Kociba et al., 1977). At the highest dose of 20 mg/kg-day in the study, benign and malignant tumors were seen in approximately 23% (9/39) of the male rats, and 15% (6/40) of the female rats. This dose exceeded the maximum tolerated dose at which increased mortality, severe renal toxicity, and significant weight loss were also observed. There were no tumors in the second highest dose of 2 mg/kg-day in this study. The conclusion from the dose response analysis is that hexachlorobutadiene is a weak carcinogen because it is carcinogenic only at cytotoxic dose.

The nephrotoxicity of HCBD in animals is dependent on a multistep bioactivation mechanism involving both kidney and liver enzymes. The ultimate step in this pathway is a β-lyase mediated degradation of a HCBD metabolite that generates a highly reactive thioketene in proximal tubule cells. *In vitro* studies suggest that cortical mitochondria are the critical subcellular target for toxicity. Covalent binding of this reactive thioketene to cellular macromolecules (e.g. proteins, mitochondrial DNA) and the resultant mitochondrial dysfunction is believed to contribute to the renal cytotoxicity and tumors observed in animals.

Limited data from *in vitro* studies suggest human have the ability to metabolize HCBD. However, the activity of HCBD metabolizing enzymes, particularly renal β-lyase, may be many fold lower in humans than the corresponding enzymes in rats. Thus, there may be less concern for toxicity in humans.

The primary target for hexachlorobutadiene is the kidney. Individuals with preexisting kidney damage may represent a potentially sensitive subpopulation for hexachlorobutadiene health effects. Studies in animals showed that the young rats and mice were more sensitve to the acute effects of hexachlorobutadiene (Hook et al., 1983; Lock et al., 1984), suggesting that infants may also be more susceptible to hexachlorobutadiene toxicity, perhaps as a result of immature organ systems.

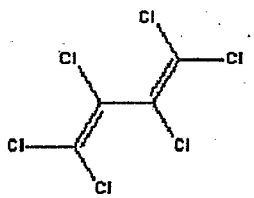
The RfD for hexachlorobutadiene is 2×10^{-4} mg/kg-day (EPA, 1998a). It is derived from a NOAEL of 0.2 mg/kg-day for renal tubular epithelial cell degeneration and regeneration from the Kociba et al. (1977) study on rats and the NTP (1991) study on mice. A composite uncertainty factor (UF) of 1,000 was used in the derivation of the RfD. The composite uncertainty factor included a factor of 10 to account for extrapolation from animals to humans; a factor of 10 for protection of sensitive subpopulation; a factor of 3 for the use of NOAEL that may be a minimal LOAEL; and a factor of 3 for database deficiencies (lack of a 2-generation reproductive study). In accordance with EPA's 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), HCBD is classified as a Group C (possible human) carcinogen. Under EPA's 1996 proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), HCBD is classified as *likely to be carcinogenic to humans*. Two different approaches were used for dose response extrapolation to estimate human cancer risk for HCBD from animal data. The linear approach calculated a slope factor of 4×10^{-2} $(mg/kg\text{-}day)^{-1}$, and a unit risk of 1.1×10^{-5} per μg/L. Using the nonlinear approach, the point of departure (Pdp) of 0.054 mg/kg-day is the human equivalent dose for the NOAEL based on absence of renal tubular degeneration and regeneration. Applying an advisory margin of exposure (MOE) of 300 yields the same value as the RfD. Thus, the RfD of 2×10^{-4} mg/kg-day will also be protective of cancer effect. In consideration of the overall evidence, the nonlinear approach was recommended by EPA (1998a).

2.0 IDENTITY: PHYSICAL AND CHEMICAL PROPERTIES

The chemical and physical properties of hexachlorobutadiene (HCBD) are summarized in Table 2-1. Synonyms for this chemical include perchlorobutadiene; 1,1,2,3,4,4-hexachloro-1,3-butadiene; 1,3-hexachlorobutadiene; Dolen-Pur; and GP-40-66:120.

HCBD is a colorless liquid at room temperature with a mild turpentine-like odor (HSDB, 2000). HCBD is poorly soluble in water, but is miscible in ethanol and ether (HSDB, 2000). HCBD has a relatively low vapor pressure of 0.15 mm Hg (U.S. EPA, 1991a). An odor threshold of 0.006 mg/L has been reported for HCBD in water (U.S. EPA, 1980). HCBD is characterized by high log K_{oc} and log K_{ow} values, 3.67 and 4.78, respectively (ATSDR, 1994), reflecting properties which strongly influence its behavior and fate in environmental media. The chemical structure of HCBD is shown in Figure 2-1.

Figure 2-1. Chemical Structure of Hexachlorobutadiene



Hexachlorobutadiene

Table 2-1. Chemical and Physical Properties of Hexachlorobutadiene

Property	Information
Chemical Abstracts Services (CAS) Registry No.	87-68-3
Chemical Formula	C_4Cl_6
Molecular Weight	260.76
Synonyms	HCBD; Perchlorobutadiene; Hexachlorbutadiene; 1,1,2,3,4,4-Hexachloro-1,3-butadiene; 1,3-Hexachlorobutadiene; Dolen-Pur; GP-40-66:120
NIOSH Registry of Toxic Effects of Chemical Substances (RTECS) No.	EJ0700000
U.S. EPA Hazardous Waste No.	U128
Oil and Hazardous Materials/Technical Assistance Data System (OHM/TADS) No.	OHM 8100011
Hazardous Substances Data Bank (HSDB) No.	2870
Boiling Point (at 760 mm Hg)	215°C
Melting Point	-21°C
Vapor Pressure (at 25°C)	0.15 mm Hg
Density (at 20°C)	1.55 g/cm^3
Water Solubility (at 20°C)	2–2.55 mg/L
Organic Solvents	Ethanol, Ether
Partition Coefficients	Log K_{ow} 4.78 Log K_{oc} 3.67
Odor Threshold (air) **Odor Threshold (water)**	12.00 mg/m^3 0.006 mg/L
Conversion Factor	1 ppm = 10.66 mg/m^3 1 mg/m^3 = 0.0938 ppm

Sources: U.S. EPA (1980, 1991a); ChemID*plus* (2000); HSDB (2000)

3.0 USES AND ENVIRONMENTAL FATE

3.1 Uses

HCBD has never been specifically manufactured as a commercial product in the United States. However, significant quantities of the chemical are generated in the U.S. as waste by-product from the chlorination of hydrocarbons, and lesser quantities are imported mostly from Germany as commercial product. HCBD is used as an intermediate product in rubber manufacturing and chlorofluorocarbon and lubricant production, as well as for transformer and hydraulic fluids, fluid for gyroscopes, heat transfer liquid, solvents, laboratory reagents, and as a wash liquor for removing C_4 and higher hydrocarbons. The chemical is also used as a fumigant in Russia, France, Italy, Greece, Spain, and Argentina (ATSDR, 1995; Howard, 1989).

Eight million pounds of HCBD were generated as a waste by-product in the U.S. in 1975, with 0.1 million pounds released into the environment. By 1982, the annual U.S. by-product generation of the chemical had jumped to 28 million pounds. In contrast, the annual import rate of HCBD dropped from 500,000 lbs/yr imported annually in the late 1970s, to 145,000 lbs/yr imported in 1981 (ATSDR, 1994; Howard, 1989).

3.2 Release to the Environment

HCBD is listed as a toxic release inventory (TRI) chemical. In 1986, the Emergency Planning and Community Right-to-Know Act (EPCRA) established the Toxic Release Inventory (TRI) of hazardous chemicals. Created under the Superfund Amendments and Reauthorization Act (SARA) of 1986, EPCRA is also sometimes known as SARA Title III. The EPCRA mandates that larger facilities publicly report when TRI chemicals are released into the environment. This public reporting is required for facilities with more than 10 full-time employees that annually manufacture or produce more than 25,000 pounds, or use more than 10,000 pounds, of TRI chemical (U.S. EPA, 1996c, 2000a).

Under these conditions, facilities are required to report the pounds per year of HCBD released into the environment both on- and off-site. The on-site quantity is subdivided into air emissions, surface water discharges, underground injections, and releases to land (see Table 3-1). For HCBD, air emissions constitute most of the on-site releases. Also, over the period for which data is available (1988–1998), surface water discharges generally increased, peaked in 1992–1993, and then decreased significantly through the late 1990s. These TRI data for HCBD were reported from eight States (CA, IL, KS, LA, NJ, NY, TX, UT); however, HCBD contamination has often been found in remote areas far from apparent physical discharge sources (U.S. EPA, 2000b; Howard, 1989).

Table 3-1. Environmental Releases (in pounds) for Hexachlorobutadiene in the United States, 1988–1998.

Year	On-Site Releases				Off-Site Releases	Total On- & Off-site Releases
	Air Emissions	Surface Water Discharges	Underground Injection	Releases to Land		
1998	2,421	5	0	0	510	2,936
1997	1,415	9	299	0	200	1,923
1996	2,381	256	952	0	310	3,899
1995	3,310	661	434	0	252	4,657
1994	1,410	351	201	0	430	2,392
1993	1,747	1,200	520	0	12	3,479
1992	4,134	1,911	738	0	5	6,788
1991	3,410	681	200	2	4,263	8,556
1990	4,906	715	330	0	45	5,996
1989	4,628	622	330	1	26,343	31,924
1988	2,508	153	220	0	19,640	22,521

source: U.S. EPA (2000b)

Although the TRI data can be useful in giving a general idea of release trends, it is far from exhaustive and has significant limitations. For example, only industries which meet TRI criteria (at least 10 full-time employees and manufacture and processing of quantities exceeding 25,000 lbs/yr, or use of more than 10,000 lbs/yr) are required to report releases. These reporting criteria do not account for releases from smaller industries. Threshold manufacture and processing quantities also changed from 1988–1990 (dropping from 75,000 lbs/yr in 1988 to 50,000 lbs/yr in 1989 to its current 25,000 lbs/yr in 1990) creating possibly misleading data trends. Finally, the TRI data is meant to reflect releases and should not be used to estimate general exposure to a chemical (U.S. EPA, 2000c, d).

While TRI releases were reported in only eight States, the use of HCBD is widespread. It is included in the Agency for Toxic Substances and Disease Registry's (ATSDR) Hazardous Substance Release and Health Effects Database (HazDat) and has been detected in site samples in fourteen States (AL, AZ, CT, IA, LA, MI, MN, NJ, NY, OH, PA, RI, SC, WA; ATSDR, 2000). These States are distributed nationwide and include 11 States and two regions (New England and the Pacific Northwest) not reporting TRI releases yet manifesting HCBD detections in the environment.

The National Priorities List (NPL) of hazardous waste sites, created in 1980 by the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), is a listing of some of the most health-threatening waste sites in the United States. HCBD was detected in eleven of the Final NPL sites in 1999. These sites are located in eight States: AK, CO, IN, LA, NJ, OH, PA, WA. Again, note that there is little overlap between these States and the eight TRI reporting States (U.S. EPA, 1999a).

In summary, although HCBD is not manufactured in the United States, both its use in industry and occurrence in the environment are widespread. Significant quantities of HCBD are generated in the United States as a waste by-product, and smaller quantities are imported for

industrial needs. HCBD is present in hazardous waste sites in at least 8 States (at NPL sites), has been detected in site samples in at least 14 States (listed in ATSDR's HazDat), and has been released into the environment directly in at least 8 States (based on TRI data).

3.3 Fate in Air

HCBD is released to air via chemical manufacturing and processing and by waste incineration (HSDB, 2000). Modeling and monitoring data suggest that the atmospheric burden of HCBD in the northern hemisphere is approximately 3.2 million kg/yr (Class and Ballschmiter, 1987). Dispersion of HCBD in the atmosphere has been confirmed by detection of HCBD at locations distant from sources of release (WHO, 1994). The high log organic carbon partition coefficient (log K_{oc}) of HCBD indicates that it will readily adsorb to airborne particulate matter with a high organic content. Thus, HCBD in air is found both as a vapor and in association with atmospheric particulates.

No specific information is available on the transformation and degradation of HCBD in air. By analogy to the structurally similar chemical tetrachloroethylene, HCBD is expected to react with photochemically-produced hydroxyl radicals and ozone via addition to double bonds (Atkinson and Carter, 1984; Atkinson, 1987). Mass-balance calculations based on monitoring data suggest that the half-life of atmospheric HCBD is about 1.6 years in the northern hemisphere (HSDB, 2000). However, comparison with tetrachloroethylene indicates that the half-life could be as short as 60 days as a result of reactions with ozone and hydroxyl radicals (HSDB, 2000).

3.4 Fate in Water

HCBD is released to surface and ground water via industrial effluents, by leaching from landfills or soil, or by urban runoff (ATSDR, 1994). Sorption to sediments and suspended particulates is an important factor in the fate of HCBD in water (U.S. EPA, 1991a). As a result of this affinity for particulates and sediments, HCBD-contaminated areas will usually have higher sediment concentrations than water concentrations of the chemical. U.S. EPA (1976) found that HCBD concentrations in the Mississippi delta water were <2 μg/L, while concentrations in mud or soil were >200 μg/L. Leeuwangh et al. (1975) observed that equilibration of initially uncontaminated sediment with HCBD-contaminated water resulted in sediment concentrations 100-fold greater than those observed in the water.

Volatilization of HCBD from water to air also occurs, although the low vapor pressure of HCBD (0.15 mmHg) suggests that this process may occur relatively slowly (U.S. EPA, 1991a). Limited data are available on the transformation and degradation of HCBD in water. Under aerobic conditions in batch culture, complete biodegradation has been observed to occur in sewage-inoculated waters after seven days (Tabak et al., 1981). These data suggest that HCBD may biodegrade in natural waters. In contrast, no degradation was observed under anaerobic conditions in a separate study (Johnson and Young, 1983). No data were available on hydrolysis or photolysis of HCBD in water. Estimates of HCBD half-life range from 3 to 30 days in rivers and 30 to 300 days in lakes and groundwater (Zoeteman et al., 1980).

The high octanol-water partition coefficient (K_{ow}) of HCBD suggests that this chemical can readily partition from water into biota. Laboratory and field investigations confirm that HCBD has bioaccumulation potential (WHO, 1994). Field-measured bioaccumulation factors range from 46 to 27,780 (U.S. EPA, 1999b). No evidence for biomagnification has been observed in laboratory or field studies (WHO, 1994).

3.5 Fate in Soil

HCBD can be released to soil by disposal of industrial waste in landfill operations (ATSDR, 1994). Volatilization from soil surfaces is expected to be a primary process for loss of HCBD from soil (Tabak et al., 1981). However, as HCBD readily adsorbs to soil organic particles, volatilization from highly organic soils is predicted to be low (HSDB, 2000).

No data regarding transformation or degradation of HCBD in soil were located. Data from experiments conducted in water (Tabak et al., 1981) suggest that biodegradation will occur if aerobic conditions are present (HSDB, 2000). Results obtained in sludge incubated under anaerobic conditions (Johnson and Young, 1983) suggest that biodegradation will not occur under anaerobic soil conditions. Soil organic matter content is likely to be an important factor in biodegradation time, since adsorption of HCBD to organic matter will significantly decrease its bioavailability to microorganisms. In the absence of significant biodegradation or other loss processes, persistence of HCBD in soil may allow migration of the compound into groundwater, particularly in sandy soils (U.S. EPA, 1984).

4.0 EXPOSURE FROM DRINKING WATER

This section of the report examines the occurrence of HCBD in drinking water. While no complete national database exists of unregulated or regulated contaminants in drinking water from public water systems (PWSs) collected under SDWA, this report aggregates and analyzes existing State data that have been screened for quality, completeness, and representativeness. Populations served by PWSs exposed to HCBD are estimated, and the occurrence data are examined for regional or other special trends. To augment the incomplete national drinking water data and aid in the evaluation of occurrence, information on the use and environmental release as well as ambient occurrence of HCBD are also reviewed.

4.1 Ambient Occurrence

To understand the presence of a chemical in the environment, an examination of ambient occurrence is useful. In a drinking water context, ambient water is source water existing in surface waters and aquifers before treatment. The most comprehensive and nationally representative data describing ambient water quality in the United States are being produced through the United States Geological Survey's (USGS) National Water Quality Assessment (NAWQA) program. NAWQA, however, is a relatively young program and complete national data are not yet available from their entire array of sites across the nation.

4.1.1 Data Sources and Methods

To examine water quality status and trends in the United States, the USGS instituted the NAWQA program in 1991. NAWQA is designed and implemented in such a manner to allow consistency and comparison between representative study basins located around the country, facilitating interpretation of natural and anthropogenic factors affecting water quality (Leahy and Thompson, 1994).

The NAWQA program consists of 59 significant watersheds and aquifers referred to as "study units." The study units represent approximately two thirds of the overall water usage in the United States and a similar proportion of the population served by public water systems. Approximately one half of the nation's land area is represented (Leahy and Thompson, 1994).

To facilitate management and make the program cost-effective, approximately one third of the study units at a time engage in intensive assessment for a period of 3 to 5 years. This is followed by a period of less intensive research and monitoring that lasts between 5 and 7 years. This way all 59 study units rotate through intensive assessment over a ten-year period (Leahy and Thompson, 1994). The first round of intensive monitoring (1991–1996) targeted 20 watersheds. This first group was more heavily slanted toward agricultural basins. A national synthesis of results from these study units and other research initiatives focusing on pesticides and nutrients is being compiled and analyzed (Kolpin et al., 1998; Larson et al., 1999).

For volatile organic chemicals (VOCs), the national synthesis will compile data from the first and second rounds of intensive assessments. Study units assessed in the second round

represent conditions in more urbanized basins, but initial results are not yet available. However, VOCs were analyzed in the first round of intensive monitoring and data are available for these study units (Squillace et al., 1999). The minimum reporting limit (MRL) for most VOCs, including HCBD, was 0.2 µg/L (Squillace et al., 1999). Additional information on analytical methods used in the NAWQA study units, including method detection limits, are described by Gilliom and others (in press).

Furthermore, the NAWQA program has compiled, by study unit, data collected from local, State, and other Federal agencies to augment its own data. The data set provides an assessment of VOCs in untreated ambient groundwater of the conterminous United States for the period 1985–1995 (Squillace et al., 1999). Data were included in the compilation if they met certain criteria for collection, analysis, well network design, and well construction (Lapham et al., 1997). They represent both rural and urban areas, but should be viewed as a progress report as NAWQA data continue to be collected that may influence conclusions regarding occurrence and distribution of VOCs (Squillace et al., 1999).

4.1.2 Results

Initial results published for the 20 NAWQA study units undergoing intensive assessment from 1991–1996 indicate that HCBD was not detected in ground water (Squillace et al., 1999). HCBD also was not detected in rural or urban wells of the local, State, and federal data set compiled by NAWQA. These data represent untreated ambient ground water of the conterminous United States for the years 1985–1995 (Squillace et al., 1999).

Furthermore, a review of highway and urban runoff studies found no detections of HCBD (Lopes and Dionne, 1998). This review was undertaken as part of the National Highway Runoff Data and Methodology Synthesis and examined 44 studies implemented since 1970.

4.2 Drinking Water Occurrence

The Safe Drinking Water Act (SDWA), as amended in 1986, required Public Water Systems (PWSs) to monitor for specified "unregulated" contaminants, on a five year cycle, and to report the monitoring results to the States. Unregulated contaminants do not have an established or proposed National Primary Drinking Water Regulation (NPDWR), but they are contaminants that were formally listed and required for monitoring under federal regulations. The intent was to gather scientific information on the occurrence of these contaminants to enable a decision as to whether or not regulations were needed. All non-purchased community water systems (CWSs) and non-purchased non-transient non-community water systems (NTNCWSs), with greater than 150 service connections, were required to conduct this unregulated contaminant monitoring. Smaller systems were not required to conduct this monitoring under federal regulations, but were required to be available to monitor if the State decided such monitoring was necessary. Many States collected data from smaller systems. Additional contaminants were added to the Unregulated Contaminant Monitoring (UCM) program in 1991 (U.S. EPA, 1991c) for required monitoring that began in 1993 [57 FR 31776] (U.S. EPA, 1992c).

HCBD has been monitored under the SDWA UCM program since 1987 [52 FR 25720]. Monitoring for HCBD under UCM continued throughout the 1990s, but ceased for small public water systems (PWSs) under a direct final rule published on January 8, 1999 (64 FR 1494). Monitoring ended for large PWSs with promulgation of the new Unregulated Contaminant Monitoring Regulation (UCMR) issued September 17, 1999 (64 FR 50556) and effective January 1, 2001. At the time the UCMR lists were developed, the Agency concluded there were adequate monitoring data for a regulatory determination. This obviated the need for continued monitoring under the new UCMR list.

4.2.1 Data Sources, Data Quality, and Analytical Methods

Currently, there is no complete national record of unregulated or regulated contaminants in drinking water from public water systems collected under SDWA. Many States have submitted their unregulated contaminant PWS monitoring data to EPA databases, but there are issues of data quality, completeness, and representativeness. Nonetheless, a significant amount of State data are available for UCM contaminants that can provide estimates of national occurrence.

The National Contaminant Occurrence Database (NCOD) is an interface to the actual occurrence data stored in a database called the Safe Drinking Water Information System (Federal version; SDWIS/FED) and can be queried to provide a summary of the data in SDWIS/FED for a particular contaminant. The data used in this report were derived from the data in SDWIS/FED and another database called the Unregulated Contaminant Information System (URCIS).

The data in this report have been reviewed, edited, and filtered to meet various data quality objectives for the purposes of this analysis. Hence, not all data from a particular source were used, only data meeting the quality objectives described below. The sources of these data, their quality and national aggregation, and the analytical methods used to estimate a given contaminant's national occurrence (from these data) are discussed in this section (for further details see U.S. EPA, 2001a,b).

UCM Rounds 1 and 2

The 1987 UCM contaminants include 34 volatile organic compounds (VOCs), divided into two groups: one with 20 VOCs for mandatory monitoring, and the other with 14 VOCs for discretionary monitoring [52 FR 25720]. HCBD was among the 14 VOCs included for discretionary monitoring. The UCM (1987) contaminants were first monitored coincident with the Phase I regulated contaminants, during the 1988–1992 period. This period is often referred to as "Round 1" monitoring. The monitoring data collected by the PWSs were reported to the States (as primacy agents), but there was no protocol in place to report these data to EPA. These data from Round 1 were collected by EPA from many States over time.

The Round 1 data were put into a database called the Unregulated Contaminant Information System, or URCIS. Most of the Phase 1 regulated contaminants were also VOCs. Both the unregulated and regulated VOCs are analyzed using the same sample and the same

laboratory methods. Hence, the URCIS database includes data on all of these 62 contaminants: the 34 UCM (1987) VOCs; the 21 regulated Phase 1 VOCs; 2 regulated synthetic organic contaminants (SOCs); and 5 miscellaneous contaminants that were voluntarily reported by some States (e.g., isomers of other organic contaminants).

The 1993 UCM contaminants include 13 SOCs and 1 inorganic contaminant (IOC) [56 FR 3526]. Monitoring for the UCM (1993) contaminants began coincident with the Phase II/V regulated contaminants in 1993 through 1998. This is often referred to as "Round 2" monitoring. The UCM (1987) contaminants were also included in the Round 2 monitoring. As with other monitoring data, PWSs reported these results to the States. EPA, during the past several years, requested that the States submit these historic data to EPA.

The details of the actual individual monitoring periods are complex. The timing of required monitoring was staggered related to different size classes of PWSs, and the program was implemented somewhat differently by different States. While Round 1 includes the period from 1988–1992, it also includes results from samples analyzed prior to 1988 that were "grandfathered" into the database (for further details see U.S. EPA, 2001a,b).

Developing a Nationally Representative Perspective

The URCIS and SDWIS/FED databases contain contaminant occurrence data from a total of 40 and 35 primacy entities (largely States), respectively. However, data from some States are incomplete and biased. Furthermore, the national representativeness of the data is questionable because the data were not collected in a systematic or random statistical framework. These State data could be heavily skewed to low-occurrence or high-occurrence settings. Hence, the data were evaluated based on pollution-potential indicators and the spatial/hydrologic diversity of the nation. This evaluation enabled the construction of a cross-section from the available State data sets that provides a reasonable representation of national occurrence.

A national cross-section from State SDWA contaminant databases was established using the approach developed for the EPA report *A Review of Contaminant Occurrence in Public Water Systems* (U.S. EPA, 1999c). This approach was developed to support occurrence analyses for EPA's Chemical Monitoring Reform (CMR) evaluation. It was supported by peer reviewers and stakeholders because it is clear, repeatable, and understandable. The approach cannot provide a "statistically representative" sample because the original monitoring data were not collected or reported in an appropriate fashion. However, the resultant "national cross-section" of States should provide a clear indication of the central tendency of the national data. The remainder of this section provides a summary description of how the national cross-sections for the URCIS (Round 1) and SDWIS/FED (Round 2) databases were developed. The details of the approach are presented in other documents (U.S. EPA, 2001a,b); readers are referred to these for more specific information.

Cross-Section Development

As a first step in developing the cross-section, the State data contained in the URCIS database (which contains Round 1 monitoring results) and SDWIS/FED database (which contains Round 2 monitoring results) were evaluated for completeness and quality. For both the URCIS (Round 1) and SDWIS/FED (Round 2) databases, some State data were unusable for a variety of reasons. Some States reported only detections, or their data had incorrect units. Datasets only including detections are obviously biased. Other problems included incomplete data sets without all PWSs reporting. Also, data from Washington, D.C. and the Virgin Islands were excluded from this analysis because it was difficult to evaluate them for the current purposes in relation to complete State data (U.S. EPA, 2001a, Sections II and III).

The balance of the States remaining after the data quality screening were then examined to establish a national cross-section. This step was based on evaluating the States' pollution potential and geographic coverage in relation to all States. Pollution potential is considered to ensure a selection of States that represent the range of likely contaminant occurrence and a balance with regard to likely high and low occurrence. Geographic consideration is included so that the wide range of climatic and hydrogeologic conditions across the United States are represented, again balancing the varied conditions that affect transport and fate of contaminants (U.S. EPA, 2001b, Sections III.A. and III.B.).

The cross-section States were selected to represent a variety of pollution potential conditions. Two primary pollution potential indicators were used. The first factor selected indicates pollution potential from manufacturing/population density and serves as an indicator of the potential for VOC contamination within a State. Agriculture was selected as the second pollution potential indicator because the majority of SOCs of concern are pesticides (U.S. EPA, 2001b, Section III.A.). The 50 individual States were ranked from highest to lowest based on the pollution potential indicator data. For example, the State with the highest ranking for pollution potential from manufacturing received a ranking of 1 for this factor and the State with the lowest value was ranked as number 50. States were ranked for their agricultural chemical use status in a similar fashion.

The States' pollution potential rankings for each factor were subdivided into four quartiles (from highest to lowest pollution potential). The cross-section States were chosen from all quartiles for both pollution potential factors to ensure representation, as follows: States with high agrichemical pollution potential rankings and high manufacturing pollution potential rankings; States with high agrichemical pollution potential rankings and low manufacturing pollution potential rankings; States with low agrichemical pollution potential rankings and high manufacturing pollution potential rankings; and States with low agrichemical pollution potential rankings and low manufacturing pollution potential rankings (U.S. EPA, 2001b, Section III.B.). In addition, some secondary pollution potential indicators were considered to further ensure that the cross-section States included the spectrum of pollution potential conditions (high to low).

The data quality screening, pollution potential rankings, and geographic coverage analysis established national cross-sections of 24 Round 1 (URCIS) States and 20 Round 2 (SDWIS/FED) States. In each cross-section, the States provide good representation of the nation's varied climatic and hydrogeologic regimes and the breadth of pollution potential for the contaminant groups (Table 4-1 and Figure 4-1).

Cross-Section Evaluation

To evaluate and validate the method for creating the national cross-sections, the method was used to create smaller State subsets from the 24-State, Round 1 cross-section and aggregations. Again, States were chosen to achieve a balance from the quartiles describing pollution potential, and a balanced geographic distribution, to incrementally build subset cross-sections of various sizes. For example, the Round 1 cross-section was tested with subsets of 4, 8 (the first 4-State subset plus 4 more States), and 13 (8-State subset plus 5) States. Two additional cross-sections were included in the analysis for comparison: a cross-section composed of the 16 biased States eliminated from the 24-State cross-section for data quality reasons and a cross-section composed of all 40 Round 1 States (U.S. EPA, 2001, Section III.B.1).

These Round 1 incremental cross-sections were then used to evaluate occurrence for an array of both high and low occurrence contaminants. The comparative results illustrate several points. The results are quite stable and consistent for the 8-, 13- and 24-State cross-sections. They are much less so for the 4-State, 16-State (biased), and 40-State (all Round 1 States) cross-sections. The 4-State cross-section is apparently too small to provide balance both geographically and with pollution potential, a finding that concurs with past work (U.S. EPA, 1999c). The CMR analysis suggested that a minimum of 6–7 States was needed to provide balance both geographically and with pollution potential, and the CMR report used 8 States out of the available data for its nationally representative cross-section. The 16-State and 40-State cross-sections, both including the biased States, provided occurrence results that were unstable and inconsistent for a variety of reasons associated with their data quality problems (U.S. EPA, 2001, Section III.B.1).

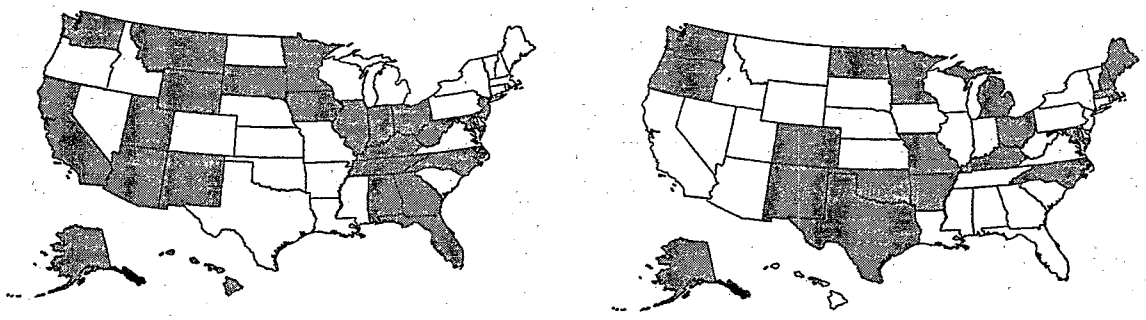
The 8-, 13-, and 24-State cross-sections provide very comparable results, are consistent, and are usable as national cross-sections to provide estimates of contaminant occurrence. Including greater data from more States improves the national representation and the confidence in the results, as long as the States are balanced related to pollution potential and spatial coverage. The 24- and 20-State cross-sections provide the best, nationally representative cross-sections for the Round 1 and Round 2 data.

Table 4-1. Cross-section States for Round 1 (24 States) and Round 2 (20 States).

Round 1 (URCIS)		Round 2 (SDWIS/FED)	
Alabama	Minnesota*	Alaska*	New Hampshire
Alaska*	Montana	Arkansas	New Mexico*
Arizona	New Jersey	Colorado	North Carolina*
California	New Mexico*	Kentucky*	North Dakota
Florida	North Carolina*	Maine	Ohio*
Georgia	Ohio*	Maryland*	Oklahoma
Hawaii	South Dakota	Massachusetts	Oregon
Illinois	Tennessee	Michigan	Rhode Island
Indiana	Utah	Minnesota*	Texas
Iowa	Washington*	Missouri	Washington*
Kentucky*	West Virginia		
Maryland*	Wyoming		

** cross-section State in both Round 1 and Round 2*

Figure 4-1. Geographic Distribution of Cross-section States for Round 1 (left) and Round 2 (right).



Data Management and Analysis

The cross-section analyses focused on occurrence at the water system level; i.e., the summary data presented discuss the percentage of public water *systems* with detections, not the percentage of *samples* with detections. By normalizing the analytical data to the system level, skewness inherent in the sample data, particularly over the multi-year period covered in the URCIS data, is avoided. System level analysis was used since a PWS with a known contaminant problem usually has to sample more frequently than a PWS that has never detected the contaminant. Obviously, the results of a simple computation of the percentage of samples with detections (or other statistics) can be skewed by the more frequent sampling results reported by the contaminated site. This level of analysis is conservative. For example, a system need only have a single sample with an analytical result greater than the MRL, i.e., a detection, to be counted as a system with a result "greater than the MRL."

Also, the data used in the analyses were limited to only those data with confirmed water source and sampling type information. Only standard SDWA compliance samples were used; "special" samples, or "investigation" samples (investigating a contaminant problem that would bias results), or samples of unknown type were not used in the analyses. Various quality control and review checks were made of the results, including follow-up questions to the States providing the data. Many of the most intractable data quality problems encountered occurred with older data. These problematic data were, in some cases, simply eliminated from the analysis. For example, when the number of data with problems were insignificant relative to the total number of observations, they were dropped from the analysis (For further details, see Cadmus, 2000).

Occurrence Analysis

To evaluate national contaminant occurrence, a two-stage analytical approach has been developed. The first stage of analysis provides a straight-forward, conservative, broad evaluation of occurrence of the Contaminant Candidate List (CCL) preliminary regulatory determination priority contaminants as described above. These descriptive statistics are summarized here. Based on the findings of the Stage 1 Analysis, EPA will determine whether more intensive statistical evaluations, the Stage 2 Analysis, may be warranted to generate national probability estimates of contaminant occurrence and exposure for priority contaminants (for details on this two stage analytical approach see Cadmus, 2000)

The summary descriptive statistics presented in Table 4-2 for HCBD are a result of the Stage 1 analysis and include data from both Round 1 (URCIS, 1987–1992) and Round 2 (SDWIS/FED, 1993–1997) cross-section States. Included are the total number of samples, the percent samples with detections, the 99th percentile concentration of all samples, the 99th percentile concentration of samples with detections, and the median concentration of samples with detections. The percentages of PWSs and population served indicate the proportion of PWSs whose analytical results showed a detection(s) of the contaminant (simple detection, > MRL) at any time during the monitoring period; or a detection(s) greater than half the Health Reference Level (HRL); or a detection(s) greater than the Health Reference Level. The Health Reference

Level, 0.9 µg/L, is a preliminary estimated health effect level used for this analysis. The HRL was derived using the 10^{-6} cancer risk as calculated by the linear method using a body weight to the three quarter power (section 8.8.2; slope factor 4×10^{-2} $(mg/kg/day)^{-1}$.

When monitoring results were compared to a value of one-half of the HRL, 0.16% of Round 1 (106 systems) and 0.08% of Round 2 (51 systems) water supplies exceeded this benchmark at least once during the reporting period. The percentages of water supplies that exceeded the HRL at least once in Round 1 and Round 2 monitoring were 0.11% (74 systems) and 0.02% (11 systems), respectively.

The 99th percentile concentration is used here as a summary statistic to indicate the upper bound of occurrence values because maximum values can be extreme values (outliers) that sometimes result from sampling or reporting error. The 99th percentile concentration is presented for both the samples with only detections and all of the samples because the value for the 99th percentile concentration of all samples is below the MRL (denoted by "<" in Table 4-2). For the same reason, summary statistics such as the 95th percentile concentration of all samples or the median (or mean) concentration of all samples are omitted because these also are all "<" values. This is the case because only 0.1 to 0.05% of all samples recorded detections of HCBD in Round 1 and Round 2.

As a convention, a value of half the MRL is often used as an estimate of the concentration of a contaminant in samples/systems whose results are less than the MRL. With a contaminant with relatively low occurrence such as HCBD in drinking water occurrence databases, the median or mean value of occurrence using this assumption would be half the MRL (0.5 × MRL). However, for these occurrence data this is not straightforward. For Round 1 and Round 2, States have reported a wide range of values for the MRLs. This is in part related to State data management differences as well as real differences in analytical methods, laboratories, and other factors.

The situation can cause confusion when examining descriptive statistics for occurrence. For example, the modal MRL value for the Round 1 samples is 0.50 µg/L—a value twice as large as the median concentration of detections for Round 1 (0.25 µg/L) (This occurs because some States and/or systems reporting detections were using a lower MRL and had positive results lower than the MRL used by other States or systems). For Round 2, most States reported non-detections as zeros resulting in a modal MRL value of zero. By definition the MRL cannot be zero. This is an artifact of State data management systems. Because a simple meaningful summary statistic is not available to describe the various reported MRLs, and to avoid confusion, MRLs are not reported in the summary table, but rather are designated as "variable" (Table 4-2).

In Table 4-2, national occurrence is estimated by extrapolating the summary statistics for the 24- and 20-State cross-sections to national numbers for systems, and population served by systems, from the *Water Industry Baseline Handbook, Second Edition* (U.S. EPA, 2000e). From the handbook, the total number of community water systems (CWSs) plus non-transient, non-community water systems (NTNCWSs) is 65,030, and the total population served by CWSs plus

NTNCWSs is 213,008,182 persons (see Table 4-2). To arrive at the national occurrence estimate for a particular cross-section, the national estimate for PWSs (or population served by PWSs) is simply multiplied by the percentage for the given summary statistic. [i.e., for Round 1, the national estimate for the total number of PWSs with detections (228) is the product of the percentage of Round 1 PWSs with detections (0.35%) and the national estimate for the total number of PWSs (65,030)].

Because the State data used for the cross-section are not a strict statistical sample, national extrapolations of these Stage 1 analytical results can be problematic, especially for contaminants with very low occurrence like hexachlorobutadiene and other CCL regulatory determination priority contaminants. For this reason, the nationally extrapolated estimates of occurrence based on Stage 1 results are not presented in the CCL Federal Register Notice. The presentation in the Federal Register Notice of only the actual results of the cross-section analysis maintains a straight-forward presentation, and the integrity of the data, for stakeholder review. The nationally extrapolated Stage 1 occurrence values are presented here, however, to provide additional perspective. A more rigorous statistical modeling effort, the Stage 2 analysis, could be conducted on the cross-section data (Cadmus, 2001). The Stage 2 results would be more statistically robust and more suitable to national extrapolation. This approach would provide a probability estimate and would also allow for better quantification of estimation error.

Round 1(1987–1992) and Round 2 (1993–1997) data were not merged because they represent different time periods, different States (only eight States are represented in both rounds), and each round has different data management and data quality problems. The two rounds are only merged for the simple spatial analysis overview presented in Section 4.2 and Figures 4-2 and 4-4.

4.2.2 Results

Occurrence Estimates

While States with detections of HCBD are widespread (Figure 4-2), the percentages of PWSs by State with detections are low (Table 4-2). In aggregate, the cross-sections show only 0.2–0.4 % of PWSs in both rounds experienced detections (>MRL), affecting 0.9–2.4% of the population served (approximately 2–5 million people). Percentages of PWSs with detections greater than half the Health Reference Level (> ½ HRL) are slightly lower: 0.1–0.2%. The percentage of PWSs exceeding the Health Reference Level (> HRL) for both rounds is very small (see also Figure 4-4). Between 0.02 and 0.1% of PWSs in Rounds 1 and 2 experienced detections >HRL, affecting a population of approximately 10,000–780,000.

There are some qualifying notes for both rounds of data that warrant discussion. The Round 1 estimates of PWSs affected by HCBD are influenced by the State of Florida (Table 4-2; Figures 4-3 and 4-4). This State reports that 5.4% of its PWSs experienced detections greater than the HRL during Round 1, a value considerably greater than the next highest State (1.5%).

This suggests that Florida's data for HCBD is incomplete and may be biased. Out of 855 Florida PWSs reporting contaminant data for Round 1 monitoring, only 112 provided data for HCBD (U.S. EPA, 2001a). Also, the 5.4% of systems reporting detections all reported concentrations greater than the Health Reference Level. These figures suggest that perhaps only systems experiencing problems submitted data for HCBD, biasing Florida's results for occurrence measures examined in this report.

The large values for the Round 2 national estimates of population served with detections greater than the MRL and greater than half the HRL are influenced by the inclusion of one PWS serving a very large population (1.5 million people). While the percentage of systems with detections of HCBD are similar (both rounds show low values, 0.2–0.4% PWSs > MRL), the difference in population served results in a larger difference in the population extrapolations.

Note that for the Round 1 cross-section, the total number of PWSs (and the total population served by the PWSs) is not the sum of the number of ground water and surface water systems (or the populations served by those systems). Because some public water systems are seasonally classified as either surface or ground water, some systems may be counted in both categories. The population numbers for the Round 1 cross-section are also incomplete. Not all of the PWSs for which occurrence data was submitted reported the population they served. However, the population numbers presented in Table 4-2 for the Round 1 cross-section are reported from 94% of the systems.

The national estimates extrapolated from Round 1 and Round 2 PWS, numbers and populations are not additive. In addition to the Round 1 classification and reporting issues outlined above, the proportions of surface water and ground water PWSs, and populations served by them, are different between the Round 1 and 2 cross-sections and the national estimates. For example, approximately 48% of the population served by PWSs in the Round 1 cross-section States are served by surface water PWSs (Table 4-2). Nationally, however, that proportion changes to 60%.

Both Round 1 and Round 2 national cross-sections show a proportionate balance in source waters. Nationally, 91% of PWSs use ground water (and 9% surface waters): Round 1 shows 89%, and Round 2 shows 90% of systems using ground water. The relative populations served are not as closely comparable. Nationally, about 40% of the population is served by PWSs using groundwater (and 60% by surface water). Round 2 data is most representative with 37% of the cross-section population served by ground water; Round 1 shows about 55%.

There are differences in the occurrence results between Round 1 and Round 2, as should be expected. The differences are not great, however, particularly when comparing the proportions of systems affected. The results range from 0.2–0.4% of PWSs with detections of HCBD and range from 0.02–0.1% of PWSs with detections greater than the Health Reference Level of 0.9 µg/L. These are not substantively different, given the data sources.

The differences in the population extrapolations appear greater, but still constitute relatively small proportions of the population. The most pronounced difference is in the estimate of the population served by PWSs with detections greater than the Health Reference Level, ranging from 10,000 to 780,000. In both cases, this is less than 0.5% of the population. The difference in this category is largely driven by the Florida data in Round 1, as discussed above.

The Round 2 cross-section provides a better proportional balance related to the national population of PWSs and may have fewer reporting problems than Round 1 (i.e., incomplete population numbers, Florida). The larger estimate of the national population served by PWSs with detections greater than the Health Reference Level using Round 1 data can also provide an upper bound estimate in considering the data.

Regional Patterns

Occurrence results are displayed graphically by State in Figures 4-2, 4-3, and 4-4 to assess whether any distinct regional patterns of occurrence are present. Combining Round 1 and Round 2 data (Figure 4-2), there are 47 States reporting. Six of those States have no data for HCBD, while another 21 have no detections of the chemical. The remaining 20 States have detected HCBD in drinking water and are well distributed throughout the United States.

The simple spatial analysis presented in Figures 4-2, 4-3, and 4-4 suggests that special regional analyses are not warranted. Florida's possible bias is notable, however. While no clear geographical patterns of occurrence are apparent, comparisons with environmental use and release information are useful (see also Section 2.2). Five of the eight Toxic Release Inventory States that reported releases of HCBD into the environment between 1988 and 1998 have also detected the chemical in PWS sampling. Of the remaining three (Kansas, Louisiana, and California), Kansas hasn't reported any data for either Round 1 or 2. Also, of the eight States with detections of HCBD at CERCLA National Priorities List (NPL) hazardous waste sites, five have detected the chemical in drinking water. Finally, six of the States detecting HCBD in PWS samples have also detected it in site samples reported to the ATSDR's HazDat database. It is interesting to note that neither Alabama nor Florida, the two States with the highest percentage of PWSs with detections greater than the Health Reference level, are Toxic Release Inventory (TRI) States for HCBD, nor do they have CERCLA NPL sites with detections of the chemical (Figure 4-4).

Table 4-2. Summary Occurrence Statistics for Hexachlorobutadiene.

Frequency Factors	24-State Cross-Section[1] (Round 1)	20-State Cross-Section[2] (Round 2)	National System & Population Numbers[3]	
Total Number of Samples	42,839	93,585	--	
Percent of Samples with Detections	0.13%	0.05%	--	
99th Percentile Concentration (all samples)	< (Non-detect)	< (Non-detect)	--	
Health Reference Level	0.9 µg/L	0.9 µg/L	--	
Minimum Reporting Level (MRL)	Variable*	Variable*	--	
99th Percentile Concentration of Detections	10 µg/L	1.5 µg/L	--	
Median Concentration of Detections	0.25 µg/L	0.30 µg/L	--	
Total Number of PWSs	12,284	22,736	65,030	
Number of GW PWSs	10,980	20,380	59,440	
Number of SW PWSs	1,385	2,356	5,590	
Total Population	71,582,571	67,075,493	213,008,182	
Population of GW PWSs	40,399,177	24,960,222	85,681,696	
Population of SW PWSs	34,418,834	42,115,271	127,326,486	
			National Extrapolation[4]	
Occurrence by System			**Round 1**	**Round 2**
% PWSs with detections (> MRL)	0.350%	0.180%	228	117
Range of Cross-Section States	0– 5.36%	0– 3.36%	N/A	N/A
GW PWSs with detections	0.301%	0.132%	179	79
SW PWSs with detections	0.722%	0.594%	40	33
% PWSs > 1/2 Health Reference Level (HRL)	0.163%	0.079%	106	51
Range of Cross-Section States	0– 5.36%	0– 0.51%	N/A	N/A
GW PWSs > 1/2 Health Reference Level	0.118%	0.064%	70	38
SW PWSs > 1/2 Health Reference Level	0.505%	0.212%	28	12
% PWSs > Health Reference Level	0.114%	0.018%	74	11
Range of Cross-Section States	0– 5.36%	0– 0.24%	N/A	N/A
GW PWSs > Health Reference Level	0.064%	0.005%	38	3
SW PWSs > Health Reference Level	0.505%	0.127%	28	7

Table 4-2 (continued)

Frequency Factors	24-State Cross-Section[1] (Round 1)	20-State Cross-Section[2] (Round 2)	National System & Population Numbers[3]	
Occurrence by Population Served			**Round 1**	**Round 2**
% PWS Population Served with detections	0.896%	2.360%	1,909,000	5,027,000
Range of Cross-Section States	0–11.38%	0– 29.93%	N/A	N/A
GW PWS Population with detections	1.458%	0.186%	1,249,000	159,000
SW PWS Population with detections	0.153%	3.649%	194,000	4,646,000
% PWS Population Served > 1/2 Health Ref Level	0.569%	2.331%	1,213,000	4,965,000
Range of Cross-Section States	0– 11.38%	0– 29.92%	N/A	N/A
GW PWS Population > 1/2 Health Ref Level	0.978%	0.177%	838,000	152,000
SW PWS Population > 1/2 Health Ref Level	0.036%	3.607%	46,000	4,593,000
% PWS Population Served > Health Ref Level	0.367%	0.005%	781,000	10,000
Range of Cross-Section States	0– 9.66%	0– 0.02%	N/A	N/A
GW PWS Population > Health Ref Level	0.619%	0.011%	531,000	9,000
SW PWS Population > Health Ref Level	0.036%	0.001%	46,000	1,000

1. Summary Results based on data from 24-State Cross-Section, from URCIS, UCM (1987) Round 1.
2. Summary Results based on data from 20-State Cross-Section, from SDWIS/FED, UCM (1993) Round 2.
3. Total PWS and population numbers are from EPA March 2000 Water Industry Baseline Handbook.
4. National extrapolations are from the 24-State data and 20-State data using the Baseline Handbook system and population numbers.

* see text for discussion
- PWS = Public Water Systems; GW = Ground Water; SW = Surface Water; MRL = Minimum Reporting Level (for laboratory analyses);
- Health Reference Level = Health Reference Level, an estimated health effect level used for preliminary assessment for this review; N/A = Not Applicable
- The Health Reference Level used for hexachlorobutadiene is 0.9 µg/L. This is a draft value for working review only.
- Total Number of Samples = the total number of analytical records for hexachlorobutadiene.
- 99th Percentile Concentration = the concentration value of the 99th percentile of either all analytical results or just the samples with detections (in µg/L).
- Median Concentration of Detections = the median analytical value of all the detections (analytical results greater than the MRL) (in µg/L).
- Total Number of PWSs = the total number of public water systems with records for hexachlorobutadiene.
- Total Population Served = the total population served by public water systems with records for hexachlorobutadiene.
- % PWS with detections, % PWS > ½ Health Reference Level, % PWS > Health Reference Level = percent of the total number of public water systems with at least one analytical result that exceeded the MRL, ½ Health Reference Level, Health Reference Level, respectively.
- % PWS Population Served with detections, % PWS Population Served >½ Health Reference Level, % PWS Population Served > Health Reference Level = percent of the total population served by PWSs with at least one analytical result exceeding the MRL, ½ Health Reference Level, or the Health Reference Level, respectively.

Figure 4-2. States with PWSs with Detections of Hexachlorobutadiene for all States with Data in URCIS (Round 1) and SDWIS/FED (Round 2).

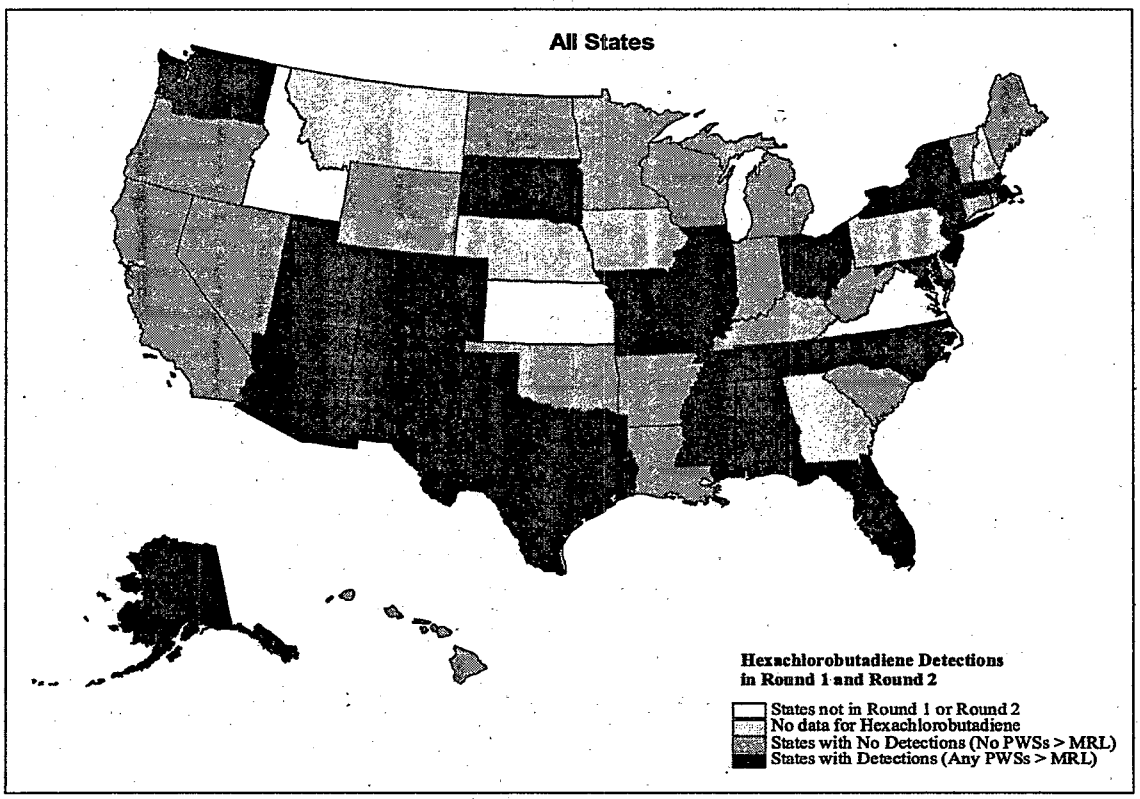
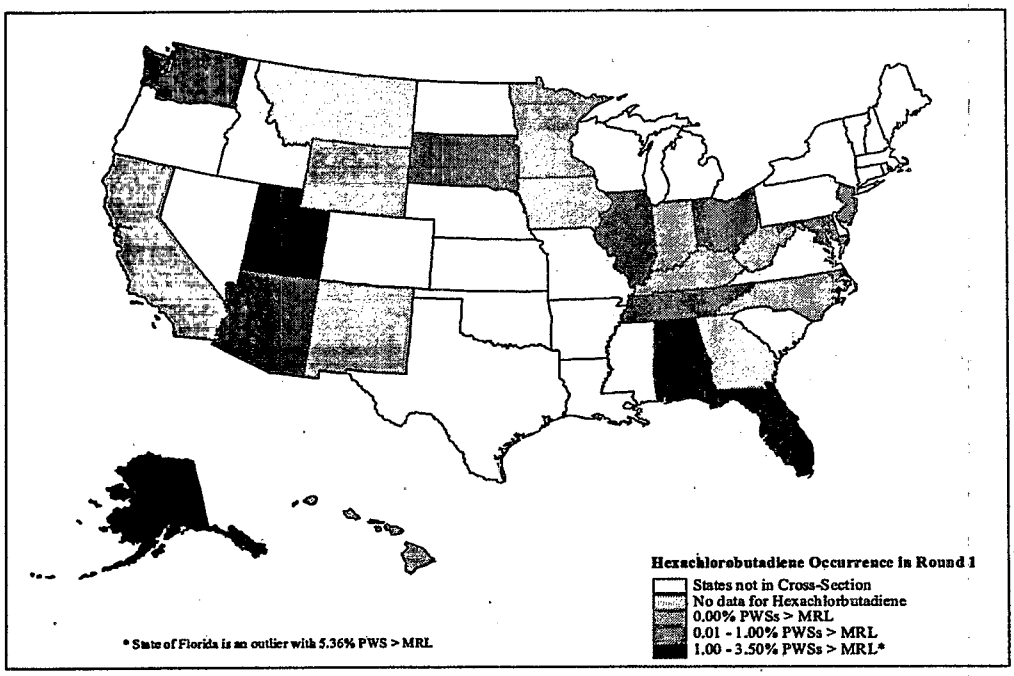


Figure 4-3. States with PWSs with Detections of Hexachlorobutadiene (any PWSs with results greater than the Minimum Reporting Level [MRL]) for Round 1 (above) and Round 2 (below) Cross-section States.



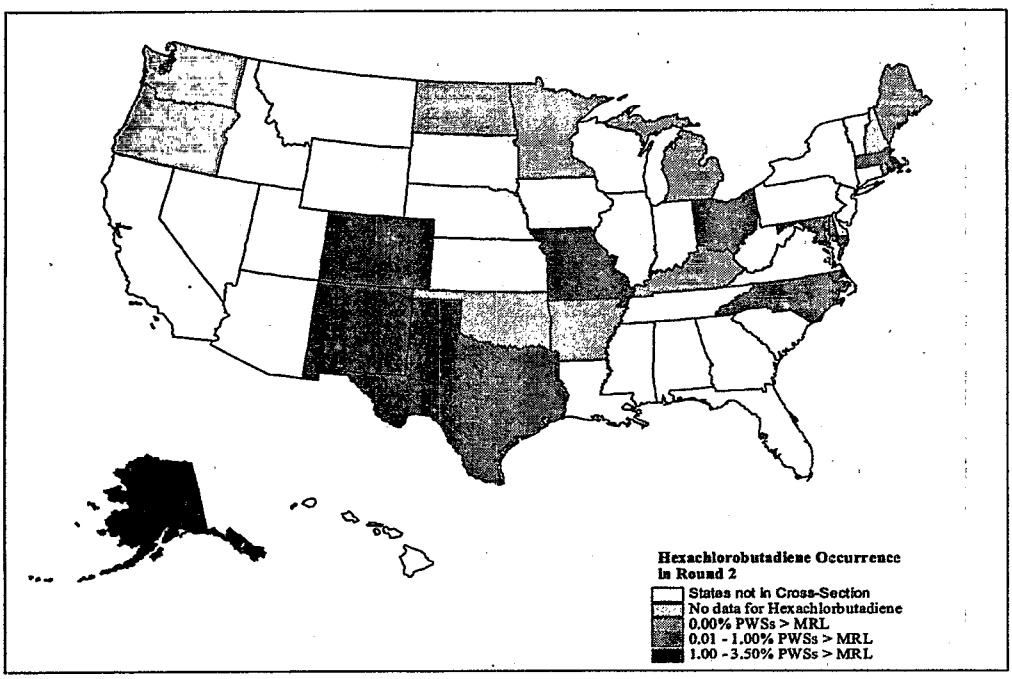
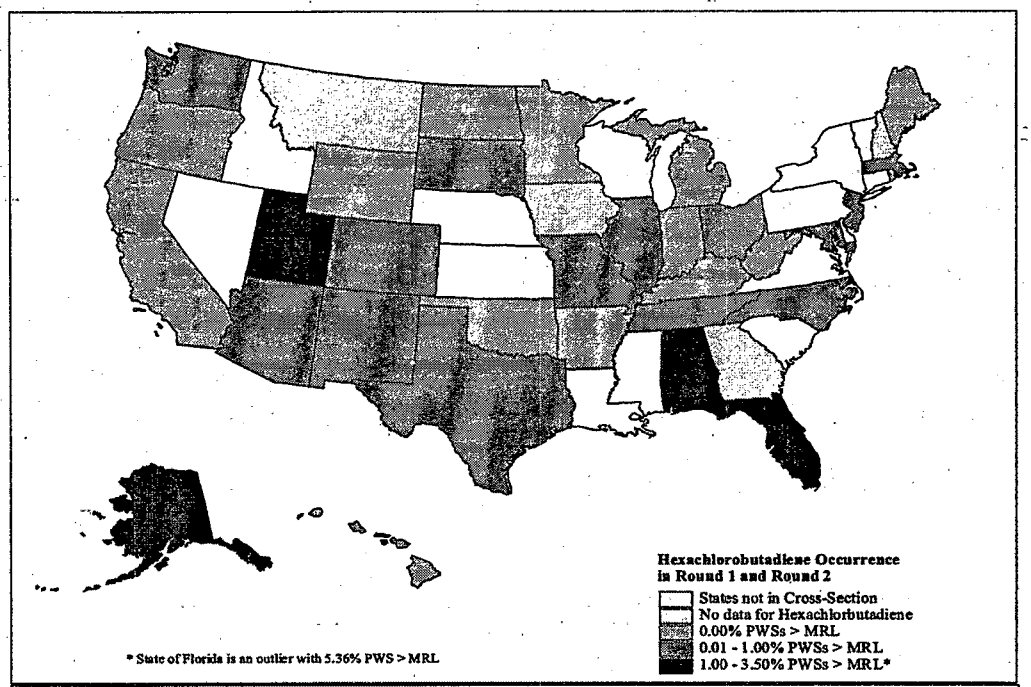
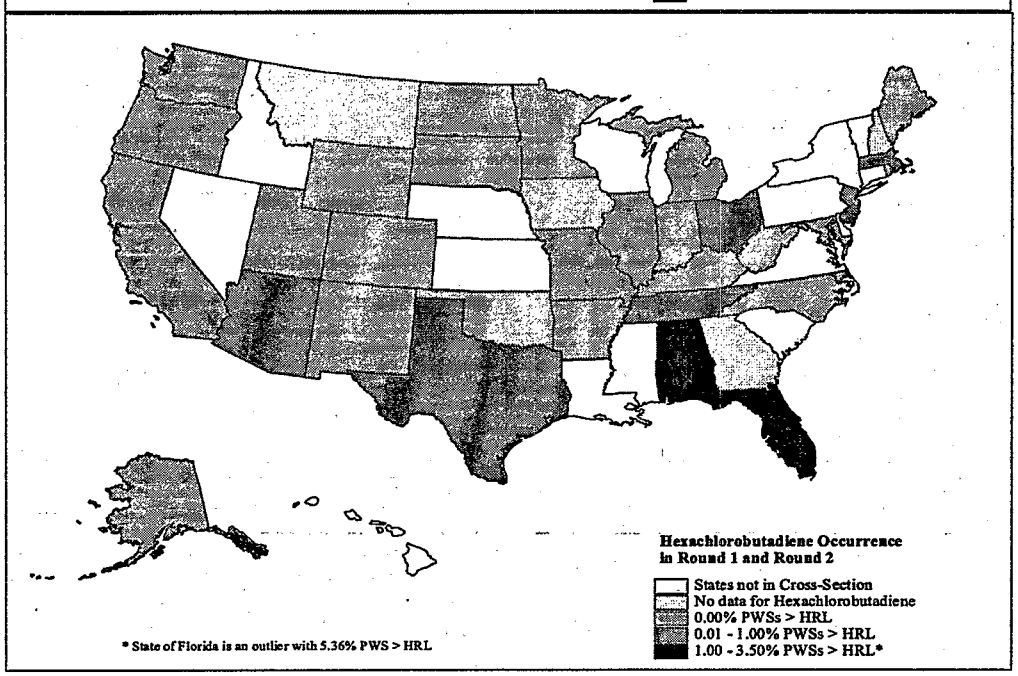


Figure 4-4. Cross-section States (Round 1 and Round 2 combined) with PWSs with Detections of Hexachlorobutadiene (above) and concentrations greater than the Health Reference Level (HRL; below).





4.3 Conclusions

While there have not been detections of the chemical in ambient water reported in USGS NAWQA studies to date, hexachlorobutadiene has been detected at a very low percentage of ATSDR HazDat sites and CERCLA NPL sites. Furthermore, releases have been reported through the TRI.

Hexachlorobutadiene has also been detected in PWS samples collected under SDWA. Occurrence estimates are low for Round 1 and Round 2 monitoring with only 0.13 % and 0.05% of all samples showing detections, respectively. Significantly, the values for the 99th percentile and median concentrations of all samples are less than the Minimum Reporting Level. For Round 1 samples with detections, the median concentration is 0.25 µg/L and the 99th percentile concentration is 10 µg/L. Median and 99th percentile concentrations for Round 2 detections are 0.30 µg/L and 1.5 µg/L, respectively. Systems with detections only constitute 0.4% of Round 1 systems and 0.2% for Round 2. National estimates for the population served by PWSs with detections are also low, especially for detections greater than the Health Reference Level. For both rounds, these estimates are less than 0.5% of the national population (Round 1: 781,076; Round 2: 9,721).

5.0 EXPOSURE FROM MEDIA OTHER THAN WATER

This section describes studies which measured concentrations of HCBD in food, air, and soil. Exposure of adults and children is estimated by combining the reported concentrations with the estimated intake of each medium. These calculations enable a comparison of exposure to HCBD from air, food, and soil with that anticipated from ingestion of drinking water (see Chapter 9.0). Estimates of human exposure to HCBD via food and air have previously been calculated by U.S. EPA (1998a).

5.1 Exposure from Food

Food may be contaminated with HCBD via environmental sources or by contact with contaminated water during food processing activity (DiNovi, 1997). According to the Food and Drug Administration (FDA), there are no approved uses of HCBD either directly or indirectly in foods, including food processing equipment (DiNovi, 1997). HCBD is not regulated in plastics.

5.1.1 Concentrations in Non-Fish Food Items

Two reports provide data for the concentration of HCBD in food items. Yip (1976) measured HCBD in food items within a 25-mile radius of tetrachloroethylene and trichloroethylene manufacturing plants that emit HCBD as a waste product. No HCBD was detected in 15 egg samples and 20 vegetable samples. One of 20 milk samples contained 1.32 mg/kg HCBD. Resampling in the same area revealed no further detections in milk, raising the possibility that the concentration of 1.32 mg/kg measured in the original data set was an artifiact. This study reported two detection limits for HCBD: 0.005 mg/kg for nonfatty foods and 0.04 mg/kg for fatty foods. Based on information supplied by Kusznesof (1997), U.S. EPA (1998a) concluded that more than 30% of foods may be considered fatty foods for the purpose of estimating exposure from food (see Section 5.1.3).

IARC (1979) reported concentrations of HCBD in foods sampled in the United Kingdom. HCBD was found at concentrations of 0.00008 mg/kg in fresh milk, 0.002 mg/kg in butter, 0.0002 mg/kg in cooking oil, 0.0002 mg/kg in light ale, 0.0008 mg/kg in tomatoes, and 0.0037 mg/kg in black grapes (IARC, 1979).

5.1.2 Concentrations in Fish

Concentrations of HCBD in fish have been reported in multiple studies. Tchounwou et al. (1998) demonstrated that aquatic organisms, particularly fish, may be a significant source of HCBD transmission from contaminated wetlands to humans. Tissue concentrations of HCBD in Louisiana were 226.33 ± 778.40 ng/g in fish collected from a contaminated study site and 6.84 ± 10.41 ng/g in fish collected from the corresponding control site.

In other studies, fish samples from the Mississippi River were reported to contain HCBD levels ranging from 100 to 4,700 ng/g (Laska et al., 1976; Yip, 1976; Yurawecz et al, 1976). Levels of HCBD generally were not detected in fish from the Great Lakes (Camanzo et al., 1987; DeVault, 1985), with the exception of trout from Lake Ontario, which were reported to contain 60 to 300 ng/g (Oliver and Nimi, 1983). HCBD was not detected in 51 biota samples catalogued in the STORET database (Staples et al., 1985).

The National Study of Chemical Residues in Fish (NSCRF), conducted by EPA's Office of Water, was undertaken to determine the occurrence of selected pollutants in fish from various locations across the United States. Pollutants were measured in bottom-feeding and game fish at nearly 400 sites between 1986 and 1989 (Kuehl et al., 1994). A complete presentation of the study plan and results is contained in a joint Office of Water and Office of Research and Development report (U.S. EPA, 1992a). To obtain nationwide coverage, samples were collected at sites near potential point and nonpoint pollution sources, at background sites in areas generally without pollution sources, and at a few sites from the U.S. Geological Survey's National Stream Quality Accounting Network (NASQAN). Targeted sites were chosen near areas of significant industrial, urban, or agricultural activities, including more than 100 sites near pulp and paper mills.

Fish species chosen for sampling were those routinely consumed by humans and/or those expected to bioaccumulate organic contaminants. At most locations, the NSCRF analyzed one composite sample of bottom-feeding fish, usually composed of whole-body samples. Some bottom-feeding fish composite samples were composed of fillets. In areas where whole-body concentrations were high, composite samples of game fish were usually composed of fillets. Each composite sample contained approximately three to five adult fish of similar size from the site. Pollutant concentrations were measured in units of wet weight (U.S. EPA, 1992a).

HCBD was detected in fish at 3% of the 362 sites sampled. Fillet samples were taken from 106 sites.[1] The mean and standard deviation of HCBD fish concentrations at all sites were 0.6 ng/g and 8.7 ng/g, respectively (Kuehl et al., 1994). These statistics represent the overall mean from all samples, not just from the positive samples. Concentrations were above 2.5 ng/g at only four sites, which were all near organic chemical manufacturing plants (U.S. EPA, 1992a). The concentrations observed at these four sites are provided in Table 5-1.

The methods for determining the mean and standard deviation for HCBD concentration and for evaluating samples below the analytical detection limit were not specifically stated by U.S. EPA (1992a). The value of the detection limit for HCBD was not given in U.S. EPA (1992a) or Kuehl et al. (1994). However, in the Kuehl et al. (1994) study, the mean concentration was calculated using one-half of the detection limit concentration when the analyte was not detected. The raw data for HCBD were not presented.

Hendricks et al. (1998) evaluated HCBD levels in zebra mussel (*Dreissena polymorpha*) and eel (*Anguilla anguilla*) from approximately 30 locations in the Rhine-Meuse river basin. In zebra mussel, HCBD levels were 240 ng/kg at a background location and ranged from 950 to 14,000 ng/kg wet weight within the study area. In eel, HCBD levels were found to range from 5,000 to 55,000 ng/kg wet weight within the study area.

[1] The total number of samples for this study is not clear. If one sample of bottom-feeding fish was taken from all sites, then the total number of composite samples was most likely 468.

Table 5-1. HCBD Tissue Concentration in Fish Collected Near Four Chemical Manufacturing Plants

CONCENTRATION (ng/g wet weight)	TYPE OF SAMPLE	LOCATION
164.0	Sea Catfish - Whole Body	Louisiana
23.0	Sea Catfish - Whole Body	Texas
10.50	Catfish - Fillet	Illinois
2.54	Catfish - Whole Body	Louisiana

source: U.S. EPA (1992a)

5.1.3 Intake of HCBD from Food

Non-fish Dietary Intake

As noted above, HCBD has been found in a variety of foods in the United Kingdom. In addition, although HCBD may have been incorrectly measured in milk by Yip (1976), it is also possible that HCBD could be found in measurable quantities in the United States. However, because HCBD was generally undetected in samples taken from areas within 25 miles of emission sources, U.S. EPA (1998a) concluded that it is appropriate to assume that, on average, HCBD will not be found in food at detectable levels. Given this observation, along with the fact that HCBD has no approved uses in food, it is anticipated that there would typically be no chronic exposure to HCBD via non-fish dietary foods (U.S. EPA, 1998a). Therefore, the average estimate of HCBD intake from non-fish foods is assumed to be zero (U.S. EPA, 1998a).

A high-end estimate of HCBD exposure may be made by assuming a concentration of one-half the detection limit (U.S. EPA, 1999b). Because the percentages of fatty or non-fatty foods in the diet are not known with certainty, a conservative estimate is made using one-half the detection limit of 0.04 mg/kg noted for fatty foods in Yip (1976). The resulting concentration of 0.02 mg/kg is multiplied by an estimate of total daily food intake of 2.6 kg/day and divided by 70 kg to obtain a total daily intake of HCBD from food of 7.4×10^{-4} mg/kg-day in adults. For children, the resulting concentration of 0.02 mg/kg was multiplied by an estimate of total daily food intake of 0.84 kg/day (U.S. EPA, 1988) and divided by a body weight of 10 kg to obtain a total daily intake of HCBD from food of 1.68×10^{-3} mg/kg-day. For the majority of regions of the United States in which HCBD is not found, using one-half the detection limit will overestimate the amount of HCBD in food (U.S. EPA, 1999b).

Because the data on concentrations in food are limited, and because the implications of assuming that HCBD occurs at one half the detection limit for fatty foods are large, further research may be required to refine this estimate.

Fish Dietary Intake

U.S. EPA (1998a) estimated HCBD intake from fish using the tissue concentration data from Kuehl et al. (1994). Because these data were taken from many monitoring stations throughout the United States, the estimate may be reasonably indicative of the magnitude of

intake from fish consumption when HCBD is present in fish tissue. An average estimate of adult exposure was obtained by multiplying the mean concentration of 0.6 ng/g from the Kuehl et al. (1994) data by a fish intake of 18 g/day for the general population and dividing by a body weight of 70 kg. The resulting estimate is 1.54×10^{-7} mg/kg-day. The maximum concentration detected in fish by Kuehl et al. (1994) can also be used to estimate the high-end intake. Following the same procedure above, but substituting a concentration of 164 ng/g, one obtains a high-end intake of 4.22×10^{-5} mg/kg-day.

An average estimate of HCBD exposure in children was determined by multiplying the mean concentration of 0.6 ng/g from the Kuehl et al. (1994) data by a fish intake of 4 g/day for the general population and dividing by a body weight of 10 kg. The resulting estimate is 2.4×10^{-7} mg/kg-day. The maximum concentration detected in fish by Kuehl et al. can also be used to calculate a high-end estimate of intake in children. Following the same procedure above, but substituting a concentration of 164 ng/g, results in an intake of 4.37×10^{-5} mg/kg-day.

5.2 Exposure from Air

5.2.1 Concentration of HCBD in Air

Concentration data for HCBD in air have previously been summarized by U.S. EPA (1998a). The largest compilation of data on ambient air concentrations is available from Shah and Heyerdahl (1988). Shah and Heyerdahl compiled ambient air monitoring data for volatile organic compounds for the period from 1970 to 1987. A total of 72 observations from six studies were reported for HCBD. In cases where more than one sample was taken per day, the concentrations were averaged and weighted by sampling time when the sampling time varied throughout the day. When more than one sample was included in the average, values less than the minimum quantifiable limit (MQL) were included as one-half the MQL when the MQL was given. When the MQL was not indicated in the Shah and Heyerdahl study, values less than the MQL were included as zeros in the average. If the resulting average was less than the MQL, a zero was included. If the average was greater than the MQL, the calculated average was used.

As reported in U.S. EPA (1998a), the average and median of all ambient HCBD concentrations measured by Shah and Heyerdahl (1988) were 0.036 parts per billion (ppb) (0.42 $\mu g/m^3$) and 0.003 ppb (0.04 $\mu g/m^3$), respectively. The 25th and 75th percentiles were 0.001 ppb (0.01 $\mu g/m^3$) and 0.006 ppb (0.07 $\mu g/m^3$). Only median values were reported for urban areas and source-dominated areas. Of 56 samples taken from urban areas, the median was 0.003 ppb (0.04 $\mu g/m^3$). Of 16 samples taken from source-dominated areas, the median was 0.002 ppb (0.02 $\mu g/m^3$). No indoor concentrations were reported (Shah and Heyerdahl, 1988).

Shah and Heyerdahl's compilation included a study conducted by Pellizzari et al. (1979), who surveyed the occurrence of halogenated hydrocarbons in various environmental media of five metropolitan areas. As part of this study, HCBD concentrations in the vapor phase of ambient air of four sites were compiled from other research programs, as well as from monitoring conducted specifically for this project. In the Niagara Falls and Buffalo, New York area, concentrations were found to range from trace levels to 389 $\mu g/m^3$, with six of 15 determinations (40%) containing detectable levels. In the Baton Rouge, Louisiana area, two of 11 determinations (18%) were positive, with concentrations of 18 and 37 $\mu g/m^3$. Sampling in Houston, Texas, and

surrounding areas showed a range of trace levels to 2,066 $\mu g/m^3$, with seven positive values from a total of 17 determinations (41%).

Class and Ballschmiter (1987) reported that the troposphere of the Northern Hemisphere contained an average concentration of 0.17 parts per trillion (ppt) (2 $\mu g/m^3$) HCBD at 18 locations sampled from 1982 to 1986. The detection limits in this survey were between 0.01 and 0.1 ppt.

HCBD concentrations in ambient air were measured in two studies included in a compilation of ambient monitoring data for the Urban Area Source Program (U.S. EPA, 1994). In the first survey, concentrations of HCBD were reported at a minimum detection level of 540 $\mu g/m^3$ when measured at six monitoring stations in Columbus, Ohio, in 1989. The second survey was conducted in Cincinnati, Ohio, from 1989 to 1991, and detected HCBD at one monitoring site at a concentration of 1,000 $\mu g/m^3$.

A number of cities had HCBD levels ranging from 2 to 11 ppt (0.02 to 0.12 $\mu g/m^3$) (Pellizzari, 1978; Singh et al., 1980, 1982). Niagara Falls had higher HCBD levels, with concentrations up to 37 ppt (0.39 $\mu g/m^3$) found in ambient air levels and up to 38 ppt (0.41 $\mu g/m^3$) found in the basement air of homes near industrial and chemical waste disposal sites (Pellizzari, 1982).

However, a study of air contaminants in Porto Alegre, Brazil (Grosjean and Rassmussen, 1999) did not find detectable levels of HCBD (detection limit = 100 ppt) at any of 46 sampling locations. A monitoring study at 6 sampling locations in Columbus, Ohio also failed to detect HCBD in the air (Spicer et al., 1996).

5.2.2 Intake of HCBD from Air

The air concentrations reported in Shah and Heyerdahl (1988) were utilized by U.S. EPA (1998a) to calculate an estimate of exposure because this data set included a fairly large number of observations (n=72). For adults, the mean concentration of 0.42 $\mu g/m^3$ was multiplied by an average air intake of 20 m^3/day (U.S. EPA, 1988). The resulting value was divided by a body weight of 70 kg, and the units were converted from μg to mg, resulting in an average intake of 1.2×10^{-4} mg/kg-day. For children, the mean concentration of 0.42 $\mu g/m^3$ was multiplied by an average air intake of 15 m^3/day (U.S. EPA, 1988). The resulting value was divided by a body weight of 10 kg and the units were converted from μg to mg, resulting in an intake of 6.3×10^{-4} mg/kg-day. As noted in U.S. EPA (1998a), these estimates may be indicative of the magnitude of HCBD intake from air in urban and source dominated areas where the chemical is present. It should be noted, however, that these concentration data are older than data from the Urban Area Source Program (U.S. EPA, 1994) and Class and Ballschmiter (1987). In addition, the number of geographic areas sampled throughout the United States by Shah and Heyerdahl is not indicated.

5.3 Exposure from Soil

5.3.1 Concentration of HCBD in Soil and Sediment

No data were located on the concentration of HCBD in either soil or dust. However, sediments have been shown to adsorb HCBD from contaminated water. As reported in U.S. EPA (1999b), HCBD was not detectable in any of the 196 sediment samples reported in the STORET database, based on a detection limit of 500 μg/kg for the analyses (Staples et al., 1985). Sediments from the Niagara River contained 2.9 to 11 μg/kg HCBD (Oliver and Bourbonniere, 1985). Sediments from the Great Lakes were reported to contain levels of HCBD typically ranging from 0.08 to 120 μg/kg (McConnell et al., 1975). Recent data for suspended solids collected from the Rhine-Meuse river basin indicate HCBD levels ranging from < 3.4 to 19 μg/kg (Hendriks et al., 1998). This concentration range is comparable to the earlier data reported for sediments collected in the United States.

Several studies have investigated HCBD levels in sediments from sites in Louisiana. HCBD levels in sediment samples from a Louisiana swamp environment ranged from less than 0.05 μg/kg to 0.40 μg/kg (Abdelghani et al,. 1995). These concentrations were well below the action levels of 4,000 μg/kg for sediment (U.S. EPA, 1991a). At a Federal Superfund site near Baton Rouge, Louisiana, preliminary data from a sampling of sediments showed HCBD levels from 2 to 3,770 mg/kg (U.S. EPA, 1992b). The HCBD level in a sediment sample from Lake Charles, Louisiana was found to be 3,500 μg/kg (Chen et al., 1999). A sediment sample collected from the intersection of an industrial canal and Bayou d'Inde (a tributary of the Calcasieu River near Lake Charles) and analyzed via Soxhlet extraction was found to contain HCBD at a level of 17,200 ± 1,000 μg/kg (Prytula and Pavlostathis, 1996). Another reported sediment sample collected from this industrial canal area had an HCBD level of 36,000 ± 6,900 μg/kg (Gess and Pavlostathis, 1997). A third study of sediments from Bayou d'Inde found levels of HCBD ranging from 1,550 to 8,220,000 μg/kg of organic carbon. Assuming an organic carbon content in the sediment of 1%, this level is equivalent to sediment concentrations of 15 to 82,200 μg/kg.

5.3.2 Intake of HCBD from Soil

Because no data were available on the concentration of HCBD in soil or dust, intake from soil was not estimated.

5.4 Other Residential Exposures

HCBD was not detected in sewage influents (Levins et al., 1979) or in sewage samples (U.S. EPA, 1990). No other information on exposure via other residential pathways was identified.

5.5 Summary

Estimated mean concentration and average intake values for HCBD in media other than water are summarized in Table 5-2. Assuming that there is no chronic exposure of the general population to HCBD from non-fish dietary sources, inspection of the data indicates that most intake of HCBD by the general population occurs via respiratory intake. However, it should be cautioned that this determination is subject to a number of uncertainties: 1) the database for the

Table 5-2. Summary of Concentration Data and Exposure Estimates for Media Other Than Water

PARAMETER	MEDIUM			
	Food		Air	
Mean Concentration in medium	Non-fish (NF): nondetect Fish (F): 0.6 ng/g		0.42 μg/m^3	
	Adult	Child	Adult	Child
Estimated average daily intake (mg/kg-day)	NF: 0 F: 1.5×10^{-7}	NF:0 F: 2.4×10^{-7}	1.2×10^{-4}	6.3×10^{-4}

occurrence of HCBD in media other than water is limited; 2) many of these data are more than 20 years old; 3) in some cases, information on the geographic location of sample collection or analytical details are lacking; and 4) data for HCBD in soil and dust were not available to estimate via this pathway.

6.0 TOXICOKINETICS

This section describes the absorption, distribution, metabolism, and excretion of hexachlorobutadiene. The information in this section focuses on findings in animals exposed primarily via the oral route. No studies were identified that evaluated the toxicokinetic behavior of HCBD in humans.

6.1 Absorption

HCBD is readily absorbed following oral administration to experimental animals. Although no studies have quantitatively determined the rate of absorption of HCBD following oral dosing, useful information has been obtained from studies that evaluated the distribution and excretion of this compound. Reichert et al. (1985) administered 1 mg/kg of ^{14}C-HCBD to female Wistar rats via gavage. The compound was administered in a tricaprylin suspension to accommodate its low water solubility. Approximately 76% of the radioactivity was excreted as metabolites in the urine, feces, or expired air within 72 hours after administration, suggesting that most of the dose was absorbed. When a higher dose of 50 mg/kg ^{14}C-HCBD was administered in the same study, 69% of the radioactivity was found in the feces and was predominantly associated with unchanged HCBD. Just 11% of the administered radioactivity was excreted in the urine for the high-dose group, compared to 31% for the low-dose animals. The study authors concluded that absorption of HCBD was saturated in animals in the higher-dose group (Reichert et al. 1985; U.S. EPA, 1991a).

Nash et al. (1984) administered 200 mg/kg ^{14}C-HCBD via oral gavage in corn oil to male Wistar-derived rats. Animals were sacrificed 2, 4, 8, or 16 hours after dosing, and the fate of the administered radioactivity was evaluated using whole-body autoradiographs. The investigators reported that absorption was virtually complete within 16 hours after dosing.

Payan et al. (1991) administered 1 mg/kg and 100 mg/kg ^{14}C-HCBD to male Sprague-Dawley rats, using an aqueous polyethylene glycol vehicle, and found that 18.5 and 8.9% of the administered radioactivity, respectively, was excreted over 72 hours in the urine. Since urinary excretion at a dose of 1 mg/kg in the Reichert et al. (1985) study was 31%, these data suggest that gastrointestinal absorption of HCBD was greater when administered in a lipophilic vehicle (tricaprylin) than with an aqueous vehicle (aqueous polyethylene glycol). As noted for other unsaturated chlorinated compounds, HCBD absorption presumably occurs by passive diffusion across the lipid portion of the intestinal matrix rather than by active or protein-facilitated transport (ATSDR, 1994).

Little information is available regarding HCBD absorption following exposure by other routes. Although no studies were located that described absorption in humans or animals after inhalation exposure to this compound, the occurrence of systemic effects following exposure indicates that absorption occurs by this route (ATSDR, 1994). With regard to dermal exposure, Duprat and Gradiski (1978) applied doses of 419 to 1,675 mg/kg HCBD to the skin of rabbits under occluded conditions, and reported that the compound was completely absorbed within 8 hours.

6.2 Distribution

HCBD has been detected in human blood (Bristol et al., 1982) and in the adipose tissue of human cadavers (Mes et al., 1985). Olea et al. (1999) detected HCBD in the adipose tissue of 13 of 50 children living in an agricultural region of southern Spain. The mean concentration in the 13 children was 0.70 μg HCBD/g of fat (range: 0.23 to 2.43 μg HCBD/g of fat). No data were available concerning the route of exposure.

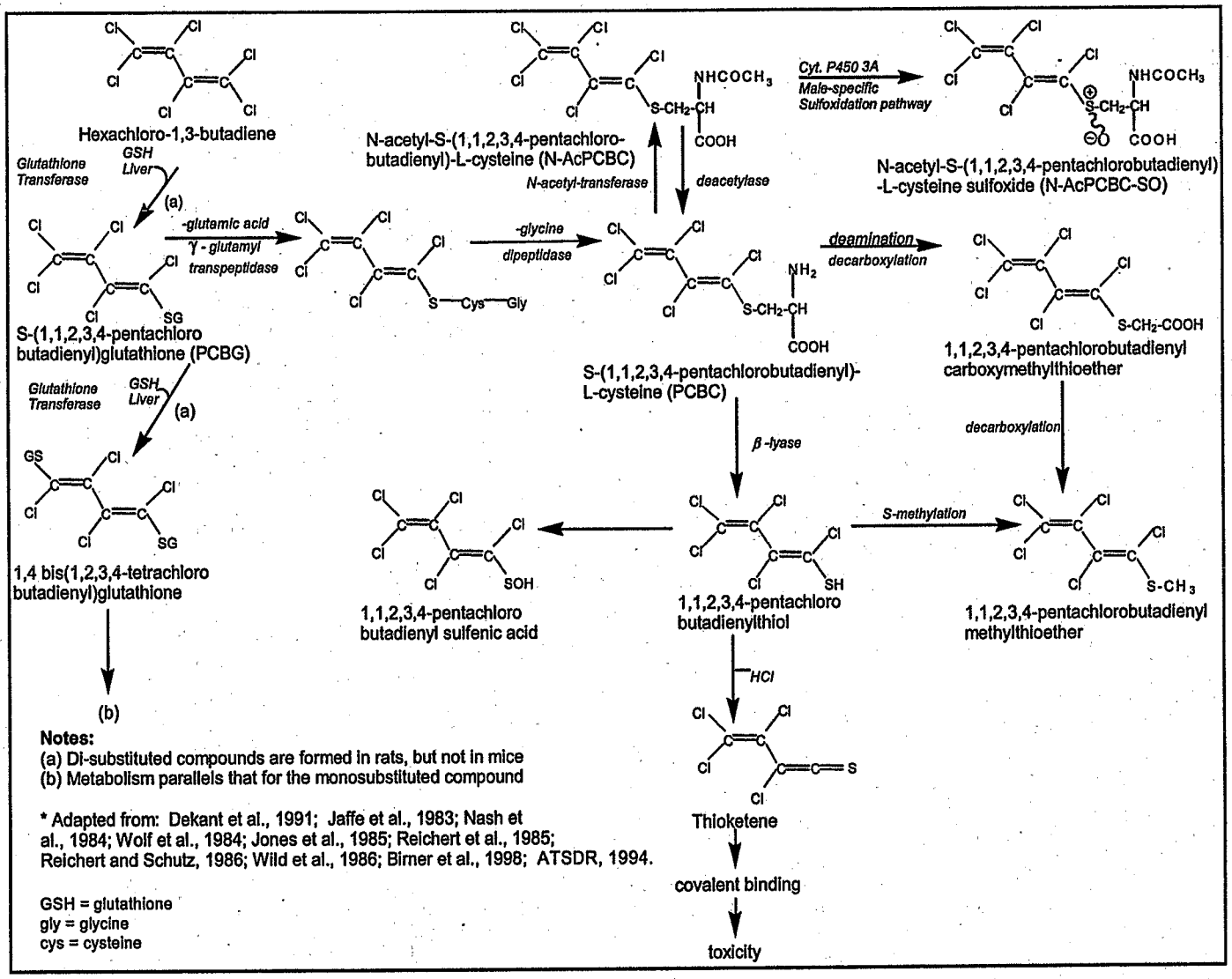
Following oral administration, HCBD and its metabolites preferentially distributed to the kidney, liver, adipose tissue and brain of experimental animals (Reichert, 1983; Reichert et al., 1985; Dekant et al., 1988a). Covalent binding of HCBD-related radioactivity to tissue proteins was highest during the first six hours after dosing, and was higher in the kidney than in the liver; and this effect was independent of dose (Reichert et al., 1985). In rats administered 1 mg/kg ^{14}C-HCBD, covalent binding of the radioactivity to protein in the kidney was about twice that in the liver 72 hours after dosing (Reichert et al., 1985). Nash et al. (1984) reported a specific localization of administered radioactivity in rats in the outer medulla of the kidney, as revealed by autoradiographic analysis following an oral dose of 200 mg/kg ^{14}C-HCBD. Payan et al. (1991) conducted a study in rats in which the bile ducts of one group of animals administered an oral dose of 100 mg/kg ^{14}C-HCBD were cannulated so that bile secretions from these animals could be infused directly into the duodenum of another group of animals. In both groups, the kidneys contained about twice as much radiolabel as the liver.

No studies were located regarding the distribution of HCBD in humans or animals after inhalation or dermal exposure. Davis et al. (1980) administered 0.1 mg/kg radiolabeled ^{14}C-HCBD as a tracer dose to male Sprague-Dawley rats (5 animals/group) via intraperitoneal injection. A nephrotoxic group received the same amount of labeled HCBD plus 300 mg/kg non-labeled HCBD. The highest concentrations of radiolabel were found in the liver, kidney and adipose tissue 48 hours after administration. Approximately 2.6 and 2.3% of the administered ^{14}C radiolabel were retained in the livers of low- and high-dose animals, respectively. The fraction of the tracer retained in kidney varied from 2.5% at the low dose to 0.5% at the high dose. The fraction of the dose found in adipose tissue was not determined. Very low levels of the radiolabel (less than 0.2%) were found in the brain, lung, heart, and muscle.

6.3 Metabolism

No available studies have characterized the metabolism of HCBD in animals following inhalation or dermal exposure. The metabolism of HCBD in animals has been studied in isolated hepatocytes (Jones et al., 1985) and by characterization of metabolites identified in urine, bile and feces following oral exposure to the compound (Figure 6-1). Following ingestion and absorption from the gastrointestinal tract, HCBD is initially transported to the liver, where it is conjugated with glutathione to form *S*-(1,1,2,3,4 -pentachlorobutadienyl)glutathione in a reaction mediated by glutathione *S*-transferase (Wolf et al., 1984; Garle and Fry, 1989; Dekant et al., 1988b; Koob and Dekant, 1992). In rats, a di-substituted glutathione conjugate, 1,4 bis(1,2,3,4-tetrachlorobutadienyl)glutathione, is also formed in the liver (Jones et al., 1985), whereas in mice, only the mono-substituted conjugate is produced (Dekant et al., 1988a). The glutathione conjugate is then excreted in bile and transported back into the gastrointestinal tract (Koob and Dekant, 1992). Nash et al. (1984), for example, collected bile excretions from cannulated rats that had been orally

Figure 6-1. Proposed Pathways for Hexachlorobutadiene Metabolism



administered 200 mg/kg ^{14}C-HCBD and determined that 40% of the bile radioactivity was associated with the glutathione conjugate. The weight of evidence suggests that oxidative reactions involving cytochrome P450 have little role in the initial metabolism of HCBD (Wolf et al., 1984; Dekant et al., 1988a).

The glutathione conjugate of HCBD can be reabsorbed intact from the gastrointestinal tract (Koob and Dekant, 1992; Gietl et al., 1991). Alternatively, a portion of it can be catabolized by γ-glutamyltranspeptidase and dipeptidases in the gastrointestinal tract to the cysteine conjugate, *S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine (Jones et al., 1985; Gietl et al., 1991; Koob and Dekant, 1992). Both the glutathione and cysteine conjugates are subject to several alternative fates. These conjugates may be reabsorbed from the gut and be translocated to the kidney (Koob and Dekant, 1992), undergo enterohepatic circulation (Nash et al., 1984; Gietl et al., 1991; Gietl and Anders, 1991), or be excreted with the feces (Dekant et al. 1988a). However, the majority of the glutathione conjugate is delivered to the kidney by systemic circulation (Koob and Dekant, 1992). Working with isolated perfused rat livers, Koob and Dekant (1992) determined that a maximum of 39% of the glutathione conjugate was recirculated to the liver of rats, whereas up to 79% of the cysteine conjugate was recirculated. Nash et al. (1984) reported that the cysteine and glutathione conjugates represented 12% and 40%, respectively, of the radioactivity excreted in the bile of cannulated rats orally administered 200 mg/kg HCBD. When the cysteine conjugate is recirculated to the liver, a minor fraction of this metabolite is converted by *N*-acetyltransferase to an acetylated cysteine conjugate, *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine (*N*-AcPCBC) (Koob and Dekant, 1992).

Further processing of both the cysteine and glutathione conjugates occurs in the kidney, which possesses high γ-glutamyltranspeptidase activities in the brush-border membrane of the proximal tubular cells (Dekant and Vamvakas, 1993; Dekant et al., 1990). Renal deacetylase, γ-glutamyltranspeptidase, and dipeptidase enzymes convert the acetylated cysteine conjugate and the glutathione conjugate to the cysteine conjugate, which accumulates in the kidney (Dekant et al., 1990). The cysteine conjugate is subsequently activated to a reactive and electrophilic thioketene intermediate (Dekant et al., 1990; Green and Odum 1985). This conversion is catalyzed by the enzyme-cysteine conjugate β-lyase, which is localized in the cytosol and mitochondria of the epithelial cells of the proximal tubule (Lash et al., 1986; Stevens, 1985; Stevens et al. 1986; Jones et al., 1988; MacFarlane et al., 1989; Kim et al., 1997).

Another pathway for metabolic disposition of the cysteine conjugate of HCBD in the kidney is the conversion of the cysteine conjugate to a mercapturic acid, *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine, by the renal enzyme *N*-acetyltransferase (Birner et al., 1997). This metabolite is excreted in the urine, accounting for 10% of urinary radioactivity in rats orally administered 100 mg/kg ^{14}C-HCBD (Reichert and Schutz, 1986). Other pathways that result in the excretion of the cysteine conjugate involve the deamination and subsequent decarboxylation of the cysteine conjugate, resulting in the formation of methylthiolated metabolites such as 1,1,2,3,4-pentachlorobutadiene methylthioether and 1,1,2,3,4-pentachlorobutadiene carboxymethylthioether (Reichert et al., 1985). In addition, 1 to 8% of the administered radioactivity is oxidized to carbon dioxide in rats (Reichert et al., 1985; Payan et al., 1991).

Evidence for a male-specific HCBD metabolic pathway in rats has been reported by Birner and colleagues (Birner et al., 1995, 1998; Werner et al.1995a). The metabolite *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine sulfoxide (*N*-AcPCBC-SO) is detected in the urine

of male, but not female, rats following oral administration of HCBD. Formation of this metabolite is mediated by cytochrome P450 3A monooxygenases, which are expressed only in male rats (Birner et al., 1995; Werner et al., 1995a). This metabolite has been found to be cytotoxic to proximal tubular cells *in vitro* without activation by β-lyase (Birner et al., 1995). When given intravenously, *N*-AcPCBC-SO produced necroses of the kidney tubules in male rats (Birner et al., 1998).

The *N*-AcPCBC-SO formed in male rats occurs as two diastereomers present in equimolar amounts: (*R*)-*N*-AcPCBC-SO and (*S*)-*N*-AcPCBC-SO (Werner et al., 1995b). These compounds are structurally analogous to unsaturated carbonyl compounds and thus may be candidates for detoxification via glutathione conjugation (Rosner et al., 1998). Experimental evidence obtained *in vitro* suggests that glutathione conjugation of the two diastereomers is catalyzed by different glutathione *S*-transferases, resulting in the formation of different products (Rosner et al., 1998). Incubation of the (*R*)-sulfoxide diastereomer with rat liver cytosol resulted in formation of (*R*)-*N*-acetyl-*S*-(4-glutathion-*S*-yl-1,2,3,4-tetrachlorobutadienyl)-L-cysteine sulfoxide. Incubation of the (*S*)-sulfoxide produced two glutathione conjugates identified as (*S*)-*N*-acetyl-*S*-(4-glutathion-*S*-yl-1,2,3,4-tetrachlorobutadienyl)-L-cysteine sulfoxide and (*S*)-*N*-acetyl-*S*-(2-glutathion-*S*-yl-1,3,4,4-tetrachlorobutadienyl)-L-cysteine sulfoxide. In the presence of rat kidney cytosol, only the (*S*)-*N*-acetyl-*S*-(2-glutathion-*S*-yl-1,3,4,4-tetrachlorobutadienyl)-L-cysteine sulfoxide conjugate was formed. Glutathione conjugation of the (*R*)-sulfoxide was not observed. The observed pattern of product formation was attributed to catalysis by different glutathione *S*-transferases in liver (α- and μ-class) and kidney (α-class). This hypothesis was confirmed by product analysis following incubation of *N*-AcPCBC-SO with purified rat α- and μ-class glutathione *S*-transferases.

Very little information is available on the toxicokinetic behavior of HCBD in humans. However, the key steps in the metabolism of HCBD have been examined *in vitro* using human tissues. The human liver microsomal glutathione transferase responsible for HCBD conjugation has been isolated and purified (McLellan et al., 1989), and the microsomal enzyme activity is 40-fold higher than the activity detected in the cytosol (Oesch and Wolf, 1989). The rate of enzymatic formation of S-(1,2,3,4,4-pentachlorobutadienyl)glutathione (PCBG) from HCBD in human liver cytosol is approximately 20 to 30% of the rates observed in rat and mouse cytosol (Dekant et al. 1998).

The enzyme γ-glutamyl transpeptidase, which catalyzes the conversion of glutathione *S*-conjugates to the corresponding cysteine conjugates, has been detected in human tissues (Shaw et al., 1978). The kidney-to-liver activity ratio for γ-glutamyl transpeptidase in human tissues is approximately 22, which is comparable to the ratios observed in pig and guinea pig. However, this ratio is much lower than in rat, where a kidney-to-liver ratio of 875 has been observed (Hinchman and Ballatori, 1990).

Cysteine conjugate β-lyase has been isolated and purified from human kidney cytosol (Lash et al., 1990), and the human β-lyase gene has been cloned and expressed (Perry et al., 1995). β-lyase activity has been demonstrated in the human kidney (Green et al., 1990) and human proximal tubular cells (Chen et al., 1990). Collectively, these human studies suggest that humans have the ability to metabolize HCBD to toxic metabolites. However, the activity of HCBD metabolizing enzymes, particularly renal β-lyase, may be many-fold lower in humans than the corresponding enzymes in rat (Lock, 1994; Lash et al., 1990; Anders and Dekant, 1998).

Werner et al. (1995b) demonstrated that human liver microsomes are capable of oxidizing *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine to the corresponding sulfoxide (*N*-AcPCBC-SO). In contrast to the male-specific formation of *N*-AcPCBC-SO in rats described above, formation of the sulfoxide was detected in human microsomes prepared from both male and female donors. Inhibitor studies suggest that formation of the sulfoxide is catalyzed by members of the cytochrome P450 3A subfamily. Since this subfamily constitutes a major fraction of cytochrome P450 content in human liver, the formation of the sulfoxide is expected to occur in humans exposed to HCBD. Incubation of *N*-AcPCBC-SO with purified human glutathione *S*-transferase M1-1 (μ-class) catalyzes the formation of (*S*)-*N*-acetyl-*S*-(4-glutathion-*S*-yl-1,2,3,4-tetrachlorobutadienyl)-L-cysteine sulfoxide and (*R*)-*N*-acetyl-*S*-(4-glutathion-*S*-yl-1,2,3,4-tetrachlorobutadienyl)-L-cysteine sulfoxide, the same products formed in the presence of rat μ-class glutathione *S*-transferase (Rosner et al., 1998).

6.4 Excretion

HCBD and its metabolites are excreted in urine, feces, and exhaled air. In rodents, oral exposure to HCBD results in fairly rapid urinary and fecal excretion. The half-life of HCBD binding to tissue proteins was 22 hours in both liver and kidney (Reichert et al., 1985). Total excretion within 72 hours was found to be at least 65% of a single oral dose of up to 100 mg/kg in rats and mice (Reichert and Schutz, 1986; Dekant et al., 1988a). At higher doses (30 to 200 mg/kg), 5 to 11% of the radiolabel was excreted in the urine (Dekant et al., 1988a; Nash et al., 1984; Reichert and Schutz, 1986; Reichert et al., 1985; Payan et al., 1991), while a dose of 1 mg/kg resulted in urinary excretion of approximately 18.5 to 30% of the administered radioactivity (Payan et al., 1991; Reichert et al., 1985). Payan et al. (1991) attributed the fractional decrease in urinary excretion with increase in dosage to a saturation of hepatobiliary excretion or a reduction of biliary metabolite reabsorption. Exhaled carbon dioxide and unchanged HCBD accounted for 4 to 8% of oral doses ranging from 1 to 100 mg/kg when measured within 72 hours of administration (Reichert et al., 1985; Dekant et al., 1988a; Payan et al., 1991).

Fecal excretion is the main pathway of elimination for HCBD and HCBD metabolites. Reichert et al. (1985) reported that elimination of HCBD in the feces represented 42 or 69% of the radioactivity orally administered to female Wistar rats at doses of 1 or 50 mg/kg, respectively. The difference in recovery was attributed to an apparent absorption saturation threshold. Dekant et al. (1988a) found that 67 to 77% of the radioactivity in an oral 30 mg/kg dose in corn oil was present in the feces of mice 72 hours after administration. Payan et al. (1991) administered oral doses of ^{14}C-HCBD in polyethylene glycol to male rats. After 72 hours, the feces and contents of the gastrointestinal tract contained 62% of a 1 mg/kg dose and 72% of a 100 mg/kg dose.

Enterohepatic circulation has been demonstrated in animals following oral administration of HCBD. Nash et al. (1984) administered 200 mg/kg ^{14}C-HCBD to rats with and without cannulated bile ducts. Feces and urine were collected over a 5-day period. Over the course of the experiment, the non-cannulated animals excreted 39% of the administered radioactivity in feces. On each of the first two days post-dosing, approximately 5% and 3.5% of the administered radioactivity were found in the feces and urine, respectively. In contrast, bile excretions collected from the cannulated animals on each of the first 2 days post-dosing contained 17–20% of the administered radioactivity (with 35% total excretion by this route over two days). These findings indicate extensive reabsorption of biliary metabolites.

Payan et al. (1991) also compared excretion patterns in bile-duct cannulated and noncannulated rats orally dosed with 1 mg/kg ^{14}C-HCBD. Urinary excretion after 72 hours accounted for 18% of the administered radioactivity in intact animals, but just 11% of the radioactivity in the cannulated rats. In comparison, fecal excretion represented 62% and 3%, of the dose administered to non-cannulated and cannulated animals, respectively. In cannulated rats, 67% of the dose was excreted into the bile. When bile excretions (isolated from bile duct-cannulated rats orally dosed with 100 mg/kg HCBD) were directly infused into the duodenum, approximately 80% of the biliary metabolites are reabsorbed, with only 20% remaining in the feces and gastrointestinal tract.

Several studies have reported the identity of excreted metabolites following exposure to ^{14}C-HCBD. Metabolites identified in the urine of treated rats or mice include *S*-(1,1,2,3,4-pentachlorobutadienyl) glutathione, *S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine, *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine, 1,1,2,3,4-pentachlorobutadienyl sulfenic acid, 1,1,2,3,4-pentachlorobutadiene methylthioether, 1,1,2,3,4-pentachlorobutadiene carboxymethylthioether, and 1,1,2,3-tetrachlorobutenoic acid (Dekant et al., 1988a; Nash et al., 1984; Reichert and Schultz, 1986; Reichert et al., 1985). As noted previously, the novel metabolite *N*-acetyl-*S*-(1,1,2,3,4,-pentachlorobutadienyl)-L-cysteine sulfoxide has been detected in the urine of male, but not female, rats following oral administration of HCBD (Birner et al., 1995).

Comparatively few data are available on the identity of fecal metabolites. Dekant et al. (1988a) administered a single 30 mg/kg gavage dose of ^{14}C-HCBD in corn oil to male and female NMRI mice. The feces were collected over a 72-hour period following dose administration. Approximately 80% of the fecal radioactivity was associated with HCBD. About 10% of the radiolabel was associated with the HCBD metabolite *S*-(1,1,2,3,4-pentachlorobutadienyl) glutathione. The remainder of the fecal radioactivity was present as polar metabolites which could not be structurally identified.

7.0 HAZARD IDENTIFICATION

7.1 Human Effects

Limited information is available on the human health effects associated with exposure to HCBD. A review of the available literature did not identify case reports describing the outcome of accidental or intentional HCBD exposure, or reports of systemic toxicity following oral or dermal HCBD exposure. A number of studies have evaluated health effects in workers occupationally exposed to HCBD via inhalation, and these studies are described below.

7.1.1 Short-Term Studies

No short-term studies describing HCBD health effects in humans were located.

7.1.2 Long-Term and Epidemiological Studies

General Population

No general population studies of HCBD toxicity were located.

Sensitive Populations

No studies concerning HCBD toxicity in sensitive populations were located.

Occupational Exposure Studies

German (1986) conducted two cytogenetic studies of workers employed in an HCBD production facility. The exposure levels reported by the manufacturer ranged from 1.6 to 16.9 mg/m^3. The investigators found an increased frequency of chromosomal aberrations in the peripheral lymphocytes of exposed workers. However, the frequency of aberrations was not associated with duration of employment in the HCBD manufacturing facility (WHO, 1994), suggesting that factors other than HCBD exposure contributed to the observed effects.

Additional occupational studies have evaluated health effects in workers exposed to HCBD. However, in each case concurrent exposure of workers to other chemicals limits the usefulness of the data for evaluation of HCBD human health effects. Krasniuk et al. (1969), for example, evaluated health effects in 153 farm workers intermittently exposed over a period of four years to soil and grape fumigants containing HCBD. When compared to a control population of 52 unexposed workers, the exposed workers exhibited increased incidence of arterial hypotension, myocardial dystrophy, chest pains, upper respiratory tract changes, liver effects, sleep disorders, hand trembling, nausea, and disordered smell functions (U.S. EPA, 1991a). Interpretation of these data is confounded by concurrent exposure of the workers to polychlorobutane-80.

Burkatskaya et al. (1982) reported adverse health effects in vineyard workers exposed to fumigants containing HCBD. However, the role of HCBD could not be evaluated because the workers were concurrently exposed to other agricultural chemicals (WHO, 1994).

Driscoll et al. (1992) determined the concentrations of individual serum or plasma bile acids in workers exposed to chlorinated hydrocarbons, including HCBD, carbon tetrachloride, and perchloroethylene. These investigators reported increases in four serum bile acid parameters in workers exposed via inhalation to 0.005–0.02 ppm HCBD. The study found no significant relation between bile acid parameters or liver function tests and exposure. As in the studies above, the specific contribution of HCBD exposure to the observed effects could not be evaluated.

7.2 Animal Studies

7.2.1 Acute Toxicity

Oral Exposure

Schwetz et al. (1977) reported a single-dose oral LD_{50} value (the dose that produces lethality in 50% of the experimental animals) of 65 mg HCBD/kg for male weanling rats and 46 mg/kg for female weanling rats. Single-dose oral LD_{50} values for adult rats ranged from 200 to 400 mg/kg for females, and 504 to 667 mg/kg for males (unpublished study cited in Schwetz et al., 1977). These data suggest that age and gender may be significant variables in the acute toxicity of HCBD. Single-dose LD_{50} values reported for other rodents were 80 to 116 mg/kg for mice and 90 mg/kg for guinea pigs (U.S. EPA, 1991a).

Three studies have evaluated the non-lethal acute effects of oral HCBD exposure. Nash et al. (1984) administered a single oral dose of 200 mg/kg HCBD in polyethylene glycol to six male Wistar-derived rats. Treatment with HCBD increased plasma urea concentration and decreased plasma alanine aminotransferase activity. Analysis of urine revealed increased levels of glucose, protein, alkaline phosphatase, *N*-acetyl-β-D-glucosaminidase (NAG), γ-glutamyl transpeptidase and alanine aminopeptidase. These biochemical changes were indicative of kidney damage.

Jonker et al. (1993a) investigated the acute oral toxicity of HCBD in 12-week-old male Wistar rats. The investigators administered single doses of 0, 10, 100, or 200 mg/kg HCBD in corn oil by gavage to five rats per treatment group. Urine was collected at intervals of 0 to 6 and 6 to 24 hours. All rats were sacrificed at 24 hours. No treatment-related effects were observed at the 10 mg/kg dose. HCBD induced a variety of adverse effects at the two highest dose levels. Kidney weight, blood plasma creatinine level, urinary pH and occult blood, number of epithelial cells in the urine, urinary lactate dehydrogenase and NAG activity were significantly increased ($p<0.05$) at 100 and 200 mg/kg. Additional effects observed in the 200 mg/kg dose group included reduced body weight, reduced food intake, elevated plasma urea level, and increased urinary volume. Increased levels of urinary protein, glucose, and potassium, and increased activity of urinary γ-glutamyltransferase and alkaline phosphatase were also observed at 200 mg/kg. Histopathological examination of the kidneys revealed limited focal necrosis at 100 mg/kg and extensive tubular necrosis at 200 mg/kg. The study authors identified 10 mg/kg and 100 mg/kg as the "No Nephrotoxic-Effect Level" and "Minimum Nephrotoxic-Effect Level", respectively.

Payan et al. (1993) administered single oral doses of 0, 100, or 200 mg/kg HCBD in polyethylene glycol to male Sprague-Dawley rats (4 to 5 animals per dose). All rats were sacrificed 24 hours after exposure, and the right kidneys were subjected to microscopic

examination. Nephrotoxicity was also evaluated by determination of urinary β_2-microglobulin, as well as γ-glutamyl transpeptidase, aspartate aminotransferase (AST), and NAG activity. A Lowest-Observed-Adverse-Effect Level (LOAEL) of 100 mg/kg was identified in this study on the basis of kidney lesions and a three-fold higher urinary AST excretion.

Lock et al. (1996) administered a single oral dose of 50 mg HCBD/kg to a calf to evaluate toxic effects on the kidney and bone marrow. The administered dose resulted in the death of the animal 5 days after treatment. Prior to death, blood urea nitrogen, plasma aspartate aminotransferase and plasma alkaline phosphatase were elevated, but no changes were observed in circulating white cells or platelets. The liver and kidneys appeared pale and swollen at necropsy. Histopathological examination revealed midzonal necrosis in the liver. Extensive areas of necrosis were evident in the kidney, and were accompanied by hyaline and granular cast formation.

Inhalation Exposure

De Ceaurriz et al. (1988) evaluated the effects of HCBD inhalation exposure on male Swiss OF1 mice (6 mice/dose). The mice were exposed to HCBD vapor at concentrations between 83 and 246 ppm (886 and 2,625 mg/m^3) for 15 minutes. Decreased respiratory rates (reflex bradypnea) were observed at concentrations of 155 ppm (1,652 mg/m^3) or greater. An EC_{50} (concentration producing an effect in 50% of the population) of 211 ppm (2,250 mg/m^3) was calculated for this effect.

De Ceaurriz et al. (1988) investigated the effects of a 4-hour whole-body exposure to HCBD at measured concentrations of 2.75, 5, 10, 25 ppm (or 29.3, 53.4, 106.7, 266.8 mg/m^3) on male Swiss OF1 mice (10 animals/dose). An HCBD-related increase in the percentage of damaged renal tubules, as determined by alkaline phosphatase staining, was observed at all exposure levels. The EC_{50} for this response was 7.2 ppm (76.8 mg/m^3).

Gehring and MacDougall (1971) exposed rats to 161 ppm (1,716 mg/m^3) HCBD for 0.88 hour or 34 ppm (362 mg/m^3) for 3.3 hours. All rats survived the treatment. Exposure of guinea pigs or cats under the same conditions resulted in the death of most animals. Inhalation exposure of rats to 133 to 150 ppm (1,418 to 1,600 mg/m^3) for 4 to 7 hours was lethal for some or all animals (NTP, 1991).

Dermal Exposure

Gradiski et al. (1975) evaluated the dermal toxicity and sensitization potential of HCBD in rabbits. Dermal application of a 10% solution of HCBD (solvent not indicated) to rabbits caused slight dermal irritation. Guinea pigs exhibited delayed allergic reactions to dermal HCBD application (U.S. EPA, 1991a).

Duprat and Gradiski (1978) evaluated the acute toxicity of dermally applied HCBD in female New Zealand rabbits (10 animals/dose) following an 8-hour exposure period. Undiluted HCBD was applied at doses of 0.25, 0.5, 0.75 and 1.0 mL/kg under occluded conditions. Four hours after termination of exposure, the epidermis and subcutaneous tissue revealed edema and polymorphonuclear leukocyte infiltration at the two highest doses. Three to five days after treatment at the three highest doses, necrotic changes were noted at the site of application. A few

animals from the two highest dose groups died within 24 hours from respiratory and cardiac failure. Indications of systemic toxicity included renal epithelial necrosis and fatty liver degeneration. The LD_{50} for the eight hour exposure was 0.72 mL/kg (Duprat and Gradiski, 1978). Based on a specific gravity of 1.675 for HCBD (U.S. EPA, 1991a), a dermal LD_{50} of 1,206 mg/kg was calculated.

Acute Ocular Toxicity

Gradiski et al. (1975) reported that instillation of a 10% solution of HCBD (solvent not reported) into rabbit eyes resulted in slight ocular mucosa irritation (U.S. EPA, 1991a).

Intraperitoneal Injection

Bai et al. (1992) exposed male Sprague-Dawley rats (4 animals/dose) by intraperitoneal injection to doses of 0, 10.4, 52.2, or 104 mg/kg-day HCBD for three days. Serum bilirubin and alkaline phosphatase activity was increased ($p<0.05$) at the two higher doses, indicating disturbance in liver function. The concentration of total serum bile acids was elevated at the highest dose. No histopathological examination of the livers was conducted.

Lock and Ishmael (1979) administered single intraperitoneal doses of HCBD in corn oil to male albino rats (3 to 19 animals/dose). The doses administered were 0, 20, 50, 100, 200, 300, 400, 500, and 1,000 mg/kg. The effects of treatment were assessed 24 hours after administration. Three of four animals treated with 500 mg/kg died. All rats in the 1,000 mg/kg dose group died. Rats in the highest dose group exhibited piloerection, sedation, hunching, incoordination, loss of muscle tone and hypothermia prior to death (ATSDR, 1993).

Hook and colleagues (Hook et al., 1982, 1983; Kuo and Hook, 1983) conducted a series of experiments to characterize HCBD toxicity in four different strains of rats. Single doses of 25 to 400 mg/kg HCBD were administered by intraperitoneal injection. Following treatment, relative kidney weight increased in all dose groups. Organic ion transport was evaluated by analysis of the anion *p*-aminohippurate (PAH) and the cation tetraethylammonium (TEA) in renal cortical slices. Renal efflux rates of PAH and TEA were unaffected. However, accumulation of the PAH anion was decreased, while accumulation of the TEA cation was unaffected. This pattern suggests a specific impact of HCBD on the renal anion uptake system (Hook et al., 1982; Kuo and Hook, 1983). Kidney-to-body weight ratios increased in all dosage groups. Blood urea nitrogen levels were elevated in all dose groups, with a more pronounced effect noted in young rats (Kuo and Hook, 1983). Adult male rats were less susceptible to HCBD-induced renal effects than were female adult rats or young male rats. The investigators attributed this pattern to age- and sex-related differences in the renal and hepatic enzymes responsible for activation and detoxification of HCBD.

Lock et al. (1984) investigated the effect of age, strain, sex, and monooxygenase inhibitors on the acute toxicity of HCBD to five different strains of mice (6 or more animals/dose group). HCBD in corn oil was administered as single intraperitoneal doses ranging from 6.3 to 50 mg/kg. Toxicity was evaluated 24 hours after treatment. Histopathological examination of the kidneys from adult Swiss-derived mice of both sexes revealed extensive, dose-dependent proximal tubular necrosis at doses of 12.5 mg/kg and above. At 6.3 mg/kg, tubular necrosis was only observed occasionally in a small number of animals. A significant increase in plasma urea occurred in adult

Swiss-derived mice at doses of 25 to 50 mg/kg. No evidence for a gender difference in response was observed. Young male mice responded to lower doses of HCBD than adults, with an increase in plasma urea and a decrease in organic ion transport in renal slices evident at 12.5 mg/kg. Prior administration of the monooxygenase inhibitor piperonyl butoxide or the monooxygenase inducers phenobarbitone or β-naphthoflavone did not modify the extent of HCBD-induced renal damage. Intraperitoneal administration of the glutathione or *N*-acetylcysteine conjugates resulted in a pattern of renal necrosis similar to that observed for HCBD. Evaluation of the comparative susceptibility of five mouse strains indicated that the BALB/c strain was slightly more susceptible to HCBD toxicity than the C57BL/10J, C3H, DBA/2J, and Swiss-derived strains.

7.2.2 Short-Term Studies

Oral exposure

Kociba et al. (1971) conducted an unpublished Dow Chemical Company study of HCBD toxicity, the results of which were summarized in Kociba et al. (1977) and Schwetz et al. (1977). Female Sprague-Dawley rats (4 animals/dose group) were fed diets containing HCBD at doses of 0, 1, 3, 10, 30, 65, or 100 mg/kg-day for 30 days. Renal toxicity in the form of increased relative kidney weight as well as renal tubular degeneration, necrosis and regeneration was observed in rats receiving doses of 30, 65 or 100 mg/kg-day. Minimal hepatocellular swelling was noted at a dose of 100 mg/kg-day. Other observed effects included decreased food consumption, reduced body weight gain, and increased hemoglobin concentration at doses of 10, 30, 65 or 100 mg/kg-day. No effects were observed in rats receiving 3 mg/kg-day. This study identified a No-Observed-Adverse-Effect Level (NOAEL) of 3 mg/kg-day and a LOAEL of 10 mg/kg-day.

Harleman and Seinen (1979) administered diets containing nominal concentrations of 0, 50, 150, or 450 ppm HCBD to weanling Wistar-derived rats (6 animals/sex/dose group) for 14 days. Based on mean body weight and food consumption data in the study, the mean HCBD doses were calculated to be 0, 4.6, 14.0 and 35.3 mg/kg-day. Body weight and food conversion efficiency were decreased in a dose-related manner. Food consumption was decreased at 35.3 mg/kg-day. Relative kidney weights were significantly increased at the two highest dose levels. A dose-related degeneration of renal tubular epithelial cells was observed in all treated animals, particularly in the straight limbs (pars recta) of the proximal tubules located in the outer medulla. No indications of liver toxicity were observed. The low dose of 4.6 mg/kg-day represented the LOAEL.

Stott et al. (1981) conducted an oral exposure study in adult male Sprague-Dawley rats. Five animals per treatment group were given daily doses of HCBD (0, 0.2 or 20 mg/kg-day) in corn oil for three weeks. In animals exposed to 20 mg/kg-day, a decrease in body weight gain and an increase in relative kidney weight were observed. Histopathological examination of the kidneys revealed damage in the middle and inner cortical regions, with loss of cytoplasm, nuclear pyknosis, increased basophilia and mitotic activity, and increased cellular debris. No indications of toxicity were observed in animals exposed to 0.2 mg/kg-day.

The National Toxicology Program (NTP) conducted a 2-week oral exposure study in B6C3F$_1$ mice (NTP, 1991; Yang et al., 1989). Groups of mice (5 animals/sex/dose group) received diets containing nominal concentrations of 0, 30, 100, 300, 1,000 or 3,000 ppm HCBD

for 15 days. Target concentrations were verified under experimental conditions by gas chromatography. The estimated daily intake calculated from feed consumption and mean body weights were 0, 3, 12, and 40 mg/kg-day for the 0, 30, 100, and 300 ppm dietary concentrations, respectively, for male mice, and 0, 5, 16, and 49 mg/kg-day for female mice. All mice provided with the 1,000 and 3,000 ppm diets died within seven days. Mice receiving 100 and 300 ppm HCBD lost weight. HCBD-related clinical signs observed at doses of 300 ppm or greater included lethargy, rough hair coats, hunched position, and incoordination. Marked reductions in thymus and heart weights were noted in mice consuming the 300 ppm diet. Kidney lesions attributed to HCBD exposure were observed in all treated mice of both sexes (Yang et al., 1989). Severe necrosis of the cortex and outer medulla was observed in the two lethal dose groups. Necrosis was less severe and regeneration was prominent in the pars recta of mice receiving lower doses of HCBD. Histopathologic changes were also observed in liver, lymphoid tissues, and testis of mice in the lethal 1,000 and 3,000 ppm dose groups, but were not clearly related to HCBD toxicity. Minimal-to-mild depletion of bone marrow in the femur was observed in 2 to 5 mice per dose group in animals receiving diets containing 300 ppm or higher levels of HCBD. This study identified a LOAEL of 3–5 mg/kg-day in male and female mice, respectively, based on renal tubular necrosis and cellular regeneration in animals in the lowest dose groups (Yang et al., 1989).

Jonker et al. (1993b) investigated the toxicity of HCBD in 10-week-old male and female Wistar rats (5 rats/sex/dose). HCBD was provided in the diet at levels of 0, 25, 100, or 400 ppm for a duration of four weeks. Based on mean body weight and food intake data in the study, these concentrations correspond to average daily doses of 0, 2.25, 8, and 28 mg/kg-day. Treatment-induced signs of toxicity were observed at doses of 8 and 28 mg/kg-day in both sexes. The observed signs included decreased liver weight, tubular cytomegaly, decreased plasma creatinine, decreased body weight (10% in males and 15% in females), and decreased adrenal weight. Increased plasma aspartate aminotransferase activity and bilirubin were observed at the 28 mg/kg-day dose. The NOAEL and LOAEL identified from this study were 2.25 and 8 mg/kg-day, respectively.

Lock et al. (1996) conducted two short-term experiments on the effects of HCBD in calves. Each experiment evaluated toxicity in a single animal. In the first experiment, a dose of 5 mg/kg-day was administered orally for 7 days. An increase in blood urea nitrogen was noted after the fifth dose, and levels remained high until the animal was euthanized nine days after initiation of treatment. Plasma levels of aspartate transaminase and alanine aminotransferase were elevated, but no changes were observed in hematological parameters. At necropsy, perirenal edema was observed in the kidneys and the liver was pale and swollen. Histopathological examination revealed midzonal necrosis of the liver and extensive swelling of the tubular epithelium and degenerative changes in the kidney. Casts were evident in the tubules of the medulla.

In the second short-term experiment conducted by Lock et al. (1996), a calf was dosed with 2.5 mg HCBD/kg-day for 10 days and the blood was monitored for 20 days for urea and platelet count. The dose was subsequently increased to 5 mg/kg-day, with 8 doses administered over 12 days. A marginal increase in blood urea nitrogen was observed on day 14. Aspartate transaminase and alanine aminotransferase were increased on day 7, and gradually decreased to normal levels on day 15. The calf was euthanized and necropsied 18 days after the start of the dosing regimen. Histopathological examination revealed slight disruption of the midzonal architecture of the liver, while mild renal tubular degeneration was evident in the kidney. The

results of the experiments conducted by Lock et al. (1996) indicate that HCBD is both a nephro- and hepato-toxicant in calves.

Nakagawa et al. (1998) exposed male Wistar rats (3 animals/dose group) to 0, 0.008, 0.04, or 0.2% HCBD in the diet for 3 weeks. Assuming a food consumption factor of 0.09 kg/kg/day (U.S. EPA, 1988), these dietary levels correspond to approximate daily doses of 0, 7.2, 36, and 180 mg/kg-day. Rats ingesting the 0.04% and 0.2% diets had lower mean body weight (decreases of 15% and 46%, respectively) at the termination of the experiment. Kidney weight was unaffected. Histopathological examination of rats in the 180 mg/kg-day group revealed indications of extensive regeneration in the straight portion (pars recta) of the proximal tubule. Similar lesions were not evident at lower doses. These data suggest a NOAEL of 7.2 mg/kg-day based on absence of effect on weight gain or renal histopathology.

Inhalation exposure

NIOSH (1981) reported 100% mortality in mice exposed to HCBD vapors for 5 days, 7 hours/day, at a concentration of 50 ppm (533 mg/m^3), but no deaths in animals exposed to 10 ppm (106.6 mg/m^3).

Gage (1970) conducted an inhalation study in Alderley Park SPF rats. Groups of adult rats (4/sex/dose) were exposed to nominal HCBD concentrations of 53, 107, or 267 mg/m^3 for 15 days, 6 hours/day; 1,067 mg/m^3 for 12 days, 6 hours/day; or 2,668 mg/m^3 for 2 days, 4 hours/day. Petroleum ether was used as a solvent for concentrations below 1,067 mg/m^3. No indications of toxicity were observed at the lowest level of exposure. Two of the four female rats exposed to 1,067 mg/m^3 HCBD died. Pale enlarged kidneys, adrenal regeneration, and renal cortical tubular degeneration with epithelial regeneration were noted at autopsy. Surviving females at this concentration were slightly anemic. The weight gain of female rats was reduced at 107 and 267 mg/m^3. At 1,067 mg/m^3, rats of both sexes lost weight. Irritation of the eyes and nose was observed at the two highest levels of exposure. Respiratory distress occurred at concentrations of 267 mg/m^3 or greater. At the termination of the experiment, enlarged pale kidneys were evident in the 267 and 1,067 mg/m^3 treatment groups. Enlarged adrenals were observed in animals exposed to 1,067 mg/m^3. Histopathological analysis revealed degeneration in the adrenal cortex and proximal tubules of the kidneys at concentrations of 267 mg/m^3 or greater (WHO, 1994).

7.2.3 Subchronic Studies

Schwetz et al. (1977) fed male and female Sprague-Dawley rats a diet containing 0.2, 2.0, or 20 mg/kg-day HCBD for evaluation of reproductive effects. HCBD was provided in the diet before and during mating, and throughout gestation and lactation, for a total study duration of 148 days. Adult rats from the 20 mg/kg-day dose level had decreased body weight gain along with decreased food consumption. At necropsy, relative kidney weights were increased in high-dose males and females. Relative liver weight was increased in high-dose males, and relative brain weight was increased in high-dose females. The kidneys of males exposed to 2 or 20 mg/kg-day were roughened and had a mottled cortex. Histopathological examination revealed dose-related increases in tubular dilation and regeneration in animals exposed to 2 or 20 mg/kg-day. These results indicate a NOAEL of 0.2 mg/kg-day for male and female rats, based on the absence of renal histopathology or other toxic effects at this dose.

Harleman and Seinen (1979) exposed groups of weanling Wistar-derived rats (10/sex/dose group) to daily oral gavage doses of 0, 0.4, 1.0, 2.5, 6.3, or 15.6 mg/kg-day HCBD in a peanut oil vehicle. The study duration was 13 weeks. Reductions in body weight gain, food consumption, and food utilization efficiency were noted in the two highest dose groups. Dose-related increases in relative kidney weights were noted in all treatment groups of male mice, and in females administered 6.3 or 15.6 mg/kg-day. Proximal tubular degeneration was noted in males treated with doses of 6.3 mg/kg-day and above, and in females treated with doses of 2.5 mg/kg-day and above. This effect was characterized by hyperchromatic nuclei, hypercellularity, vacuolation and focal necrosis of renal epithelial cells, and a diminished brush border. Polyuria and decreased urine osmolarity were noted in females receiving doses equal to or greater than 2.5 mg/kg-day, and in males receiving 15.6 mg/kg-day. Relative liver weights were increased in females at 15.6 mg/kg-day and in males at 6.3 and 15.6 mg/kg-day. Histological examination of the liver revealed increased cytoplasmic basophilia only in males treated with 6.3 mg/kg-day and above. Relative spleen weights were increased in males at 15.6 mg/kg-day, and in females at the two highest doses. The study authors identified NOAEL values of 1.0 mg/kg-day for females and 2.5 mg/kg-day for males.

NTP (1991) conducted a 13-week oral exposure study in B6C3F$_1$ mice. Groups of mice (10 animals/sex/dose group) received diets containing 0, 1, 3, 10, 30, or 100 ppm HCBD. Target concentrations were verified under experimental conditions by gas chromatographic analysis. Based on average food consumption and body weight data, these concentrations corresponded to dose levels of 0, 0.1, 0.4, 1.5, 4.9 or 16.8 mg/kg-day for males and 0, 0.2, 0.5, 1.8, 4.5 or 19.2 mg/kg-day for females. No HCBD-related clinical signs or deaths were observed. Food consumption of treated and control animals was similar. Reduced body weight gain was reported in males exposed to diets containing 30 or 100 ppm HCBD (decreases of 49% and 56%, respectively) and in females exposed to the 100 ppm diet (47%). Relative kidney weight was significantly decreased ($p<0.01$) in the three highest dose groups of males, and in females in the highest dose group. High-dose males also exhibited decreased relative heart weight, although no histologic lesions were reported in this organ.

Histopathological changes were noted in the kidneys of treated animals. Necropsy revealed treatment-related increases in renal tubular cell regeneration. This lesion was characterized as a diffuse increase in epithelial nuclei and increased basophilic staining. Female mice appeared to be more susceptible than male mice to the formation of this lesion following exposure to HCBD, with occurrence noted at dose levels of 0.2 mg/kg-day and above. Lesions were observed in male mice at dose levels of 4.9 mg/kg-day and above (NTP, 1991; Yang et al., 1989). In contrast to results from the 2-week study conducted by the same investigators, no evidence of necrosis was observed. Based on the histopathologic evaluation of the kidney, the authors identified a NOAEL of 1.5 mg/kg-day for male mice. Because tubular regeneration occurred in 1 of 10 females in the lowest dose group (0.2 mg/kg-day), the study authors concluded that a NOAEL for female mice could not be identified from these data (NTP, 1991). However, others have concluded that the effect observed at 0.2 mg/kg-day is not statistically significant, and therefore considered this dose to be the NOAEL for female mice (WHO, 1994; U.S. EPA, 1998a).

Nakagawa et al. (1998) administered 0.1% HCBD in the diet to male Wistar rats (21 rats/group) for 30 weeks in conjunction with a cancer promotion study (discussed in Section 7.2.7). Assuming a food consumption factor of 0.09 kg/kg/day (U.S. EPA, 1988), this dietary level

corresponds to an average daily dose of 90 mg/kg-day. HCBD treatment resulted in decreased final body weight, and increased relative kidney weight. No significant differences were noted in serum and urine biochemical parameters. Simple hyperplasia of renal tubular structures was observed, but the incidence did not differ significantly from the control. Histopathological examination did not reveal adenomatous hyperplastic foci or renal tumors.

7.2.4 Neurotoxicity

Data from distribution studies indicate that HCBD accumulates in brain tissue (Reichert et al., 1985). This observation raises the possibility that HCBD exposure may affect neurological function. Studies designed to specifically evaluate neurotoxicological endpoints following HCBD exposure were not identified in the available literature. However, neurological effects have been observed in a number of oral and dermal exposure studies. Kociba et al. (1977) reported increased relative brain weights in female rats fed 20 mg/kg-day HCBD for 2 years. This increase occurred in a dose group with depressed body weights and was not accompanied by histopathological changes in the brain. Similarly, Schwetz et al. (1977) noted depressed body weight and increased relative brain weight in female rats fed 20 mg/kg-day for 148 days in a reproductive study. Concurrent changes in behavior or brain histopathology were not observed in the affected animals. An increase in relative brain weights with decreased body weights was also observed in male and female B6C3F$_1$ mice fed 16.8 to 19.2 mg/kg-day HCBD in their diet in a 90-day subchronic study (NTP, 1991).

Treatment-associated neurotoxic effects were observed by Harleman and Seinen (1979), who provided female Wistar rats (6 animals/dose) with diets containing 0, 150, or 1,500 ppm HCBD (corresponding to average daily doses of 0, 15 or 150 mg/kg-day). The duration of exposure ranged from 10 to 18 weeks. Indications of neurotoxicity observed at the 150 mg/kg-day dose included ataxia, incoordination, weakness of the hind legs, and unsteady gait. Histopathological examination revealed demyelination and fragmentation of femoral nerve fibers in high-dose animals. No treatment-related histopathological changes were observed in the brain.

Badaeva (1983) and Badaeva et al. (1985) observed that daily oral administration of 8.1 mg/kg-day HCBD to pregnant rats throughout gestation resulted in histopathological changes in nerve cells and myelin fibers of the spinal cord in the dams and their offspring (WHO, 1994). Increased levels of free radicals were detected in the brain and spinal cord of the offspring of treated dams (U.S. EPA, 1991a).

Neurotoxicity has also been observed following HCBD exposure by the dermal route. Duprat and Gradiski (1978) observed central nervous system depression manifested as stupor in rabbits following application of 0.25 to 1.0 ml/kg (418 to 1,675 mg/kg) in an acute dermal toxicity test. Stupor was observed throughout the 8-hour exposure period, and during a 2-hour period immediately following exposure.

7.2.5 Developmental/Reproductive Toxicity

Oral Exposure

Schwetz et al. (1977) provided male and female Sprague-Dawley rats (30 to 51 animals/dose group) with a diet containing HCBD at levels corresponding to doses of 0, 0.2, 2.0, or 20 mg/kg-day HCBD. The HCBD-containing diet was administered for 90 days prior to mating, 15 days during mating, 22 days during gestation, and 21 days during lactation. Adults in the two higher dose groups exhibited multiple signs of toxicity, including decreased food consumption, decreased body weight gain, and renal tubular degeneration. No HCBD-related effects were observed on pregnancy rate, time to delivery, neonatal survival, neonate sex ratio, weanling histopathology, or incidence of neonatal external, visceral, or skeletal anomalies. Slightly decreased pup weight ($p<0.05$) was observed in the 20 mg/kg-day treatment group at postnatal day 21. The identified NOAEL and LOAEL for developmental effect were 2 and 20 mg/kg-day, respectively.

Harleman and Seinen (1979) provided female rats (6 animals/dose) with a diet containing HCBD at levels of 0, 150, or 1,500 ppm for 3 weeks prior to mating, 3 weeks during mating, and throughout gestation and lactation. Assuming a food consumption factor of 0.1 kg/kg/day (U.S. EPA, 1988), these concentrations correspond to average daily doses of 0, 15, or 150 mg/kg-day. High-dose females were sacrificed at week 10 of the study, and low-dose females were sacrificed at week 18. Maternal toxicity occurred in both dose groups, and observed effects included reduced body weight gain, increased relative kidney weight, and histopathological changes in kidneys. Neurological effects, including ataxia, incoordination, weakness of the hind legs, and unsteady gait, were observed in the dams at the 150 mg/kg-day dose. Furthermore, no conceptions occurred, the ovaries showed little follicular activity, and no uterine implantation sites were observed at the 150 mg/kg-day dose. At 15 mg/kg-day, fertility and litter size were reduced, but the effect was not statistically significant. Pup weights in this treatment group were significantly reduced on days 0, 10, and 20. No gross abnormalities were noted. The LOAEL identified was 15 mg/kg-day.

Badaeva and colleagues (Badaeva, 1983; Badaeva et al., 1985) conducted two studies in which pregnant female rats were orally administered 8.1 mg/kg-day HCBD throughout gestation. Offspring of HCBD-treated dams had lower body weight and shorter crown-rump length when compared to controls (U.S. EPA, 1991a). Histological changes in the nerve cells and myelin fibers of the spinal cord were noted in both dams and offspring (WHO, 1994). Neurological changes reported in the offspring included ultrastructural changes in neurocytes and increased levels of free radicals in the brain and spinal cord (U.S. EPA, 1991a).

In addition to the reproductive and developmental toxicity studies discussed above, two longer-term toxicity studies have evaluated reproductive endpoints following oral exposure to HCBD. No treatment-related lesions in reproductive organs were observed in rats that received lifetime exposures of up to 20 mg/kg-day HCBD (Kociba et al., 1977). No significant changes were noted in sperm count, the incidence of abnormal sperm, estrual cyclicity, or the average estrous cycle length in mice administered 100 ppm HCBD in the diet for 13 weeks. Sperm motility in treated mice was significantly lower, though not in a dose-related manner, than that observed for controls (NTP, 1991).

Inhalation Exposure

Saillenfait et al. (1989) exposed pregnant Sprague-Dawley rats (24 animals/dose) to HCBD vapor at nominal concentrations of 0, 21, 53, 107, or 160 mg/m^3 (0, 2, 5, 10, or 15 ppm) for 6 hours/day from gestation days (GD) 6–20. Concentrations were monitored by gas chromatography. Animals were sacrificed on GD 21. Decreased body weight gain was occurred in dams exposed to 53 or 160 mg/m^3. Body weight was decreased ($p<0.01$) in male (9.5%) and female (12.9%) fetuses in the 160 mg/m^3 treatment group. Fetal body weight was unaffected at lower doses. The mean number of implantation sites, total fetal losses, resorptions, number of live fetuses, pregnancy rate, and fetal sex ratio were comparable in the treated and control groups. No exposure-related external, visceral, or skeletal anomalies were noted in any dose group.

In dominant lethal tests in CD (Sprague-Dawley-derived) rats, exposure to HCBD vapors at 10 or 50 ppm (107 or 533 mg/m^3) for 5 consecutive days, 7 hours/day, did not affect fertility indices, number of corpora lutea or implantations, or the frequency of early death (NIOSH, 1981).

For B6C3F$_1$ mice that were exposed to HCBD vapors at 107 or 533 mg/m^3, all animals in the high-dose group died during the 5-week post-treatment period (NIOSH, 1981). The frequency of abnormal sperm morphology in the low-dose group did not differ significantly from controls.

Intraperitoneal Injection

Mated female Sprague-Dawley rats (10–15 animals/group) received 10 mg/kg-day HCBD in corn oil via intraperitoneal injection, during gestation days 1 to 15 (Hardin et al., 1981). Maternal toxicity consisted of changes in two organ weights (no further details provided). Decreased pre- and post-implantation survival was also noted. Developmental effects included decreased fetal weight or length, a 1-to-2-day delay in heart development, and dilated ureters. Gross external and internal examinations revealed no malformations (WHO, 1994).

Harris et al. (1979) exposed pregnant female rats to 10 mg/kg-day HCBD from gestation days 1 to 15 via intraperitoneal injection. A 3-fold increase in soft tissue anomalies was reported in offspring of treated dams. No particular type of anomaly predominated (U.S. EPA, 1991a).

7.2.6 Chronic Toxicity

Data from a single chronic oral exposure study are available in the published literature. Kociba et al. (1977) provided male and female Sprague-Dawley rats (39 to 40/sex/dose level; 90/sex for controls) with diets that contained 0, 0.2, 2, or 20 mg/kg-day HCBD (99% pure) for 22 months (males) or 24 months (females). Parameters monitored included appearance and demeanor, body and organ weights, food consumption, hematologic and urine analysis, urinary porphyrins, and serum clinical chemistry, and histopathology of major organs. The investigators reported significantly increased mortality in high-dose males ($p<0.05$). Decreased body weight gain was noted in high-dose males and females, with significant differences ($p<0.05$) from controls evident after day 27 (females) or day 69 (males). There were no apparent treatment-related effects on food consumption. High-dose animals had increased relative brain weights (females) and relative testes weights (males).

An important observation in the Kociba et al. (1977) study is the clear dose-response relationship for HCBD-induced renal toxicity. No discernible effects were noted at the 0.2 mg/kg-day dose. Effects noted at the intermediate dose of 2 mg/kg-day included increased urinary coproporphyrin excretion (females only), and increased renal tubular epithelial hyperplasia. Lifetime ingestion of the 20 mg/kg-day dose resulted in increased urinary excretion of coproporphyrin and increased terminal weight of the kidneys in rats of both sexes. Microscopic examination revealed histopathological changes in the kidney, including hyperplasia and neoplasia of the renal epithelium. HCBD-related neoplastic changes are further discussed in Section 7.2.7. The lowest dose of 0.2 mg/kg-day was identified as the NOAEL in this study. The LOAEL was 2 mg/kg-day.

7.2.7 Carcinogenicity

Oral Exposure

Kociba et al. (1977) observed the tumorigenic potential of HCBD in male and female rats fed 0, 0.2, 2, or 20 mg/kg-day in a 2-year oral exposure study. No adverse effects attributable to HCBD were observed in the low-dose group. Ingestion of the intermediate 2 mg/kg-day dose resulted in signs of renal tubular epithelial hyperplasia, but no evidence of neoplasia was observed. Ingestion of 20 mg/kg-day for 2 years resulted in development of renal tubular adenomas and adenocarcinomas. Neoplasms were observed in approximately 23% (9/39) of the males and 15% (6/40) of the females. Combined incidence of renal tubular benign and malignant tumors was significantly increased when compared to controls ($p<0.05$) for both males and females. Metastasis to the lungs was observed in two of the treated animals. An important observation in this study was that HCBD-induced neoplasms occurred only at a dosage level that caused substantial renal tissue injury. Additional details of this study are provided in Section 7.2.6.

Chudin et al. (1985) conducted a 1-year HCBD oral exposure study in rats. The average daily doses ranged from 0.6 to 37 mg/kg-day. Benign liver and kidney tumors were noted, but malignant tumors were not observed (U.S. EPA, 1991a).

Nakagawa et al. (1998) investigated the effect of HCBD on renal carcinogenesis in male Wistar rats pre-treated with *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN). EHEN is a known nephrocarcinogen in rats, where it selectively induces renal tubular cell tumors. The purpose of this study was to evaluate the ability of HCBD to act as a promoting stimulus following subthreshold exposure of EHEN. HCBD was administered for 30 weeks at a concentration of 0.1% by weight (1,000 mg/kg) in the diet to rats (12/treatment group) that had previously received 0.1% EHEN in the drinking water. The combined treatment with HCBD and EHEN resulted in a significantly higher renal tumor incidence than did administration of EHEN alone. Rats treated with HCBD alone did not develop renal tumors under the conditions used in this investigation. Significantly increased levels of bromodeoxyuridine (BrdU) labeling indicated increased cell proliferation in the outer stripe and cortex of kidneys from HCBD-treated rats. In a parallel experiment, immunostaining for proliferating cell nuclear antigen (PCNA) was used to estimate nuclear DNA synthesis in defined renal tubular segments of HCBD-treated rats. A significant increase in the number of PCNA-positive cells was noted only in the outer stripe. These results are consistent with the outer stripe as a site for renal lesions induced by HCBD.

Nakagawa et al. (1998) concluded that the ability of HCBD to induce EHEN-initiated carcinogenesis appears to be associated with nephropathy and subsequent cell proliferation.

Dermal Exposure

Van Duuren et al. (1979) evaluated the carcinogenicity of dermally applied HCBD in female Swiss mice (30 animals/group). The investigators applied 6.0 mg of HCBD in acetone to shaved dorsal skin three times per week for a duration of 440 to 594 days. The treatment did not increase the incidence of papillomas or carcinoma at the site of application, or the incidence of tumors at distant sites such as the lung, stomach or kidney.

Van Duuren et al. (1979) also evaluated HCBD in an initiation-promotion experiment. Female Swiss mice (30 animals/group) received a single application of 15.0 mg HCBD in acetone on shaved dorsal skin. Fourteen days after HCBD application, dermal applications of 5 μg of the tumor promoter 12-*o*-tetradecanoylphorbol-13-acetate (TPA) were administered to the test site three times per week for a total duration of 428 to 576 days. The incidence of skin papillomas in HCBD-treated animals was comparable to that in controls.

Intraperitoneal Injection

Theiss et al. (1977) investigated the carcinogenic potential of HCBD by assessment of the pulmonary tumor response in male strain A/St mice. Twenty animals per dose group were given intraperitoneal injections of 4 or 8 mg/kg HCBD in tricaprylin, three times per week for a total of 13 and 12 injections, respectively. The total injected dose was 52 or 96 mg HCBD per animal. All surviving animals were killed 24 weeks after the first injection, and were examined for pulmonary surface adenomas. The tumor incidences were similar in treated and control groups. However, the use of this study for the evaluation of the carcinogenicity of HCBD is limited by the use of a mouse strain that is highly predisposed to spontaneous lung cancer, the small number of animals per dose group, the parenteral route of administration, and the limited scope of histopathological evaluation (WHO, 1994).

7.3 Other Key Data

7.3.1 Mutagenicity/Genotoxicity

The mutagenicity of HCBD has been evaluated in an array of *in vivo* and *in vitro* assays. The results of these tests are summarized below by category. No information was located regarding the genotoxic effects of HCBD in humans.

Bacterial Test Systems

Test results from bacterial assays of mutagenicity are summarized in Table 7-1. Most tests in standard *S. typhimurium* reverse mutation assays have been negative, with or without S9 activation (Rapson et al., 1980; Reichert et al., 1983; Stott et al., 1981; DeMeester et al., 1981; Haworth et al., 1983; Chudin et al., 1985) except for a study by Simmon (1977) in which a positive response in *Salmonella typhimurium* was reported in the presence of metabolic activation induced by rat liver S9 fraction (U.S. EPA, 1991a). Conflicting results in standard assays may be due to contaminants in technical and even analytical grade HCBD (Reichert et al., 1984;

Vamvakas et al., 1988). Vamvakas et al. (1988) observed 98% pure HCBD was a direct-acting mutagen in *S. typhimurium* TA 100; but after HCBD was purified to 99.5%, a negative mutagenic response was obtained.

Positive results have been reported for HCBD when specialized pre-incubation conditions that included rat liver microsomes and glutathione were utilized (Vamvakas et al., 1988; Roldan-Arjona et al., 1991). The mutagenic response was increased with additional inclusion of rat kidney microsomes as a γ-glutamyl transpeptidase and dipeptidase source (Vamvakas et al., 1988). Reichert et al. (1984) also reported a positive response in *S. typhimurium* when a "fortified" S9 mix, containing 3-fold more S9 protein than standard Ames test protocols, was utilized.

Test results for mutagenicity assays of HCBD metabolites are summarized in Table 7-2. Positive results in bacterial reverse mutation assays have been obtained for the mono-glutathione and mono-cysteine conjugates of HCBD (Green and Odum, 1985; Dekant et al., 1986; Vamvakas et al., 1988) and the mercapturic acid, *N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine (Reichert and Schutz, 1986; Wild et al., 1986). Other HCBD metabolites that gave positive results in reverse mutation assays were pentachloro-3-butenoic acid and pentachloro-3-butenoic acid chloride (Reichert et al., 1984). When rat kidney fractions were used for metabolic activation, the addition of a specific inhibitor of β-lyase (aminooxyacetic acid) to the system reduced the mutagenic response (Vamvakas et al., 1988), indicating that HCBD metabolites mediated mutagenesis in these

Table 7-1. Mutagenicity of HCBD in *Salmonella typhimurium* Test Systems

Strain	Conditions[a]	Results[b]		Reference
		With S9 Activation	Without S9 Activation	
TA100 TA 1535	-	+	+	Simmon (1977)
TA100	purity not reported	nd	-	Rapson et al. (1980)
TA100	purity >99%	-	-	Stott et al. (1981)
TA98 TA100 TA1530 TA1535 TA1538	purity 98%, plate incorporation	-	-	De Meester et al. (1981)
TA100	purity > 99%, plate incorporation	-	-	Stott et al. (1981)
TA98 TA100	purity not reported, suspension test	-	-	Reichert et al. (1983)
TA98 TA100 TA1535 TA1537	purity not reported, preincubation test	-	-	Haworth et al. (1983)
	+ fortified S9*, purity > 99.5%, preincubation test	+	nd	
TA100 TA1535 TA1538	+/- rat liver S9, purity not reported, plate incorporation	-	-	Chudin et al. (1985)
TA100	+/- rat liver S9, preincubation test	-	-	Reichert et al. (1984)
	+ rat liver S9*, preincubation test	+	-	
TA100	+ rat liver S9*, purity 99.5%, preincubation test	+[c]	nd	Wild et al. (1986)
TA100	purity 99.5%	nd	+	Vamvakas et al. (1988)
	purity 99.5%, preincubation test	nd	-	

Table 7-1 (continued)

Strain	Conditions[a]	Results[b]		Reference
		With S9 Activation	Without S9 Activation	
TA100	+ rat liver microsomes without additional GSH	-	nd	Vamvakas et al. (1988)
	+ rat liver microsomes and additional GSH, purity 99.5%, plate incorporation	+[d]	nd	
TA100	purity 98%, preincubation test	-	+	Roldan-Arjona et al. (1991)

Source: adapted from WHO (1994)

[a] S9* = a fortified S9 mix containing 3 times the normal protein concentration; GSH = reduced glutathione
[b] + = ≥ twice the background rate or, in the case of bacterial studies, a reproducible dose-related increase in the number of revertants per plate; - = negative; nd = not determined
[c] 0.23 revertants per nmol
[d] Addition of rat kidney microsomes further increased the number of revertants; positive results were inhibited by the β-lyase inhibitor aminooxyacetic acid

Table 7-2. Mutagenicity of HCBD Metabolites

Metabolite[a]	Conditions[b]	Result[c]		Reference
		With S9 Activation	Without S9 Activation	
PCBG	no activation	nd	-	Green & Odum (1985)
	+ rat kidney S9	+	nd	Green & Odum (1985)
	+/- rat kidney fractions	+[d]	+[d]	Vamvakas et al. (1988)
TCBG	+/- rat kidney fractions	-	-	Vamvakas et al. (1988)
TCBC	+/- rat kidney fractions	-	-	Vamvakas et al. (1988)
PCBC	+/- rat kidney S9	+[e]	+[e]	Green & Odum (1985)
	no activation	nd	+[f]	Dekant et al. (1986)
N-AcPCBC	- rat liver S9	nd	-	Wild et al. (1986)
	+ rat liver S9	+	nd	
	+ rat liver S9	+	nd	Reichert and Schutz (1986)
PCCMT	+ rat liver S9*	-	nd	Wild et al. (1986)
PCMT	+ rat liver S9	-	nd	Wild et al. (1986)
PCBA	+ rat liver S9	+	+	Reichert et al. (1984)
PCBAC	+ rat liver S9	+	+	Reichert et al. (1984)

Source: modified from WHO (1994)

[a]Abbreviations:
PCBG *S*-(1,1,2,3,4-pentachlorobutadienyl) glutathione
TCBG 1,4 bis(1,2,3,4-tetrachlorobutadienyl) glutathione
TCBC 1,4 bis (1,2,3,4-tetrachlorobutadienyl)-L-cysteine
PCBC *S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine
N-AcPCBC *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine
PCCMT 1,1,2,3,4-pentachlorobutadiene carboxymethylthioether
PCMT 1,1,2,3,4-pentachlorobutadiene methylthioether
PCBA 2,2,3,4,4-pentachloro-3-butenoic acid
PCBAC 2,2,3,4,4-pentachloro-3-butenoic acid chloride

[b] S9* = a fortified S9 mix containing 3 times the normal protein concentration; GSH = reduced glutathione

[c] + = twice background rate; - = negative; nd = not detectable
[d] Mutagenic potency enhanced by rat kidney microsomes or mitochondria and less so by cytosol; positive results
[e] Mutagenic potency enhanced by rat kidney microsomes or mitochondria and less so by cytosol; positive results
[f] Mutagenic potency enhanced by the β-lyase inhibitor aminooxyacetic acid

assays. These results are consistent with the proposed mechanism for bioactivation of HCBD in animals (Figure 6-1, Section 6.3).

In Vitro Mammalian Cell Test Systems

Data for mutagenicity assays in mammalian test systems are summarized in the upper portion of Table 7-3. Treatment with HCBD did not increase the frequency of chromosome aberrations in Chinese hamster ovary (CHO) cells (Galloway et al., 1987) or cultured human lymphocytes (German, 1988). However, Galloway et al. (1987) observed a significant increase in sister chromatid exchange in CHO cells treated with HCBD. Schiffman et al. (1984) reported unscheduled DNA synthesis (UDS) activity and morphological transformation in Syrian hamster embryo fibroblasts with and without metabolic activation. Stott et al. (1981) reported negative results in a rat primary hepatocyte UDS assay.

Vamvakas et al. (1989) evaluated the genotoxicity of the HCBD metabolite *S*-(1,2,3,4,4-pentachlorobutadienyl)glutathione (PCBG) in cultured porcine kidney LLC-PK cells. Incubation of confluent monolayers with PCBG resulted in a dose-dependent induction of DNA repair. Addition of either acivicin, an inhibitor of γ-glutamyl transpeptidase, or aminooxyacetic acid, an inhibitor of cysteine conjugate β-lyase, prevented PCBG-induced genotoxicity. These results are consistent with the hypothesis that renal metabolism plays a key role in PCBG-induced genotoxicity.

In Vivo Test Systems

Results of *in vivo* HCBD genotoxicity tests are summarized in the lower portion of Table 7-3. Both negative (NIOSH, 1981; Schwetz et al., 1977) and positive (German, 1988) results have been reported for chromosome aberration assays conducted in HCBD-treated mice or rats. Negative findings have been reported in a dominant lethal assay in rats (NIOSH, 1981), and in the *Drosophila melanogaster* sex-linked recessive lethal mutation assay with exposure via either injection or feeding (NIOSH, 1981; Woodruff et al., 1985). However, Stott et al. (1981) reported a small (1.25 to 1.54-fold) increase in UDS activity and DNA alkylation (0.78 alkylation per 10^6 nucleotides) in kidney cells from rats fed 20 mg/kg-day HCBD in the diet for 3 weeks, suggesting that HCBD exhibited a minor degree of renal genotoxicity.

Schrenk and Dekant (1989) evaluated the covalent binding of HCBD metabolites to renal and hepatic DNA in NMRI mice. A low level of covalent binding (covalent binding index (CBI) = 27) was observed in nuclear DNA (nDNA) isolated from the kidney, while covalent binding was undetectable in nDNA isolated from liver. Significantly higher levels of covalent binding were observed in mitochondrial DNA (mtDNA), with CBIs of 513 and 7,506 determined for liver and kidney, respectively. Analysis of covalent binding to renal mtDNA identified three ^{14}C-labeled compounds that appeared to be DNA bases altered by HCBD metabolites.

Table 7-3. Genotoxicity of HCBD in Eukaryotic Assay Systems

Species/Strain/ Cell Type	Compound	End Point	Comments	Results[a]	Reference
In Vitro Assays					
Chinese hamster ovary cells	HCBD	Chromosome aberrations	+/- rat liver S9	-	Galloway et al. (1987)
Human lymphocytes	HCBD	Chromosome aberrations	+/- rat liver S9	-	German (1988)
Chinese hamster ovary cells	HCBD	Sister chromatid exchange	+/- rat liver S9	+	Galloway et al. (1987)
Syrian hamster embryo fibroblast	HCBD	Unscheduled DNA synthesis	+/- activation	+	Schiffmann et al. (1984)
Porcine kidney cells	PCBG	Unscheduled DNA synthesis	Addition of acivicin or aminooxyacetic acid gave negative results	+	Vamvakas et al. (1989)
Syrian hamster embryo fibroblasts	HCBD	Morphological transformation	+/- activation	+	Schiffmann et al. (1984)
Rat primary hepatocyte	HCBD	Unscheduled DNA synthesis		-	Stott et al. (1981)
In Vivo Assays					
Mouse; bone marrow cells	HCBD	Chromosome aberrations	Inhalation, 4h	+	German (1988)
Mouse; bone marrow cells	HCBD	Chromosome aberrations	Oral gavage	+	German (1988)
Rat; bone marrow cells	HCBD	Chromosomal aberration	Dietary doses of up to 20 mg/kg-day for 148 days	-	Schwetz et al. (1977)
Rat; bone marrow cells	HCBD	Chromosomal aberrations	10 or 50 ppm, 7 hrs/day, 1 or 5 days	-	NIOSH (1981)

Table 7-3 (continued)

Species/Strain/ Cell Type	Compound	End Point	Comments	Results[a]	Reference
Rat	HCBD	Dominant lethality	10 or 50 ppm, 7 hrs/day, 5 days	-	NIOSH (1981)
Rat kidney cells	HCBD	DNA alkylation	20 mg/kg by gavage	+	Stott et al. (1981)
Rat kidney cells	HCBD	DNA repair	20 mg/kg by gavage	+	Stott et al. (1981)
Mouse	HCBD	DNA binding	Single dose, 30 mg/kg	+[b]	Schrenk and Dekant (1989)
Drosophila melanogaster	HCBD	Gene mutation (sex-linked recessive lethal)		-	NIOSH (1981)
Drosophila melanogaster	HCBD	Sex-linked lethals	Feeding or injection	-	Woodruff et al. (1985)

Source: modified from ATSDR (1994) and WHO (1994).

[a] + = ≥ twice the background rate or, in the case of bacterial studies, a reproducible dose-related increase in the number of revertants per plate; - = negative; nd = not determined;
[b] Binding was predominately to mitochondrial DNA

7.3.2 Immunotoxicity

The immunological effects of HCBD have not been systematically evaluated in humans, and there are currently no case reports that describe immunological abnormalities occurring in humans exposed to HCBD.

Animal data on the immunological effects of HCBD are limited. In a 2-week oral exposure study conducted by NTP (1991), depletion (atrophy) and necrosis of lymphoid tissues was observed in $B6C3F_1$ mice administered lethal doses of 1,000 and 3,000 ppm of HCBD. However, the investigators noted that these lesions may have been secondary to chemical-induced stress. Similar lesions were not observed in mice administered 19.2 mg/kg-day in a subsequent 13-week study conducted by NTP (1991).

In a 13-week gavage study, relative spleen weights were significantly increased in male rats orally administered HCBD at 15.6 mg/kg-day and in females at 6.3 mg/kg-day and above (Harleman and Seinen, 1979). Treatment-related lesions in lymphoid organs (thymus, lymph nodes, spleen) have not been reported in terminal necropsy of mice or rats in other HCBD subchronic and chronic oral exposure studies at doses up to 100 mg/kg-day (Harleman and Seinen, 1979; Kociba et al. 1977; Schwetz et al., 1977). Performance of immune function screening batteries in HCBD-treated animals has not been evaluated.

Delayed hypersensitivity reaction was exhibited in guinea pigs to dermal HCBD application (Gradiski et al., 1975).

7.3.3 Hormonal Disruption

No studies were identified that associate HCBD exposure with endocrine disruption.

7.3.4 Physiological or Mechanistic Studies

The proximal tubule-specific toxicity of HCBD is likely determined by two factors: 1) the distribution of enzymes required for its bioactivation, and 2) the ability of this region to concentrate precursors of the ultimate toxic species (Dekant et al., 1990). The enzyme cysteine conjugate β-lyase is believed to catalyze the conversion of HCBD-cysteine conjugates to a highly reactive thioketene metabolite (Figure 6-1, Section 6.3). Multiple investigators have addressed the localization of β-lyase and its relationship to nephrotoxicity. MacFarlane et al. (1989) demonstrated by immunocytochemical technique that the region of highest cytosolic β-lyase activity in untreated rats coincides with the site of HCBD-induced necrosis in the pars recta region of the proximal tubule. However, Jones et al. (1988) and Kim et al. (1997) detected β-lyase in the entire proximal tubule. Trevisan et al. (1998) detected histopathological changes and increased levels of β-lyase activity in the urine following treatment of rats with S_3 and S_1-S_2 specific nephrotoxicants, which were cited as evidence for distribution of the enzyme along the entire length of the proximal tubule. These data suggest that additional factors may contribute to selective damage in the pars recta.

The ability of the proximal tubule to concentrate HCBD metabolites has been investigated as a factor in renal toxicity. Nash et al. (1984) administered a single dose of radiolabeled HCBD and observed that radioactivity was concentrated in the renal cortex shortly after dosing. Renal

cells that concentrated the radiolabeled compounds were subsequently observed to undergo necrosis. In mammals, γ-glutamyltranspeptidase, the enzyme that together with dipeptidase catalyzes the conversion of glutathione conjugate to cysteine conjugate, is concentrated in the brush-border membrane of the proximate tubular cells. The distribution of this enzyme may also contribute to an increase in the concentration of cysteine conjugates in the proximal renal tubules.

The probenecid-sensitive organic anion transporter that is present on the basolateral side of proximal tubule cells appears to play a role in the accumulation of HCBD metabolites (Dekant 1996). Probenecid is a competitive inhibitor of organic anion transport, and is reported to be without effect on energy metabolism, transport carrier synthesis, or uptake of other substances actively transported by the kidney (Lock and Ishmael, 1985; Dekant, 1996). Haloalkene-derived mercapturates have the highest affinity for the organic anion transporter, but glutathione and cysteine *S*-conjugates with lipophilic substituents on sulfur are also substrates for transport (Dekant, 1996).

The effect of probenecid on development of HCBD-induced renal toxicity has been investigated in *in vivo* and *in vitro* studies. Lock and Ishmael (1985) administered a single intraperitoneal dose of 16 or 64 μmol/kg ^{14}C-radiolabeled *N*-acetyl-pentachlorobutadienyl-L-cysteine to female Alpk/AP rats and observed acute renal necrosis within four hours. Prior administration of up to 500 μmol/kg probenecid reduced renal cortical concentrations of radioactivity and provided protection against nephrotoxicity in a dose-dependent manner as assessed by plasma urea concentration and renal histopathology. Pretreatment with probenecid also reduced or prevented the toxic effects of intraperitoneally injected HCBD and its glutathione and cysteine conjugates. Thus, the selective toxicity to the pars recta in rats is thought to result in part from transport of HCBD metabolites into cells of this region via a probenecid-sensitive transport system.

Bach et al. (1986) confirmed the protective effect of probenecid against HCBD metabolite-induced toxicity in freshly isolated proximal tubule fragments. Incorporation of ^{3}H-proline into acid precipitable protein was utilized as an indicator of synthetic capacity of the tubular fragment. Addition of 2 mM *N*-acetyl-pentachlorobutadienyl-L-cysteine to the incubation medium reduced ^{3}H-proline incorporation to 34% of the control value. The inclusion of 400 μM probenecid in the incubation medium almost completely restored (to 95%) ^{3}H-proline incorporation.

Multiple studies suggest that renal cortical mitochondria are a primary subcellular target for HCBD toxicity. Jones et al. (1986) investigated the toxic effects of pentachlorobutadienyl-glutathione (PCBG) in isolated rat renal epithelial cells. Exposure to PCBG decreased cell viability and reduced the concentration of intracellular thiols. Other PCBG-related effects included depletion of Ca^{2+} from the mitochondrial compartment, an elevation of cytosolic Ca^{2+} concentration, inhibition of respiration, and decreased levels of ATP. Prevention of PCBG bioactivation by inhibition of γ-glutamyl transpeptidase or β-lyase provided complete protection against cytotoxicity. The authors hypothesized that PCBG-induced renal cell injury results from selective effects on mitochondrial function, including inhibition of respiration, depression of ATP synthesis, and release of mitochondrial calcium (II) ions.

Wallin et al. (1987) studied *S*-pentachlorobutadienyl-L-cysteine (PCBC) toxicity in mitochondria isolated from the rat kidney cortex. Respiring mitochondria exposed to PCBC

showed a dose-dependent loss of ability to retain calcium. This effect was associated with a collapse of mitochondrial membrane potential. A slow nonenzymatic depletion of mitochondrial glutathione was also observed. Preincubation with aminooxyacetic acid, an inhibitor of β-lyase, effectively counteracted the loss of glutathione, suggesting that an interaction of the reactive thioketene with the mitochondrial inner membrane was responsible for the observed effects.

Schnellmann et al. (1987) investigated the mechanism of PCBC-induced toxicity in renal proximal tubules isolated from New Zealand rabbits. Addition of 20 to 500 μM PCBC to renal tubule suspension initiated a specific sequence of toxic effects. Fifteen minutes of exposure to 200 μM PCBC caused an increase in basal and ouabain-insensitive respiration. Sixty minutes of PCBC exposure inhibited basal, nystatin-stimulated and ouabain insensitive respiration, and resulted in a 79% decrease in glutathione concentration. In addition, an 11% decrease in lactate dehydrogenase retention was observed after 60 minutes of PCBC exposure, suggesting that cell viability was decreased as a result of treatment. Analysis of mitochondrial function indicated that the initial increase in respiration resulted from uncoupling of oxidative phosphorylation. The resulting ATP deficiency may have limited energy-dependent active transport processes in the tubules, thus inhibiting reabsorption processes. The changes in respiration observed at 60 minutes appeared to result from gross mitochondrial damage characterized by inhibition of state 3 respiration, depression of cytochrome c/cytochrome oxidase activity, and inhibition of electron transport. The results of these studies suggest that alterations in mitochondrial function are an early event in PCBC-mediated toxicity.

A similar pattern of events was observed by Groves et al. (1991). These workers investigated the relationship between uptake and covalent binding of the HCBD metabolite pentachlorobutadienyl-L-cysteine (PCBC) in rabbit renal proximal tubules, renal membrane vesicles, and isolated renal cortical mitochondria. Their findings confirmed the PCBC-induced pattern of mitochondrial dysfunction previously observed by Schnellmann et al. (1987) in rabbit proximal tubule suspensions. Furthermore, Groves et al. (1991) demonstrated the rapid accumulation of ^{35}S-PCBC in renal proximal tubule cells and its metabolism to a reactive intermediate that bound to tubular protein. An estimated 70 to 90% of the intracellular radioactivity was bound to protein. Mitochondria isolated from renal proximal tubules also metabolized ^{35}S-PCBC to a reactive intermediate that bound to mitochondrial protein, consistent with the mitochondrion being a critical subcellular target for HCBD-induced toxicity. Addition of the β-lyase inhibitor aminooxyacetic acid (AOAA) reduced covalent binding to tubular proteins, and blocked the toxic effects of PCBC on isolated mitochondria. However, AOAA decreased but did not prevent the toxic effects PCBC on respiration and cellular ATP levels induced by PCBC exposure.

Additional studies have investigated interactions between the reactive intermediate generated by metabolism of PCBC and cellular macromolecules. Lock and Schnellmann (1990) examined the ability of reactive thiols formed by the action of β-lyase on cysteine conjugates of several haloalkenes, including HCBD, to inhibit renal enzymes. The activities of glutathione reductase (a cytosolic enzyme) and lipoyl dehydrogenase (a mitochondrial enzyme) were assayed for this purpose. Administration of 200 mg/kg HCBD to male rats by intraperitoneal injection resulted in inhibition of both enzymes. The authors suggested that such inhibition is a general outcome of PCBC exposure, and is likely to occur with a diverse range of renal enzymes.

Schrenk and Dekant (1989) investigated covalent binding of ^{14}C-labeled HCBD metabolites to mouse DNA after a single oral dose of 30 mg/kg ^{14}C-HCBD. HCBD metabolites bound extensively to mitochondrial DNA. In contrast, little binding to nuclear DNA was observed. The study authors suggested that proximity to high β-lyase concentration in the mitochondrial membrane and the absence of associated histones make mitochondrial DNA a more vulnerable target for reactive HCBD metabolites.

As noted above (Section 6.3), the cysteine derivative of HCBD is a substrate for cysteine conjugate β-lyase. The activity of β-lyase leads to formation of an enethiol intermediate which is rapidly converted to thioketene, a potent acylating agent (Dekant et al., 1990). In rats, the enethiol intermediate may be detoxified by methylation to form pentachlorobutadienyl-methylthioether. Morel et al. (1999) investigated the role of *S*-adenosyl methionine (SAM)-dependent thiol methylation in prevention of HCBD-induced nephrotoxicity in male Swiss OF1 mice. The mice were treated with a single intraperitoneal dose of periodate-oxidized adenosine (ADOX) prior to administration of a single intraperitoneal dose of 80 or 100 mg HCBD/kg. Pretreatment with ADOX increased the level of SAM in the liver and kidney approximately four-fold, but did not modify the nephrotoxicity of HCBD as determined by histopathological evaluation of renal proximal tubules. This result was interpreted by the study authors as evidence that SAM-dependent thiol methylation does not play a role in detoxification of HCBD-derived enethiol in mice.

Chemically-induced $\alpha_{2\mu}$-globulin nephropathy represents a potential alternative mechanism for HCBD toxicity in rats. Since $\alpha_{2\mu}$-globulin synthesis is androgen-dependent in the liver, this form of nephropathy occurs exclusively in male rats, and is characterized by the accumulation of hyaline droplets in proximal tubule cells. Binding of the chemical to $\alpha_{2\mu}$-globulin is a prerequisite for development of nephrotoxicity. Because HCBD-induced renal toxicity occurs in both male and female rats, it is evident that $\alpha_{2\mu}$-globulin nephropathy is not required for nephrotoxicity. However, there is limited evidence to suggest that the $\alpha_{2\mu}$-globulin mechanism may contribute to the nephrotoxicity observed in male animals. Birner et al. (1995) observed that unmetabolized HCBD was excreted in the urine of male, but not female, Wistar rats following exposure to a single gavage dose of ^{14}C-HCBD in corn oil. The study authors also noted more pronounced necrotic changes in the proximal tubules of male rats when examined 48 hours after treatment. Slight liver damage was observed only in male rats. In a subsequent experiment in the same laboratory, Pähler et al. (1997) orally administered 200 mg/kg ^{14}C-HCBD in corn oil to Sprague-Dawley (SD) and NCI Black-Reiter rats (NBR), an $\alpha_{2\mu}$-globulin-deficient strain. ^{14}C-HCBD was present only in the urine of male SD rats, but not NBR rats. The study authors determined that the excreted HCBD detected in the urine of male SD rats was associated with its binding to $\alpha_{2\mu}$-globulin. Histopathological examination 48 hours after treatment revealed the formulation of hyaline droplets indicative of $\alpha_{2\mu}$-globulin accumulation in renal epithelial cells. In addition, microscopic examination confirmed the occurrence of more extensive nephropathy in male than in female animals as previously observed in Wistar rats.

Saito et al. (1996) established that dose-dependent levels of kidney-type $\alpha_{2\mu}$-globulin (αG-K) in the urine are a reliable predictor of $\alpha_{2\mu}$-globulin accumulation in the kidney. These investigators subsequently administered 100 mg/kg-day HCBD to adult male Sprague-Dawley rats for five consecutive days. No increase in urinary αG-K was detected following exposure, suggesting that HCBD treatment did not induce a marked accumulation of $\alpha_{2\mu}$-globulin. However, histopathological examination revealed some epithelial cells showing hyaline droplet-

related degeneration. The size of the hyaline droplets formed following HCBD-treatment were generally smaller than those observed after treatment with the well-characterized $\alpha_{2\mu}$-globulin nephropathy-inducing agent *d*-limonene.

No significant increase in $\alpha_{2\mu}$-globulin was observed in kidney cytosol prepared from Fischer 344/N rats treated with a single oral dose of 200 mg HCBD/kg when assayed by Western blot and capillary electrophoresis (Pähler et al., 1999).

7.3.5 Structure-Activity Relationship

HCBD appears to share a common mechanism of toxicity with several structurally-related haloalkenes, including perfluoropropene, trichloroethene, tetrachloroethene, and trichlorotrifluoropropene. All of these chemicals are selectively toxic to the proximal tubule. The common basis for toxicity is bioactivation of these compounds by a multistep pathway which is initiated by conjugation with glutathione, resulting in the formation of a glutathione *S*-conjugate. Metabolism to the corresponding cysteine *S*-conjugates, and subsequent degradation by renal cysteine conjugate β-lyase, yields reactive electrophiles that are believed to be ultimately responsible for renal toxicity. These electrophiles alkylate mitochondrial macromolecules, resulting in cellular energy deficit, loss of membrane potential, and disruption of calcium homeostasis.

When haloalkenes are considered as a group, the extent of conjugation is much higher with liver microsomes than with liver cytosol, in contrast to results observed with most other substrates. This effect is attributed to the preferential distribution of the highly lipophilic haloalkenes into lipid membranes, thus providing high substrate concentrations for membrane-bound glutathione *S*-transferase (Dekant et al., 1990). *In vitro* studies suggest that rates of haloalkene conjugation correlate well with the chemical reactivity of the individual compounds (Dekant et al., 1990). For example, substitution with chlorine results in stabilization of a π-bond. Chloroalkenes are thus reported to be more resistant to metabolism by glutathione conjugation than are fluoroalkenes.

Multiple investigations have compared the toxicity of structurally-related haloalkene conjugates. Anders et al. (1987) and Lock (1988) evaluated the toxicity of structural analogues of HCBD conjugates and noted remarkable similarity. Lock and Schnellmann (1990) investigated the effect of HCBD and other haloalkene cysteine conjugates on renal glutathione reductase and lipoyl dehydrogenase activity, and concluded that inhibition of these enzymes by the reactive thiols formed by β-lyase cleavage of haloalkene cysteine conjugates represented a general mechanism of toxicity.

Green and Odum (1985) investigated the nephrotoxicity and mutagenicity of the cysteine conjugates of halogenated alkenes in rat kidney slices. Compounds investigated included the chloroalkenes HCBD, trichloroethylene and perchloroethylene, and the fluoroalkenes hexafluoropropene (HFP) and tetrafluoroethylene (TFE). All of these conjugates had a marked effect on the uptake of both the organic anion *p*-aminohippuric acid (PAH) and the cation tetraethylammonium bromide (TEA) into rat kidney slices. This observation was considered to be consistent with the known nephrotoxicity of HCBD, TFE and HFP *in vivo*. Each of the conjugates was metabolized by rat kidney slices and by semi-purified rat kidney β-lyase to pyruvate, ammonia, and an unidentified reactive metabolite. Although all of the conjugates were

activated by β-lyase and had a similar effect on ion transport, their mutagenicity differed. The conjugates of HCBD, trichloroethylene and perchloroethylene were mutagenic in the Ames bacterial mutation assay when activated by rat kidney S9 fraction. In contrast, the conjugates of TFE and HFP were not mutagenic either in the presence or absence of rat kidney S9 fraction.

Birner et al. (1997) compared the nephrotoxicity of cysteine *S*-conjugates derived from trichloroethene, tetrachloroethene, and HCBD. Male and female rats received identical intravenous doses of *S*-(1,2-dichlorovinyl)-L-cysteine (1,2-DCVC), *S*-(2,2-dichlorovinyl)-L-cysteine (2,2-DCVC), *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), or *S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine (PCBC). Assessment of the relative nephrotoxic potency of the conjugates by histopathological examination and excretion of γ-glutamyltranspepidases in urine indicated a decrease in the order TCVC > 1,2-DCVC > PCBC ≥ 2,2-DCVC.

7.4 Hazard Characterization

7.4.1 Synthesis and Evaluation of Major Noncancer Effects

There are no reliable reports of human health effects following HCBD exposure by any route. Oral exposure studies of HCBD toxicity in animals are summarized in Table 7-4. A distinctive feature of HCBD toxicity in animals is its selective effect on the kidney, regardless of the route of administration. Toxicity within the kidney is also selective, with damage restricted to the proximal tubule. In rats, damage is further localized to the pars recta region of the proximal tubule.

Subchronic and chronic studies in rodents present a clear picture of dose-related renal damage. Progressive events over time include changes in kidney weight, increased excretion of coproporphyrin, renal tubular degeneration, necrosis and regeneration, hyperplasia, focal adenomatous proliferation, and tumor formation. Tumor formation occurs exclusively in the kidney, and only at doses that cause extensive cytotoxicity.

Evidence from metabolic enzyme inhibitor studies, cannulation experiments, and analysis of urinary metabolites indicates that the nephrotoxicity of HCBD is dependent on a multistep bioactivation mechanism involving both liver and kidney enzymes. The initial step in HCBD metabolism is the glutathione-*S*-transferase mediated biosynthesis of a glutathione conjugate (PCBG) in the liver. After elimination into the bile, PCBG undergoes subsequent metabolism to a cysteine conjugate (PCBC) in the bile, gut or kidneys. PCBC may be acetylated by renal *N*-acetyltransferases to form a *N*-acetyl cysteine conjugates (*N*-AcPCBC). Both PCBC and *N*-AcPCBC are concentrated in renal cells via an active transport system (Dekant, 1990). *N*-AcPCBC can be excreted in the urine or de-acetylated to regenerate PCBC. PCBC is a substrate for β-lyase-dependent activation to a highly reactive thioketene in the kidney. Covalent binding of this reactive species to cellular macromolecules is believed to initiate the damage that ultimately results in renal cell toxicity.

Potential molecular targets for binding of the reactive thioketene include enzymes, membrane proteins, glutathione, phospholipids, and mitochondrial DNA. Localized damage to the proximal tubule is believed to reflect high β-lyase concentration in this region. Evidence from studies using the selective inhibitor probenecid suggests that accumulation of the cysteine and *N*-acetyl cysteine conjugates via anion transport systems localized in this segment of the proximal

tubule may account for this selective pattern of toxicity.

In vitro studies suggest that cortical mitochondria are the critical subcellular target for toxicity of the bioactivated sulfur conjugates of HCBD. Susceptibility of mitochondria to HCBD toxicity is linked to high concentrations of β-lyase associated with mitochondrial membranes. The reactive metabolite formed by β-lyase cleavage of sulfur conjugates is thought to interact with components of the inner mitochondrial membrane. Disruption of respiration and uncoupling of oxidative phosphorylation leads to a marked reduction of ATP levels in susceptible kidney cells, and ultimately necrosis.

The mechanism described above is believed to contribute to the renal damage observed in both male and female rats. However, additional mechanisms may contribute to nephrotoxicity of HCBD in male rats. Current evidence suggests that at least two discrete male-specific pathways may participate in the more pronounced necrotic changes observed in the renal tubules of male rats in some studies. Formation of hyaline droplets indicative of $\alpha_{2\mu}$-globulin accumulation has been observed in the kidney of HCBD-treated male rats. The significance of this finding for HCBD-induced nephrotoxicity remains to be determined. A second potential mechanism for male specific toxicity involves the cytochrome P450 3A-mediated formation of an *N*-acetylated cysteine conjugate sulfoxide.

Other noncancer effects associated with HCBD exposure in animals include developmental effects and neurotoxicity. Reproductive effects were observed only at maternal toxic dose. In one study, female Wistar rats were administered a diet containing 0, 15 or 150 mg/kg-day) HCBD for 3 weeks prior to mating, 3 weeks during mating and throughout gestation and lactation. Maternal toxicity was evident in treated groups. No conceptions occurred for the high dose group, the ovaries showed little follicular activity, and no uterine implantation sites were observed. At 15 mg/kg-day, pups exhibited lower birth weights and reduced growth compared to controls (Harleman and Seinen, 1979). In another study, pregnant rats administered 8.1 mg/kg-day of HCBD during gestation gave birth to pups with lower body weights and shorter crown-rump lengths (Badaeva, 1983).

Harleman and Seinen (1979) observed ataxia, incoordination, weakness of the hind legs, and unsteady gait in conjunction with demyelination and fragmentation of femoral nerve fibers in female rats consuming dietary dose of 150 mg/kg-day HCBD for 10 to 18 weeks. No neurotoxic effects were reported for rats consuming 15 mg/kg-day. Daily oral administration of 8.1 mg/kg-day HCBD to pregnant rats throughout gestation resulted in histopathological changes in nerve cells and myelin fibers of the spinal cord in treated dams and their offspring (Badaeva et al., 1985).

The mode of action for these effects have not been studied. Toxicokinetic studies in animals following oral administration demonstrated that HCBD and its metabolites distributed to the brain and adipose tissues in addition to the kidney and the liver (Reichert, 1983; Reichert et al., 1985; Dekant et al., 1988a). Thus, reported toxicity at targets other than the kidney is probably related to the distribution of HCBD and its reactive metabolites to these targets and subsequent covalent binding of the reactive metabolites to cellular macromolecules.

An important issue in the evaluation of the hazard posed by HCBD concerns the applicability of mechanistic data obtained in rodent studies to humans. Limited data from *in vitro*

studies with human renal cytosol and cultured human proximal tubule cells suggest that humans have the ability to form HCBD glutathione conjugates and to metabolize HCBD cysteine conjugates to a toxic metabolite. However, the rate of metabolism, particularly for the reaction catalyzed by β-lyase, appears to be much lower than that observed in rats.

7.4.2 Synthesis and Evaluation of Carcinogenic Effects

No studies of HCBD carcinogenicity in humans have been reported. In animals, one lifetime exposure carcinogenicity study has been performed. Kociba et al. (1977) observed increased incidence of renal tumor formation in male and female rats following lifetime exposure to HCBD in the diet. Neoplastic changes occurred only at the highest dose, which exceeded the maximum tolerated dose (MTD). There was increased mortality, significant weight loss (greater than 10%), and severe renal toxicity were also observed. This pattern suggests that tumor formation may be secondary to HCBD-induced cytotoxicity. This conclusion is supported by the study of Nakagawa et al. (1998), who found increased cell proliferation and increased DNA synthesis in the outer stripe and cortex of kidneys from HCBD-treated rats.

However, these data must be considered as too limited to support a conclusion of high confidence. The widely-spaced doses (a 10-fold spacing between the highest and next lower dose) in the Kociba et al. (1977) study, for example, did not provide the opportunity to confirm that pronounced cytotoxicity is a prerequisite for tumorigenesis. Additional limitations in the database include the absence of cell proliferation studies and limited *in vivo* data for mutagenesis.

Results from mutagenicity studies with HCBD are mixed. In the presence of appropriate metabolic activation conditions, HCBD and its metabolites are mutagenic in some, but not all, studies. Thus, a genotoxic mode of action must be considered. The observation that HCBD metabolites can bind to DNA *in vivo* in mice (Schrenk and Dekant, 1989) strengthens this conclusion.

Table 7-4. Summary of Principal HCBD Toxicity Studies

Species/Strain	Number/ Sex/Dose	Route	Frequency/ Duration	Doses (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect(s)	Reference
ACUTE EXPOSURE								
Rat	6 M	G	Single Dose 24 hours	200	--	200	Increased plasma urea; increased proteins and metabolites in urine	Nash et al. (1984)
Rat / Wistar	5 M	G	Single Dose 24 hours	0, 10, 100, 200	10	100	Limited focal necrosis of the kidneys and other signs of renal damage in urine and blood	Jonker et al. (1993a)
Rat / Sprague-Dawley	4-5 M	G	Single Dose 24 hours	0, 100, 200	--	100	Histological kidney lesions and impaired kidney function	Payan et al. (1993)
SHORT-TERM EXPOSURE								
Rat / Sprague-Dawley	4 F	F	Daily 30 days	0, 1, 3, 10, 30, 65, 100	3	10 (reduced body wt. gain; increased hemoglobin concentration)	Increased relative kidney weight; renal tubular degeneration, necrosis and regeneration; increased hemoglobin concentration reduced body weight gain	Kociba et al. (1971)
Rat / Wistar-derived	6 M 6 F	F	Daily 14 days	0, 4.6, 14, 35.3	--	4.6	Degeneration of renal tubular epithelial cells, localized to pars recta	Harleman and Seinen (1979)
Rat / Sprague-Dawley	5 M	G	Daily 3 weeks	0, 0.2, 20	0.2	20	Histopathological indications of renal cortical damage; decreased body weight gain; increased relative kidney weight	Stott et al. (1981)
Mouse / $B6C3F_1$	5 M 5 F	F	Daily 15 days	M: 0, 3, 12, 40 F: 0, 5, 16, 49	-	3 5	Renal necrosis and cellular regeneration	NTP (1991) Yang et al. (1989)

Table 7-4 (continued)

Species/Strain	Number/ Sex/Dose	Route	Frequency/ Duration	Doses (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect(s)	Reference
Rat / Wistar	5 M 5 F	F	Daily 4 weeks	0, 2.25, 8, 28	2.25	8	Renal tubular cytomegaly; decreased plasma creatine; decreased body weight, decreased liver and adrenal weight	Jonker et al. (1993b)
Rat / Wistar	12 M	F	Daily 3 weeks	0, 7.2, 36, 180	7.2	36 (body weight) 180 (kidney lesions)	Lower mean body weight (15% decrease); extensive regeneration at 180 but not 36 mg/kg-day	Nakagawa et al. (1998)
SUBCHRONIC EXPOSURE								
Rat / Wistar-derived	10 M 10 F	G	Daily 13 weeks	0, 0.4, 1.0, 2.5, 6.3, 15.6	2.5 (M) 1.0 (F)	6.3 (M) 2.5 (F)	Proximal tubular degeneration	Harleman and Seinen (1979)
Mouse / $B6C3F_1$	10 M 10 F	F	Daily 13 weeks	M: 0, 0.1, 0.4, 1.5, 4.9, 16.8 F: 0, 0.2, 0.5, 1.8, 4.5, 19.2	1.5 (M) 0.2? (F)	4.9 (M) 0.5 (F)	Renal tubular regeneration	NTP (1991)
Rat / Sprague-Dawley	10-12 M, 20-24 F (HCBD) 17M, 34 F (control)	F	Daily 148 days	0.2, 2.0, 20	0.2	2	Renal tubular hyperplasia	Schwetz et al. (1977)
Rat / Wistar	21 M	F	Daily 30 weeks	90	--	90	Decreased final weight and increased relative kidney weight	Nakagawa et al. (1998)

Table 7-4 (continued)

Species/Strain	Number/ Sex/Dose	Route	Frequency/ Duration	Doses (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect(s)	Reference
CHRONIC EXPOSURE								
Rat / Sprague-Dawley	39M, 40F	F	Daily 22 months (M) 24 months (F)	0, 0.2, 2, 20	0.2	2	Increased urinary coproporphyrin excretion (females); increased kidney weight; renal tubular epithelial hyperplasia	Kociba et al. (1977)
DEVELOPMENTAL AND REPRODUCTIVE STUDIES								
Rat / Sprague-Dawley	10-12 M, 20-24 F (HCBD) 17M, 34 F (control)	F	Daily 148 days (90 days prior to mating through postnatal day 21)	0, 0.2, 2, 20	2	20	Slightly decreased neonatal weight	Schwetz et al. (1977)
Rat	6 F	F	Daily 3 weeks prior to mating; 3 weeks during mating; throughout gestation and lactation	0, 15, 150	--	15	Maternal toxicity (renal, neurological). Reduced pup weights on days 0, 10, and 20	Harleman and Seinen (1979)
Rat	F	NS	NS	8.1	--	8.1	Reduced body weight; shorter crown-rump length; ultrastructural chances in neurocytes; increased levels of free radicals in brain and spinal cord	Badaeva (1983)

Abbreviations: Sex: M = male, F = female Route: G = gavage, F = feed study NS = Not specified

7.4.3 Mode of Action and Implications in Cancer Assessment

Both sustained cytotoxic damage and irreversible DNA binding have been proposed as events in HCBD carcinogenesis (Stott et al., 1981). An oral carcinogenic study in rats showed kidney tumors at a very high dose that exceeded the MTD, suggesting that HCBD-induced cytotoxicity may lead to tumor formation (see Section 7.4.2). Studies in rats and mice indicate that kidney is the target organ. Progressive toxicological changes are observed in kidney over time: decreased and increased kidney weight, increased excretion of coporphyrin (kidney dysfunction), renal tubular degeneration, necrosis and regeneration, hyperplasia, focal adenomatous proliferation, and finally tumor formation.

On the other hand, in the presence of metabolic activation, HCBD and its reactive metabolites are mutagenic in some (Simmon, 1977; Reichert et al., 1984; Reichert and Schutz, 1986; Wild et al., 1986), but not all, studies (See Section 7.3.1). Thus, a mutagenic mode of action cannot be ruled out (Dekant et al., 1990; Lock, 1994).

The hypothesis that both cytotoxicity and mutagenic mode of action may be operating is consistent with the findings that the adverse effects of HCBD are dependent on a multistep pathway of bioactivation. The ultimate step in this pathway is a β-lyase-mediated degradation of a HCBD metabolite that generates a highly reactive thioketene in proximal tubule cells. Covalent binding of this thioketene to DNA, proteins and other macromolecules is considered to be the mechanism responsible for the observed cytotoxic and mutagenic effects of HCBD and its metabolites. Restriction of these effects to the proximal tubule most likely reflects both uptake processes that concentrate the cysteine conjugate substrate in epithelial cells, and localization of γ-glutamyltranspeptidase and β-lyase activity to this region of the kidney.

In vitro studies (Schnellman et al., 1987; Groves et al., 1991; Jones et al., 1986; Wallin et al., 1987) indicate that mitochondria in renal tubular epithelial cells are the major target for HCBD metabolite-induced toxicity. Interaction of highly reactive metabolites with components of the mitochondrial inner membrane, such as enzymes related to cell function, may result in mitochondrial dysfunction. Other studies indicate that the reactive species generated by β-lyase-mediated degradation of HCBD metabolites interact directly with mitochondrial DNA (mtDNA) from mouse kidney (Schrenk and Dekant, 1989). Renal mtDNA may be the preferential target due to the high concentration of β-lyase in the mitochondrial membrane, the lack of protective histones associated with mitochondrial DNA (Borst & Grivell, 1978), and an inadequate repair function (Mansouri et al., 1997). Mutations in the mtDNA can lead to a respiratory chain deficiency and cell dysfunction when the percentage of the mutants reach a certain level (Schapira, 1999).

Three important aspects of mitochondrial oxidative phosphorylation involved in mitochondrial dysfunction are: generation of cellular energy in the form of ATP; generation of reactive oxygen species (ROS); and regulation of apoptosis or programmed cell death (Wallace, 1999). The process of oxidative phosphorylation produces significant amounts of ROS which are toxic byproducts of respiration. Chronic exposure to ROS can result in oxidative damage to mitochondrial and cellular proteins, and mutations in the mtDNA. Because mtDNA codes for important proteins involved in the oxidative phosphorylation, 22 transfer RNAs (tRNA) and 2 ribosomal RNAs (rRNA), functional mtDNA is critical to the normal function of a cell.

Mutations in mtDNA may lead to overexposure to ROS and decreased energy production. Apoptosis is initiated when the mitochondrial permeability transition pore (mtPTP) in the inner membrane opens and cell death-promoting factors such as the caspases are released (Wallace, 1999). Opening of the mtPTP and the accompanying cell death can be initiated by the mitochondrion's excessive uptake of Ca^{2+}, increased exposure to ROS, or decline in energetic capacity. Therefore, a marked reduction in mitochondrial energy production and a chronic increase in oxidative stress could activate the mtPTP and initiate apoptosis.

Numerous mtDNA mutations have been associated with human mitochondrial disease. Mitochondrial disease is a disruption of the proper function of the mitochondria, resulting in a variety of clinical manifestation. This disruption can include an inhibition of the electron transport chain, a disruption of oxidative phosphorylation and an increase in the production of reactive oxygen species. MtDNA mutations could contribute to neoplastic transformation by changing cellular energy capacities, mitochondrial oxidative stress, and/or modulating apoptosis (Wallace, 1999). Thus, it may be postulated that mutations of renal mtDNA induced by HCBD may result in reduction in energy production, increase in oxidative stress, and initiation of apotosis, leading to tumor formation.

Mitochondrial dysfunction may also result from interaction of highly reactive HCBD metabolites with components of the mitochondrial inner membrane, such as enzymes related to cell function. Subsequent energy depletion may trigger the renal cytotoxicity that is the putative mechanism for HCBD-mediated carcinogenesis. Thus, HCBD induced cytotoxicity and tumorigensis may be ultimately the consequence of mitochondrial dysfunction resulting from exposure.

Recent evidence suggests that HCBD-induced $\alpha_{2\mu}$-globulin accumulation contributes to renal injury in male rats. However, renal tubular necrosis and renal tubular tumors were observed in both male and female rats following HCBD exposure (Kociba et al., 1977), and renal necrosis and regeneration were also observed in male and female mice (NTP, 1991). Therefore, $\alpha_{2\mu}$-globulin accumulation cannot be the sole mechanism for HCBD-induced carcinogenesis.

7.4.4 Weight of Evidence Evaluation for Carcinogenicity

No human carcinogenicity data are available for HCBD. A single lifetime study of HCBD carcinogenicity in rats (Kociba et al., 1977) is available for evaluation. This study revealed statistically significant increases in the incidence of tumors in male and female rats following oral HCBD exposure. Although human carcinogenicity data are unavailable, evidence exists that the metabolic enzymes responsible for conversion of HCBD to the reactive and toxic thioketene occur in humans, albeit at levels lower than that in the rat (see Section 6.3). In accordance with EPA's 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), HCBD is best classified as Group C, possible human carcinogens, based on limited evidence of carcinogenicity in one animal study, and no data in humans. Based on the proposed guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), HCBD is classified as *likely to be carcinogenic to humans*. This descriptor is considered appropriate when there are no or inadequate data in humans, but the combined experimental evidence demonstrates the production or anticipated production of tumors in animals by modes of action that are relevant or assumed to be relevant to humans.

7.4.5 Sensitive Populations

Sensitive populations are those which experience more adverse effects at comparable levels of exposure, or which experience adverse effects at lower exposure levels, than the general population. The enhanced response of these sensitive subpopulations may result from intrinsic or extrinsic factors. Factors that may be important include, but are not limited to: impaired function of detoxification, excretory, or compensatory processes that protect against or reduce toxicity; differences in physiological protective mechanisms; genetic differences in metabolism; developmental stage; health status; gender; or age of the individual.

Human populations that exhibit greater sensitivity to HCBD have not been identified. However, it has been generally observed that existing nephropathy or age-related kidney degeneration can increase the risk of renal injury or exacerbate nephrotoxicity in humans (WHO, 1991). Evidence that existing nephropathy increases sensitivity to HCBD toxicity has been obtained in a study conducted in male Wistar rats (Kirby and Bach, 1995). Nephrosis was induced by pretreatment with adriamycin (ADR), and rats were subsequently exposed to HCBD. Damage to the proximal tubule was more severe and renal cortical repair capacity was decreased in ADR-treated rats when compared to rats exposed to HCBD without prior ADR exposure. These results suggest that individuals with existing kidney damage or the elderly may be potentially sensitive populations for HCBD exposure.

Studies in animals showed that the young rats and mice experience acute effects at significantly lower doses than do adults (Hook et al., 1983; Lock et al., 1984), suggesting that infants may represent a potentially sensitive subpopulation for acute HCBD exposure, perhaps as a result of immature organ systems.

8.0 DOSE-RESPONSE ASSESSMENT

8.1 Dose-Response for Noncancer Effects

8.1.1 RfD Determination

The reference dose (RfD) for a chemical is "an estimate (with uncertainty spanning approximately an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects over a lifetime" (U.S. EPA, 1993). Data on the non-cancer effects of HCBD from chronic and subchronic studies were used to estimate a RfD value using the NOAEL/LOAEL approach.

Choice of Principal Study and Critical Effect

There are no reliable dose-response data for humans exposed to HCBD. The Kociba et al. (1977) study on rats and the NTP (1991) study on mice are chosen as co-principal studies. The RfD for HCBD is derived from a NOAEL of 0.2 mg/kg-day for renal tubular epithelial cell hyperplasia/regeneration from these two studies.

In the Kociba et al. (1977) lifetime oral exposure study of rats to HCBD, a NOAEL of 0.2 mg/kg-day and a LOAEL of 2 mg/kg-day were identified, based on an increase in renal tubular epithelial cell hyperplasia/regeneration and altered renal function (increased urinary coproporphyrin excretion). In the 13-week feeding study by NTP (1991), the study authors identified a NOAEL of 1.5 mg/kg-day for male mice, and did not identify a NOAEL for female mice because renal tubular regeneration occurred in 1 of 10 females in the lowest dose group (0.2 mg/kg-day). However, others (U.S. EPA, 1998a; WHO, 1994) have concluded that the effect observed at 0.2 mg/kg-day is not statistically significant, and therefore considered this dose to be the NOAEL. The lowest dose of 0.2 mg/kg-day in this study may be a minimal LOAEL for renal injury.

Application of Uncertainty Factors

A composite uncertainty factor (UF) of 1,000 was used in the derivation of the RfD. The composite UF included a factor of 10 to account for extrapolation from animals to humans; a factor of 10 for protection of sensitive subpopulations; a factor of 3 for the use of a NOAEL that may be a minimal LOAEL; and a factor of 3 for database deficiencies (lack of a 2-generation reproductive study).

Calculation of RfD

Using the NOAEL of 0.2 mg/kg-day from the Kociba et al. (1977) and NTP (1991) studies, the RfD is derived as follows:

$$\text{RfD} = \frac{(0.2 \text{ mg/kg-day})}{1000} = 2 \times 10^{-4} \text{ mg/kg-day}$$

where:

0.2 mg/kg-day = NOAEL, based on the absence of histopathological effects in kidneys of rats and mice exposed to HCBD in the diet for up to 24 months (Kociba et al., 1977; NTP, 1991).

1,000 = uncertainty factor. This is based on a factor of 10 to account for extrapolation from animals to humans; a factor of 10 for protection of potentially sensitive human subpopulations; a factor of 3 for database deficiencies (lack of a two-generation reproductive study); and a factor of 3 for the use of a NOAEL that may be a minimal LOAEL.

8.1.2 RfC Determination

RfC for HCBD is not derived. No subchronic or chronic inhalation exposure studies are available for the determination of RfC.

8.2 Dose-Response for Cancer Effects

8.2.1 Choice of Study

As noted previously, only one lifetime oral carcinogenicity study of HCBD was located (Kociba et al., 1977). In this study, Sprague-Dawley rats (40 animals/sex/dose group and 90 animals/sex in the control group) were dosed with 0, 0.2, 2 or 20 mg/kg-day HCBD via the diet for 22 months (males) or 24 months (females).

Neoplastic changes were found only at the highest dose, which exceeded the maximum tolerated dose. There was a significant increase in mortality in males, a greater than 10% decrease in body weights for both sexes, and other severe renal toxicity effects were observed. The incidence of renal tubular neoplasms was increased only in the high-dose group of both males and females, as shown in Table 8-1.

Increased renal tubular hyperplasia and renal tubular adenomas and adenocarcinomas (some of which metastasized to the lungs), were found in rats exposed to 20 mg/kg-day of HCBD for up to 2 years. Lesser degrees of toxicity, including an increase in urinary coproporphyrin excretion and an increase in renal tubular hyperplasia, were found in rats ingesting 2 mg/kg-day for up to 2 years. A composite dose-related change in the rodent kidney leading to tumor formation is shown in Table 8-2. This pattern is consistent with the hypothesis that renal tumor formation may require, and be secondary to, renal cytotoxicity induced by exposure to HCBD.

Table 8-1. Incidence of Renal Tubular Neoplasms in Rats Treated with HCBD for 2 Years.

Test Organism	Administered Dose (mg/kg-day)	Human Equivalent Dose[a] (mg/kg-day)	Renal Tubular Neoplasm Incidence
Male rats	0	0	1/90 (1.1%)
	0.2	0.062	0/40 (0%)
	2.0	0.62	0/40 (0%)
	20	5.8	9/39 (23%)
Female rats	0	0	0/90 (0%)
	0.2	0.054	0/40 (0%)
	2.0	0.55	0/40 (0%)
	20	5.3	6/40 (15%)

[a] Human Equivalent Dose = Animal dose ·(Animal body weight/Human body weight)$^{1/4}$

source: Kociba et al. (1977)

Table 8-2. Dose-Related Changes in the Rodent Kidney after Oral Exposure to HCBD, Chronic Study - Rat (Kociba et al., 1977)

Dose (mg/kg-day)	0.2	2	20
coproporphyrin increase	–	+ (♀ only)	+
terminal kidney weight increase (abs. & rel.)	–	–	+
hyperplasia - multi focal	–	?	+
hyperplasia-adenomatous	–	+ (♀ only)	+
tumors	–	–	+

8.2.2 Dose-Response Characterization

The Kociba et al. (1977) study was used to quantify the cancer risk from ingested HCBD, as discussed below.

1986 Guidance: Linearized Multistage Model

The current IRIS file contains a carcinogenicity assessment of HCBD based on EPA's 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986). The dose-response data for

male rats were fitted to the linearized multistage model. To estimate human equivalent dose from an animal study, the doses administered to animals were adjusted by a scaling factor of (body weight)$^{2/3}$. The resulting cancer slope factor is 7.8×10^{-2} (mg/kg-day)$^{-1}$. This slope factor corresponds to a drinking water unit risk of 2.2×10^{-6} per µg/L (U.S. EPA, 1997), and the drinking water concentration that corresponds to a lifetime excess cancer risk of 1×10^{-6} is 0.5 µg/L.

1996 Proposed Guidance

The draft Ambient Water Quality Criteria for hexachlorobutadiene (U.S. EPA, 1998a) utilized the methodology discussed in EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a) to evaluate the carcinogenicity of HCBD. Under the proposed guidelines, two approaches can be used for dose-response extrapolation for quantification of cancer risk, depending on what is known about the mode of action for carcinogenicity and the shape of the dose-response curve. A linear approach is used for a chemical when available evidence indicates the chemical has direct DNA mutagenic activity or is DNA-reactive, or when the evidence supports another mode of action that is anticipated to be linear. An inference of linearity may also be supported if existing human exposure is high and near doses associated with key events in the carcinogenesis process. The linear approach is used as a matter of policy if there is an absence of sufficient mode-of-action information on tumorogenesis. The nonlinear approach may be used when the tumor mode-of-action supports nonlinearity (e.g., some cytotoxic and hormonal agents) and the chemical does not demonstrate mutagenic effects consistent with linearity. The nonlinear approach is also selected when a mode of action supporting nonlinearity has been demonstrated, and the chemical has some indication of mutagenic activity, but is judged not to play a significant role in tumor causation. As a matter of science policy, nonlinear probability functions are not fitted to tumor response data to extrapolate quantitative low-dose risk estimates because different models can lead to a wide range of results, and there is currently no basis to choose among them. In these cases, a margin of exposure analysis is used to evaluate concern for levels of exposure.

Because both linear (mutagenic) and nonlinear (toxicity associated) mode of action for carcinogenicity of HCBD may be operating *in vivo*, both of these approaches have been evaluated in the draft Ambient Water Quality Criteria for hexachlorobutadiene (U.S. EPA, 1998a) for characterizing the carcinogenic hazard of HCBD, as discussed below.

Linear Approach

Because there are limited data which suggest that HCBD might be genotoxic and mutagenic (see Section 7.3.1), this approach is considered in the dose-response extrapolation for HCBD.

Under the proposed guidelines, the cancer risk from a chemical is assessed in two steps. The first step involves curve-fitting of the cancer dose-response data within the observable range to derive a point-of-departure (Pdp) (U.S. EPA, 1999). The point-of-departure employs the human equivalent dose. The dose that causes a 10% increase in extra risk is referred to as the ED_{10}. The point-of-departure is defined as the 95% lower confidence limit on the ED_{10}, and is

referred to as the LED_{10}. The second step in the process is linear extrapolation of the dose-response curve from the LED_{10} to the origin.

The LED_{10} for HCBD was calculated by fitting the quantal polynomial model[2] to the tumor dose response data reported by Kociba et al. (1977). Since the mortality rate was significantly increased in the male rats exposed at the high dose (which is the only dose with an increased tumor incidence in animals), the tumor data from the female rats were used. In accordance with current guidance (U.S. EPA, 1992d, 1999), the human equivalent dose was calculated by assuming dose equivalency based on body weight raised to the 3/4 power. The best fit to the data is shown in Figure 8-1. The ED_{10} was found to be 4.9 mg/kg-day, and the LED_{10} was 2.5 mg/kg-day. Linear extrapolation from the LED_{10} to the origin yields a slope of 4×10^{-2} $(mg/kg\text{-}day)^{-1}$.

Non-linear Margin of Exposure (MOE) Approach

The MOE approach is used when available data indicate that the dose-response curve for tumor induction is nonlinear, and that cancer may not be the result of a direct DNA-damage mechanism. As discussed previously, data from the study by Kociba et al. (1977) indicate that the dose response curve is strongly non-linear, and that renal tumors only occur at HCBD doses that cause frank renal toxicity and increased mortality. Therefore, tumor data from this study is

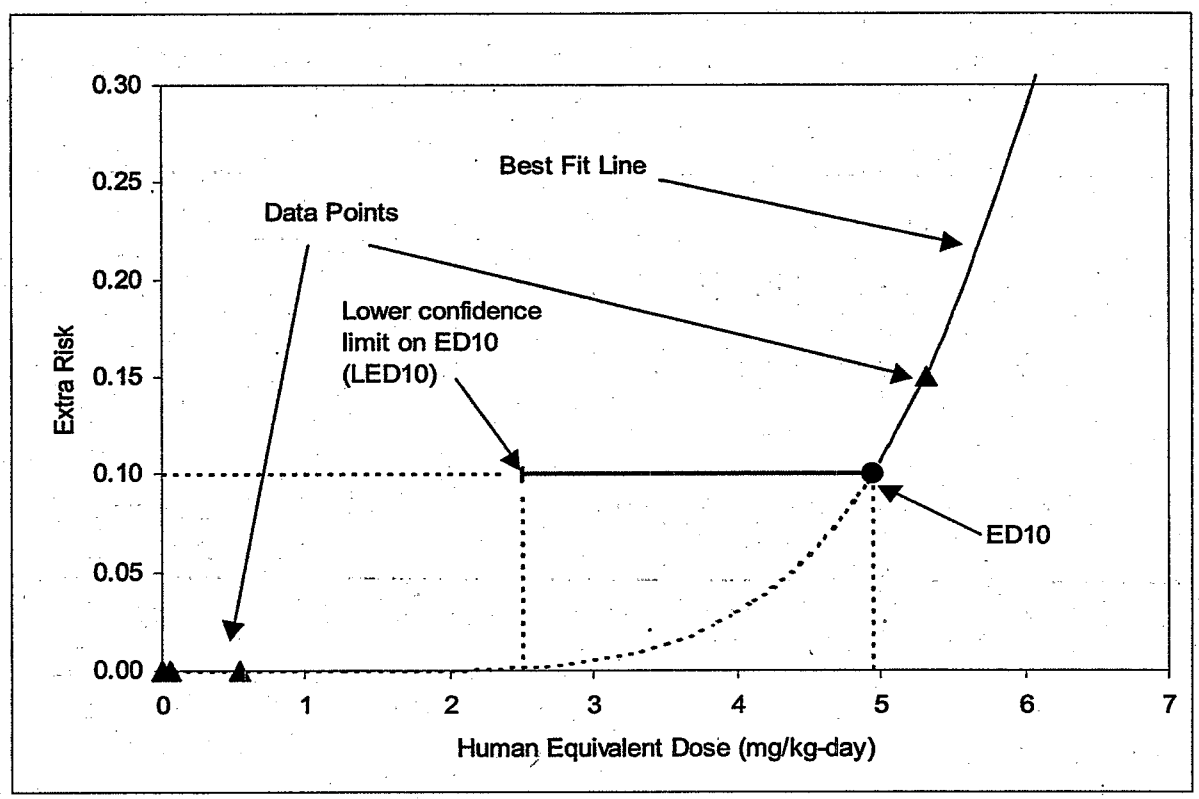


Figure 8-1. Renal Tumor Dose Response Curves

[2] This modeling was carried out using the Global 86 multistage model software.

not considered suitable for dose-response extrapolation, and the MOE approach should be evaluated.

For HCBD, mode-of-action considerations suggest carcinogenicity is secondary to renal toxicity, which has a threshold (see Section 7.4.3, Mode of Action), and the MOE analysis becomes an RfD derivation in accordance with the proposed cancer guidelines (U.S. EPA, 1996). The point-of-departure (Pdp) selected for use was the NOAEL for renal tubular histological lesions in female rats. This is because a Pdp based on a sensitive key precursor of the neoplastic response is more protective and more reliable than a Pdp based on the neoplastic response itself (U.S. EPA, 1996). The NOAEL for renal tubular damage was selected rather than an LED_{10} for renal damage because quantitative data on incidence and severity of renal non-cancer histopathological changes were not reported.

The NOAEL for renal damage reported by Kociba et al. was 0.2 mg/kg-day in female rats. The human equivalent dose for the NOAEL was calculated to be 0.054 mg/kg-day, using the new scaling factor of body weight raised to the 3/4 power. Based on this, the adjusted point-of-departure is 0.054 mg/kg-day.

An advisory MOE (which is the composite uncertainty factor for HCBD) is calculated from appropriate uncertainty factors. The advisory MOE includes: a factor of 10 for protection of sensitive human subpopulations; a factor of 3 for extrapolation from animals to humans (since human equivalent dose is used as the Pdp); a factor of 3 for database deficiency (lack of a 2-generation reproductive study); and a factor of 3 for use of a NOAEL that may be a minimal effect LOAEL. The advisory MOE is calculated as follows:

Advisory MOE

$10 \cdot 3 \cdot 3 \cdot 3 = 300$ (rounded)

Applying this advisory MOE to the Pdp (NOAEL) of 0.054 mg/kg-day yields a dose of 2×10^{-4} mg/kg-day, which is equal to the oral RfD. Based on this, the RfD is considered to be protective not only for non-cancer but also for cancer effects from ingestion of HCBD.

8.2.3 Extrapolation Model and Rationale

In Section 8.2.2., the carcinogenicity of HCBD was evaluated using both linear and non-linear approaches. Because of the lack of data, it is not certain which method of cancer risk evaluation is most appropriate for HCBD. On the one hand, some tests indicate that one or more of the metabolites of HCBD are mutagenic, suggesting direct damage to renal mitochondrial DNA by its reactive metabolites. On the other hand, direct observations on cancer dose-response clearly support a nonlinear curve, with no observable increase in tumors at doses that do not induce significant renal necrosis and regeneration. This is supported by the observation that tumors occur only in the kidney and not in other tissues that are not significantly injured by HCBD. Therefore, the tumor data from the Kociba et al. (1977) study are not considered suitable for linear dose-response extrapolation.

Although HCBD metabolites have some indication of mutagenic activity, they are judged not to play a significant role in tumor causation due to their weak activity. Moreover, mutations of mitochondrial DNA may result in mitochondrial dysfunction (See Sectioin 7.4.3), which would support cytotoxicity and nonlinear approach. There should also be decreased concern over genotoxicity for humans because the activity of HCBD metabolizing enzymes, particularly renal β-lyase, may be many fold lower in humans than the corresponding enzymes in rats (see section 6.3). In addition, human exposure levels (see Section 9.3) are about 4 orders of magnitude lower than the human equivalent dose corresponding to the dose at which tumor incidence was reported in Kociba et al. (1977) study. In consideration of the overall evidence, the MOE approach may be more appropriate for HCBD. The draft Ambient Water Quality Criteria Document for Hexachlorobutadiene (U.S. EPA, 1998a) has also recommended using the nonlinear approach for carcinogenicity assessment of HCBD.

8.2.4 Cancer Potency and Unit Risk

Table 8-3 summarizes the cancer values derived for HCBD. Analysis of tumor dose-response information from the Kociba et al. (1977) study using the linear extrapolation approach from the proposed carcinogen risk assessment guidelines (U.S. EPA, 1999) resulted in a slope factor of 4×10^{-2} (mg/kg-day)$^{-1}$. This value is about half of the slope factor of 7.8×10^{-2} (mg/kg-day)$^{-1}$ derived previously using the linearized multistage (LMS) model (U.S. EPA, 1997), but most of the apparent difference may be attributable to the different methods used to calculate human equivalent doses from the animal doses (the scaling factor used in the LMS approach assumed body weight to the 2/3 power, while a factor of body weight raised to the 3/4 was used for the Pdp method). Based on the slope factor of 4×10^{-2} (mg/kg-day)$^{-1}$ derived using the LED_{10} approach with linear extrapolation, the unit risk is 1.1×10^{-6} per (μg/L) and the drinking water concentration that corresponds to a lifetime excess risk of 1×10^{-6} is 0.9 μg/L.

Table 8-3. Summary of Cancer Risk Values for HCBD

Approach	Parameter	Value
LMS[a] (U.S. EPA, 1991)	Slope	7.8×10^{-2} (mg/kg-day)$^{-1}$
	Unit Risk	2.2×10^{-6} per (μg/L)
	Risk of 1×10^{-6}	0.5 μg/L
LED_{10}, linear extrapolation	LED_{10} (tumors)[b]	2.5 mg/kg-day
	Slope	4×10^{-2} (mg/kg-day)$^{-1}$
	Unit Risk	1.1×10^{-6} per (μg/L)
	Risk of 1×10^{-6}	0.9 μg/L
Nonlinear	Pdp (NOAEL)	0.054 mg/kg-day
	Advisory MOE	300

[a] Animal to human dose extrapolation based on body weight$^{2/3}$
[b] Animal to human dose extrapolation based on body weight$^{3/4}$

8.2.5 Discussion of Confidence

The available database associating HCBD and carcinogenicity is limited. There are no human data. The evidence is obtained only in one chronic dietary study in a single species (Sprague-Dawley rats) (Kociba et al., 1977), where rats developed severe renal toxicity preceding tumor formation. The tumors were seen only at a high dose which exceeded the maximum tolerated dose (MTD, i.e., greater than 10% body weight depression) in both sexes of rats and produced high mortality in the males. Similar renal toxicity observed in a 30-day study of HCBD in rats by the same laboratory and in another 90-day subchronic study in mice (NTP, 1991) strengthens the idea that the tumor formation is induced by cytotoxicity. Both the NTP (1991) and Kociba et al. (1977) studies tested a sufficient number of animals.

A limitation of the Kociba et al. (1977) study is the selection and spacing of doses. Although the study employed an adequate number of animals, the doses selected for testing were separated by a factor of 10 (0, 0.2, 2, and 20 mg/kg-day). Thus, there are no observations between the dose of 2 mg/kg-day (causing no tumors), and the dose of 20 mg/kg-day (causing a 15% tumor response in females and a 23% tumor response in males). More doses between 2 and 20 mg/kg-day would better delineate the shape of the dose-response curve.

A weight-of-evidence analysis of the available data as a whole indicates that the confidence in using either the linear or nonlinear approach is not high; this is particularly true for the linear method which is based on only one data point at a high-dose exceeding the MTD.

9.0 REGULATORY DETERMINATION AND CHARACTERIZATION OF RISK FROM DRINKING WATER

9.1 Regulatory Determination for Chemicals on the CCL

The Safe Drinking Water Act (SDWA), as amended in 1996, required the Environmental Protection Agency (EPA) to establish a list of contaminants to aid the Agency in regulatory priority setting for the drinking water program. EPA published a draft of the first Contaminant Candidate List (CCL) on October 6, 1997 (62 FR 52193, U.S. EPA, 1997). After review of and response to comments, the final CCL was published on March 2, 1998 (63FR 10273, U.S. EPA 1998). The CCL grouped contaminants into three major categories as follows:

Regulatory Determination Priorities - Chemicals or microbes with adequate data to support a regulatory determination,

Research Priorities - Chemicals or microbes requiring research for health effects, analytical methods, and/or treatment technologies,

Occurrence Priorities - Chemicals or microbes requiring additional data on occurrence in drinking water.

The March 2, 1998 CCL included one microbe and 19 chemicals in the regulatory determination priority category. More detailed assessments of the completeness of the health, treatment, occurrence and analytical method data led to a subsequent reduction of the regulatory determination priority chemicals to a list of 12 (one microbe and 11 chemicals) which was distributed to stakeholders in November 1999.

SDWA requires EPA to make regulatory determinations for no fewer than five contaminants in the regulatory determination priority category by August 2001. In cases where the Agency determines that a regulation is necessary, the regulation should be proposed by August 2003 and promulgated by February 2005. The Agency is given the freedom to also determine that there is no need for a regulation if a chemical on the CCL fails to meet one of three statutory criteria established by SDWA and described in Section 9.1.1.

9.1.1 Criteria for Regulatory Determination

These are the three criteria used to determine whether or not to regulate a chemical on the CCL:

The contaminant may have an adverse effect on the health of persons,

The contaminant is known to occur, or there is a substantial likelihood that the contaminant will occur, in public water systems with a frequency and at levels of public health concern,

In the sole judgment of the administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

The findings for all criteria are used in making a determination to regulate a contaminant. As required by SDWA, a decision to regulate commits the EPA to publication of a Maximum Contaminant Level Goal (MCLG) and promulgation of a National Primary Drinking Water Regulation (NPDWR) for that contaminant. The agency may determine that there is no need for a regulation when a contaminant fails to meet one of the criteria. A decision not to regulate a contaminant is considered a final Agency action and is subject to judicial review. The Agency can choose to publish a Health Advisory (a nonregulatory action) or other guidance for any contaminant on the CCL independent of the regulatory determination.

9.1.2 National Drinking Water Advisory Council Recommendations

In March 2000, the EPA convened a Working Group under the National Drinking Water Advisory Council (NDWAC) to help develop an approach for making regulatory determinations. The Working Group developed a protocol for analyzing and presenting the available scientific data, and recommended methods to identify and document the rationale supporting a regulatory determination decision. The NDWAC Working Group report was presented to and accepted by the entire NDWAC in July 2000.

Because of the intrinsic difference between microbial and chemical contaminants, the Working Group developed separate but similar protocols for microorganisms and chemicals. The approach for chemicals was based on an assessment of the impact of acute, chronic, and lifetime exposures, as well as a risk assessment that includes evaluation of occurrence, fate, and dose-response. The NDWAC protocol for chemicals is a semi-quantitative tool for addressing each of the three CCL criteria. The NDWAC requested that the Agency use good judgement in balancing the many factors that need to be considered in making a regulatory determination.

The EPA modified the semi-quantitative NDWAC suggestions for evaluating chemicals against the regulatory determination criteria and applied them in decision making. The quantitative and qualitative factors for hexachlorobutadiene (HCBD) that were considered for each of the three criteria are presented in the sections that follow.

9.2 Health Effects

The first criterion asks if the contaminant may have an adverse effect on the health of persons. Because all chemicals have adverse effects at some level of exposure, the challenge is to define the dose at which adverse health effects are likely to occur, and estimate a dose at which adverse health effects are either not likely to occur (threshold toxicant), or have a low probability for occurrence (non-threshold toxicant). The key elements that must be considered in evaluating the first criterion are the mode of action, the critical effect(s), the dose-response for critical effect(s), the RfD for threshold effects, and the slope factor for non-threshold effects.

A description of the health effects associated with exposure to HCBD is presented in Chapter 7 of this document and summarized below in Section 9.2.2. Chapter 8 and Section 9.2.3

present dose-response information, where applicable, for threshold and non-threshold health effects.

9.2.1 Health Criterion Conclusion

The available toxicological data indicate that HCBD has the potential to cause adverse health effects in animals, and probably in humans. The available human data involve inhalation exposure and are confounded by simultaneous exposures to other chemicals in an occupational setting; thus, attributing observed effects to specific levels of HCBD exposure is not possible. In rodents, there is clear evidence of renal damage resulting from acute, subchronic, and chronic HCBD oral exposures. A few animal studies have also reported liver effects and neurotoxicity. Review of animal dose-response data endpoints indicates that subchronic and chronic LOAEL values for HCBD toxicity are generally at 2 mg/kg-day and above. The RfD for HCBD is 2×10^{-4} mg/kg-day (U.S. EPA, 1998a). Limited evidence of carcinogenic potential in rodents suggests that HCBD may be carcinogenic secondary to renal tubular epithelial cell cytotoxicity. However, data in humans are lacking. Using the non-linear margin of exposure approach, the RfD is also the acceptable dose for protection of potential carcinogenic effect.

9.2.2 Hazard Characterization and Mode of Action Implications

Data for the human health effects of HCBD are limited to a few studies of occupational exposure to HCBD. A relationship could not be established from these studies between HCBD exposure and toxic or cytogenetic effects either because of concurrent exposure to other chemicals or because of equivocal results.

Studies in animals show the selective effect of HCBD on the kidney, specifically the proximal tubule. Renal toxicity in rodents has been shown with single acute exposures to 100–200 mg HCBD/kg, and with short-term exposures to 3 mg/kg-day and above. Subchronic and chronic studies in rodents show clear dose-related renal damage at 2 mg/kg-day and above. Progressive events over time include changes in kidney weight, increased urinary excretion of coproporphyrin, and increased renal tubular epithelial hyperplasia.

Othe noncancer effects associated with HCBD exposure in animals include developmental effects and neurotoxicity (Harleman and Seinen, 1979; Badeva, 1983; Badaeva et al., 1985). However, these effects were observed at higher doses than for renal toxicity. Pups with lower birth weights and reduced growth were reported at maternal dose of 8.1-15 mg/kg-day in rats (Badaeva, 1983; Harleman and Seinen, 1979).

Results from mutagenicity studies with HCBD are ambiguous. In the presence of appropriate metabolic activation conditions, HCBD and its metabolites are mutagenic in some (Vamvakas et al., 1988; Reichert et al., 1984), but not all studies. HCBD metabolites have been shown to bind to mitochondrial DNA *in vivo* in mice (Schrenk and Dekant, 1989), and induce DNA repair in cultured porcine kidney cells (Vamvakas et al., 1989), suggesting its genotoxic potential. No human studies of HCBD carcinogenicity have been reported and only one lifetime animal study has been performed (Kociba et al., 1977). In this study, neoplastic changes occurred only at the highest dose which exceeded the maximum tolerated dose (MTD), i.e. there was increased mortality, greater than 10% decrease in body weight and severe renal toxicity. Because

these significant adverse effects were observed at the high dose, tumor formation may be secondary to cytotoxicity.

The nephrotoxicity of HCBD is dependent on a multistep bioactivation mechanism involving both kidney and liver enzymes. The ultimate step in this pathway is a β-lyase mediated degradation of a HCBD metabolite that generates a highly reactive thioketene in proximal tubule cells. *In vitro* studies suggest that cortical mitochondria are the critical subcellular target for toxicity of the bioactivated sulfur conjugates of HCBD. Covalent binding of this reactive HCBD metabolite to cellular macromolecules (e.g. proteins, mitochondria DNA), and the resultant mitochondrial dysfunction is believed to contribute to the renal cytotoxicity and tumors observed in animals. Recent evidence suggests that HCBD-induced $\alpha_{2\mu}$-globulin accumulation contributes to renal injury in male rats. However, renal tubular necrosis and renal tubular tumors were observed in both male and female rats following HCBD exposure (Kociba et al., 1977), and renal necrosis and regeneration were also observed in male and female mice (NTP, 1991). Therefore, $\alpha_{2\mu}$-globulin accumulation cannot be the sole mechanism for HCBD-induced carcinogenesis.

One important issue in the evaluation of the hazard posed by HCBD is the applicability of rodent mechanistic data to humans. *In vitro* studies with human renal cytosol and cultured human proximal tubule cells suggest that humans have the potential to form the HCBD-glutathione conjugates and to metabolize HCBD cysteine conjugates to toxic metabolites. However, the rate of metabolism, particularly for the reaction catalyzed by β-lyase, appears to be much lower for humans than rodents (Lock, 1994; Lash et al., 1990).

It has been generally observed that existing nephropathy or age-related kidney degeneration can increase the risk of renal injury or exacerbate nephrotoxicity in humans. Therefore, sensitive populations for HCBD exposure may include people with pre-existing kidney or liver damage or the elderly. Although it is unlikely that human newborns would be acutely exposed to significant doses of HCBD, acute exposures for young rats and mice cause toxicity at lower doses than for adults (Hook et al., 1983; Lock et al., 1984).

9.2.3 Dose-Response Characterization and Implications in Risk Assessment

Dose-response information from several key studies of HCBD toxicity in animals is summarized in Table 9-1. These studies currently provide the most reliable information on threshold levels for HCBD toxicity in animals exposed via the oral route.

Noncancer effects

In short-term studies, a LOAEL of 10 mg/kg-day and a NOAEL of 3 mg/kg-day were identified for reduced body weight gain and food consumption in female Sprague-Dawley rats administered HCBD in their diets for 30 day. Renal tubular degeneration, necrosis and regeneration were observed at 30 mg/kg-day (Kociba et al., 1971; Schwetz et al., 1977). A LOAEL of 8 mg/kg-day and a NOAEL of 2.25 mg/kg-day were identified for decreased body weight gain and renal tubular effects in Wistar rats given HCBD in their diets for 4 weeks (Jonker et al., 1993b). A 3-week oral exposure with male Sprague-Dawley rats identified a LOAEL of 20 mg/kg-day and a NOAEL of 0.2 mg/kg-day for kidney damage and increased

Table 9-1. Dose-Response Information from Several Key Studies of HCBD Toxicity (Oral Exposure).

Study	Species	No./ Sex	Doses mg/kg-day	Duration	NOAEL mg/kg-day	LOAEL mg/kg-day	Effects
Short-term Studies							
Kociba et al. (1977)	Rat Sprague-Dawley	4 F	0 1 3 10 30 65 100	30 days	3	10	Reduced body weight gain, food consumption; increased hemoglobin concentration, relative kidney weight; renal tubular degeneration, necrosis, regeneration.
Jonker et al. (1993b)	Rat Wistar	5 M 5 F	0 2.25 8 28	4 weeks	2.25	8	Decreased liver weight, plasma creatinine, body weight, adrenal weight; renal tubular cytomegaly.
Harleman and Seinen (1979)	Rat Wistar	6 M 6 F	0 4.6 14.0 35.3	14 days	--	4.6	Decreased body weight gain and food conversion efficiency; renal tubular epithelial cell degeneration.
Stott et al. (1981)	Rat Sprague-Dawley	5 M	0 0.2 20	3 weeks	0.2	20	Decreased body weight gain; increased relative kidney weight; kidney damage.
NTP (1991)	Mouse $B6C3F_1$	5 M 5 F	0 M 0 F 3 M 5 F 12 M 16 F 40 M 49 F	2 weeks	--	3 M 5 F	Renal tubular necrosis.
Subchronic Studies							
NTP (1991)	Mouse $B6C3F_1$	10 M 10 F	0 M 0 F 0.1 M 0.2 F 0.4 M 0.5 F 1.5 M 1.8 F 4.9 M 4.5 F 16.8 M 19.2 F	13 weeks	1.5 M 0.2 F	4.9 M 0.5 F	Renal tubular cell regeneration (increased epithelial nuclei and basophilic staining)

Table 9-1 (continued)

Study	Species	No./ Sex	Doses mg/kg-day	Duration	NOAEL mg/kg-day	LOAEL mg/kg-day	Effects
Chronic Studies							
Kociba et al. (1977)	Rat Sprague-Dawley	39–40 M, F	0 0.2 2 20	22–24 months	0.2	2	Increased kidney weight; renal tubular epithelial hyperplasia and neoplasia.

M = male; F = female

relative kidney weight (Stott et al., 1981), and a 2-week feeding study in Wistar rats identified a LOAEL of 4.6 mg/kg-day (the lowest dose tested) for renal tubular epithelial cell degeneration (Harleman and Seinen, 1979). A 2-week oral exposure study in $B6C3F_1$ mice reported a LOAEL of 3–5 mg/kg-day (the lowest dose tested) for renal tubular necrosis (NTP, 1991). Thus, renal effects in rodents resulting from short-term exposure to HCBD appear to have LOAELs of around 5–20 mg/kg-day, depending on the species and strain used, the length of exposure, and the method of administration.

In a subchronic oral exposure study of HCBD in $B6C3F_1$ mice, a NOAEL of 1.5 mg/kg-day was identified for male mice based on renal tubular cell regeneration (NTP, 1991). Tubular regeneration occurred in 1 of 10 females in the lowest dose group (0.2 mg/kg-day). The study authors concluded that a NOAEL for female mice could not be identified from these data (NTP, 1991). However, EPA (U.S. EPA, 1998a) and others (WHO, 1994) have concluded that the effect observed at 0.2 mg/kg-day is not statistically significant, and therefore consider this dose to be the NOAEL for female mice. Because tubular regeneration occurred in 1 of 10 females at 0.2 mg/kg-day, this NOAEL may be close to a minimal LOAEL for renal injury.

Only one study of lifetime oral exposure to HCBD was located (Kociba et al., 1977). This study identified a NOAEL of 0.2 mg/kg-day and a LOAEL of 2 mg/kg-day in rats, based on an increase in renal tubular epithelial cell hyperplasia/regeneration and altered renal function (increased urinary coproporphyrin excretion). The value of this NOAEL from a chronic study is the same as the equivocal NOAEL of 0.2 mg/kg-day identified in the 13-week NTP study in female mice (NTP, 1991), indicating the female mice may be more sensitive than rats to HCBD.

The Reference Dose (RfD) for HCBD is 2×10^{-4} mg/kg-day (U.S. EPA, 1998a). The RfD is "an estimate (with uncertainty spanning approximately an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects over a lifetime" (U.S. EPA, 1993). The RfD is derived from a NOAEL of 0.2 mg/kg-day for renal tubular epithelial cell hyperplasia/regeneration from the Kociba et al.(1977) and NTP (1991) studies. A composite uncertainty factor of 1,000 was used in the derivation of the RfD to account for: extrapolation from animals to humans (factor of 10); protection of sensitive subpopulations (factor of 10); use of a NOAEL that may be closer to a LOAEL (factor of 3); and database deficiency (factor of 3) because of lack of a 2-generation reproductive study.

Cancer effects

The single lifetime exposure study in rats is also a source of data on tumor formation (Kociba et al., 1977). Only at the highest dose, 20 mg/kg-day, were tumors seen in both sexes. This dose exceeded the level at which significant noncancer effects were seen, such as mortality, renal toxicity, and body weight depression. In this study, the second highest dose was 2 mg/kg-day and there were no tumors in this exposed group. The shape of the dose-response curve cannot be determined from this data set.

Under EPA's 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), HCBD is classified as Group C, possible human carcinogen. Using the linearized multistage model, a slope factor of 7.8×10^{-2} per mg/kg-day was calculated at the 95th upper confidence level (U.S. EPA, 1991c). Under EPA's 1996 proposed Guidelines for Carcinogen Risk Assessment (USPEA, 1996), HCBD is classified as *likely to be carcinogenic to humans.* Both the linear and nonlinear dose-response extrapolation approaches were used to quantify cancer risk (U.S. EPA, 1998a) because both cytotoxicity and mutagenic mode of action may be involved. The linear approach yields a slope of 4×10^{-2} per mg/kg-day. Using the non-linear approach, the point-of-departure selected for use was the NOAEL of 0.2 mg/kg-day for renal tubular damage. The adjusted point of departure of 0.054 mg/kg-day is the human equivalent dose for the NOAEL of 0.2 mg/kg-day. Applying a margin of exposure (MOE) of 300 (which consists of uncertainty factors of 10 for protection of sensitive human subpopulations, 3 for extrapolation from animals to human, 3 for data base insufficiency, and 3 for use of a NOAEL that may be a minimal effect LOAEL) to the adjusted point-of-departure (0.054 mg/kg-day), the resulting dose is 2×10^{-4} mg/kg-day, which is the same as the RfD. EPA's draft Ambient Water Quality Criteria for hexachlorobutadiene (U.S. EPA, 1998a) recommended using the non-linear approach for dose-response extrapolation. As discussed previously, data from Kociba et al. (1977) indicated that the tumor dose response curve is strongly non-linear, and that renal tumors only occur at HCBD doses that cause frank toxicity. Therefore, the non-linear margin of exposure approach may be more appropriate for HCBD.

The conclusion from the dose response analysis is that HCBD is a weak carcinogen because it is carcinogenic only at cytotoxic dose.

9.3 Occurrence in Public Water Systems

The second criterion asks if the contaminant is known to occur or if there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern. In order to address this question, the following information was considered:

- Monitoring data from public water systems
- Ambient water concentrations and releases to the environment
- Environmental fate

Data on the occurrence of HCBD in public drinking water systems were the most important determinants in evaluating the second criterion. EPA looked at the total number of systems that reported detections of HCBD, as well as those that reported concentrations of HCBD above an estimated drinking water health reference level (HRL). For noncarcinogens, the estimated HRL level was calculated from the RfD assuming that 20% of the total exposure would come from drinking water. For carcinogens, the HRL was the 10^{-6} risk level. The HRLs are benchmark values that were used in evaluating the occurrence data while the risk assessments for the contaminants were being developed.

The available monitoring data, including indications of whether or not the contamination is a national or a regional problem, are included in Chapter 4 of this document and summarized below. Additional information on production, use, and fate are found in Chapters 2 and 3.

9.3.1 Occurrence Criterion Conclusion

HCBD has never been specifically manufactured as a commercial product in the United States, but is generated as waste by-product from the chlorination of hydrocarbons. The available data for HCBD use indicate an overall downward trend. The ten-year pattern of TRI releases to surface water is variable but generally decreasing within the ranging from 5 to 1,911 pounds. The physicochemical properties of HCBD and the available data for environmental fate indicate that HCBD in surface water is likely to be rapidly degraded by biotic and abiotic processes although it has the potential for bioaccumulation. Monitoring data indicate that HCBD is infrequently detected in public water supplies. When HCBD is detected, it very rarely exceeds the HRL or a value of one-half of the HRL. Chemical treatment of drinking water and leaching from drinking water surfaces are not expected to contribute to significantly elevated levels of HCBD in drinking water.

9.3.2 Monitoring Data

Drinking Water

HCBD has been detected in a small percentage of public water supply (PWS) samples collected under the authority of the Safe Drinking Water Act. Occurrence data for HCBD in drinking water are presented and analyzed in Chapter 4 of this document. Data from two monitoring periods were available for analysis. Data from Round 1 were collected during the period 1987 to 1992. Data from Round 2 were collected during the period 1993 to 1997. Round 1 and 2 monitoring detected HCBD in only 0.13% and 0.05% of all samples analyzed, respectively. When data are expressed on a PWS basis, Round 1 and Round 2 monitoring detected HCBD at least once in 0.35% (228 systems) and 0.18% (117 systems) of the tested water supplies, respectively.

The median and 99th percentile concentrations for all samples (i.e., samples with and without detectable levels of HCBD) were below the minimum reporting level (MRL). When subsets of the data containing only samples with detectable levels of HCBD were analyzed, the median and 99th percentile concentrations for Round 1 were 0.25 µg/L and 10 µg/L, respectively. The median and 99th percentile for Round 2 detections were 0.30 µg/L and 1.5 µg/L, respectively.

When monitoring results were compared to a value of one-half of the HRL, 0.163% of Round 1 (106 systems) and 0.079% of Round 2 (51 systems) water supplies exceeded this benchmark at least once during the reporting period. The percentages of water supplies that exceeded the HRL at least once in Round 1 and Round 2 monitoring were 0.114% (74 systems) and 0.018% (11 systems), respectively.

PWSs with detected levels of HCBD were widely distributed throughout the United States (see Figures 4-2 and 4-3 in this document), and no clear patterns of regional geographic occurrence were evident.

Ambient Water

HCBD has not been detected in the ground water samples reviewed and/or analyzed under the U.S. Geological Survey National Ambient Water Quality Assessment (NAWQA) program. The first round of intensive monitoring in the ongoing NAWQA was conducted from 1991 to 1996 and targeted 20 watersheds. Data from each NAWQA study unit were augmented by additional data from local, state, and federal agencies that met specified criteria.(See Section 4.1.1). HCBD was not detected in rural and urban wells of the local, State, and federal data set compiled by NAWQA. These data represent untreated ground water of the conterminous United States for the years 1985-1995.

A review of highway and urban runoff studies also found no detections of HCBD.

9.3.3 Use and Fate Data

Significant quantities of HCBD are generated in the United States as waste by-product from the chlorination of hydrocarbons, although HCBD has never been specifically manufactured as a commercial product domestically. No recent estimate could be found on the by-product amounts, but in 1982, it was estimated that about 28 million pounds were generated (ATSDR, 1994). HCBD imports dropped during the late 1970s, the period for which data are reported (Howard, 1989).

In all environmental media, HCBD binds strongly to particles (ATSDR, 1994). It is readily adsorbed to airborne particulate matter, to sediments in water, and to soil organic particles. Volatilization from soil or water to air appears to occur relatively slowly (U.S. EPA, 1991a).

Very little information is available on degradation or transformation of HCBD. Under aerobic conditions, HCBD in sewage contaminated waters showed complete biodegradation (Tabak et al., 1981). Under anaerobic soil conditions, biodegradation will not occur based on results obtained in sludge incubated under anaerobic conditions (Johnson and Young, 1983). Estimates of the half-life of HCBD in water range from 3 to 30 days in rivers and from 30 to 300 days in lakes and ground water (Zoeteman et al., 1980). It is expected that airborne HCBD, like the structurally similar compound tetrachloroethylene, will react with atmospheric ozone and hydroxyl radicals, leading to degradation (Atkinson and Carter, 1984; Atkinson, 1987). Estimates of the atmospheric half-life of HCBD based on this assumption range from 60 days to 1.6 years (HSDB, 2000).

HCBD may readily partition from the water into biological tissues, as suggested by its high log octanol:water partition coefficient (K_{ow} of 4.78). Laboratory and field studies have confirmed its bioaccumulation potential (WHO, 1994; U.S. EPA, 1998a). There is no evidence that HCBD has biomagnification potential (WHO, 1994).

HCBD is not used as a drinking water treatment chemical, and leaching from drinking water contact surfaces is not likely. Therefore, these factors are not expected to contribute to elevated levels of HCBD in drinking water.

9.4 Risk Reduction

The third criterion asks if, in the sole judgment of the Administrator, regulation presents a meaningful opportunity for health risk reduction for persons served by public water systems. In evaluating this criterion, EPA looked at the total exposed population, as well as the population exposed above the estimated HRL. Estimates of the populations exposed and the levels to which they are exposed were derived from the monitoring results. These estimates are included in Chapter 4 of this document and summarized in Section 9.4.2 below.

In order to evaluate risk from exposure through drinking water, EPA considered the net environmental exposure in comparison to the exposure through drinking water. For example, if exposure to a contaminant occurs primarily through ambient air, regulation of emissions to air provides a more meaningful opportunity for EPA to reduce risk than does regulation of the contaminant in drinking water. In making a preliminary regulatory determination, the available information on exposure through drinking water (Chapter 4) and information on exposure through other media (Chapter 5) were used to estimate the fraction that drinking water contributes to the total exposure. The EPA findings are discussed in Section 9.4.3 below.

In making its preliminary regulatory determination, EPA also evaluated effects on potential sensitive populations, including the fetus, infants and children. Sensitive population considerations are included in section 9.4.4.

9.4.1 Risk Reduction Criterion Conclusion

Approximately 2 to 5 million people are served by systems with detections of HCBD. An estimated 10,000 of these individuals may be served by systems with detections greater than the HRL, based on Round 2 monitoring data. Sensitive populations to HCBD may include people with preexisting kidney damage and infants, though it is unlikely for human newborns to be acutely exposed to significant doses of HCBD. When average daily intakes from drinking water are compared with intakes from air, drinking water accounts for a relatively small proportion of total HCBD intake. Relative intake rates from food may be higher, however, and intakes from soil are not known. On the basis of these observations, the impact of regulating HCBD concentrations in drinking water on health risk reduction is likely to be small.

9.4.2 Exposed Population Estimates

National population estimates for HCBD exposure were derived using summary statistics for Round 1 and Round 2 PWS cross-sectional data (see Table 4-2 of this document) and

population data from the *Water Industry Baseline Handbook* (U.S. EPA, 2000e). Summary data for exposed population estimates are provided in Table 9-2 below. An estimated 1.9 to 5 million people are served by PWSs that have detected HCBD. Of this population, approximately 1.2 million people could be exposed at one-half of the HRL, based on data from Round 1 sampling; and about 5 million people could be exposed to over one-half the HRL, based on Round 2 sampling. Based on the data from Round 1 sampling, about 781,000 individuals were exposed to concentrations at or above the HRL. Based on Round 2 sampling results, an estimated 10,000 persons could be exposed at or above the HRL. The Round 2 based estimate is probably a better estimate of possible exposure since the database is more recent, and more representative of the cross-section population served by groundwater.

Table 9-2. National Population Estimates for HCBD Exposure via Drinking Water

Population of Concern	Round 1	Round 2
Served by PWS with detections	1,909,000	5,027,000
Served by PWSs with detections > (1/2 HRL)	1,213,000	4,965,000
Served by PWSs with detections > HRL	781,000	10,000

Source: Data taken from Table 4-2 of this document.
HRL = Health Reference Level

9.4.3 Relative Source Contribution

Relative source contribution analysis compares the magnitude of exposure expected via drinking water to the magnitude of exposure from intake of HCBD in other media, such as food, air, and soil. To perform this analysis, intake of HCBD from drinking water must be estimated. Occurrence data for HCBD in water and other media are presented in Chapter 4 and 5 of this document.

As shown in Table 4-2, the 99th percentile concentration for all samples (i.e., those with detectable and nondetectable levels of HCBD) from Round 1 and Round 2 PWS sampling is below the MRL. As a convention, a value of half the MRL is often used as an estimate of the concentration of a contaminant in samples/systems whose results are less than the MRL. However, for Round 1 and Round 2, States have reported a wide range of values for the MRLs (See Section 4.2.1), and a single estimate of the MRL for HCBD is unavailable.

As an alternative, the median concentration (0.3 µg/L) for HCBD in samples with detectable levels from both rounds was used to estimate intake from drinking water. The exposure estimate for an average individual is determined by multiplying the drinking water concentration by daily water intake (2 liters/day) and dividing by average adult body weight (70 kg), and is estimated to be 8.6×10^{-6} mg/kg-day. For children, assuming a daily water intake of 1 liter/day and body weight of 10 kg, the exposure estimate is 3.0×10^{-5} mg/kg-day.

The estimated average daily intakes of HCBD from drinking water (based on median concentration of detected samples) and other sources are shown in Table 9-3. The estimated food:drinking water exposure ratio is 0.03 for an adult and 0.02 for a child (Table 9-4). The

estimated air:drinking water exposure is 14 for an adult and 21 for a child. Collectively, these data indicate that intake from drinking water is low when compared to intake from air, though not necessarily when compared to possible intake from food.

Table 9-3. Comparison of Average Daily Intakes from Drinking Water and Other Media[a]

Medium	Adult (ng/kg-day)	Child (ng/kg-day)
Drinking Water[b]	8.6	30
Food	0.15	0.24
Air	120	630

[a] See Chapter 5 for derivation of intakes from media other than water
[b] Based on half the median values for detected hexachlorobutadiene concentrations in Round 1 and Round 2

Table 9-4. Ratios[a] of Exposures from Various Media to Exposures from Drinking Water

Exposure Ratio	Adult	Child
Food:Drinking Water	0.02	0.008
Air:Drinking Water	14	21

[a] Calculated from estimated daily intakes in Table 9-3

9.4.4 Sensitive Populations

The target organ for HCBD is primarily the kidney. Sensitive populations to HCBD exposure may include people with preexisting kidney damage. Though it is unlikely that human newborns would be acutely exposed to significant doses of HCBD, acute exposure for young rats causes toxicity at lower levels than for adults (Hook et al., 1983; Lock et al., 1984).

Calculation of medium-specific exposure ratios (Table 9-4) indicates that HCBD intake from air is about 14- 20 fold greater than intake from water. Therefore, regulation of HCBD in drinking water would be unlikely to significantly reduce the risk to sensitive populations.

9.5 Regulatory Determination Summary

While there is evidence that HCBD may have adverse health effects in humans at moderate-to-high doses, it is unlikely that: 1) this contaminant will occur with a frequency or at concentrations that are of public health concern; or 2) regulation of this contaminant represents a meaningful basis for health risk reduction in persons served by public water systems. For these

reasons, EPA may not propose to regulate HCBD with a NPDWR. All final determinations and future analysis will be presented in the Federal Register Notice covering CCL proposals.

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APPENDIX A: Abbreviations and Acronyms

ATSDR	- Agency for Toxic Substances and Disease Registry
CAS	- Chemical Abstract Service
CCL	- Contaminant Candidate List
CERCLA	- Comprehensive Environmental Response, Compensation & Liability Act
CMR	- Chemical Monitoring Reform
CWS	- Community Water System
DWEL	- Drinking Water Equivalent Level
EPA	- Environmental Protection Agency
EPCRA	- Emergency Planning and Community Right-to-Know Act
FDA	- Food and Drug Administration
GW	- ground water
HRL	- Health Reference Level
IRIS	- Integrated Risk Information System
MCL	- Maximum Contaminant Level
MRL	- Minimum Reporting Level
NAWQA	- National Water Quality Assessment Program
NCOD	- National Drinking Water Contaminant Occurrence Database
NIOSH	- National Institute for Occupational Safety and Health
NPDWR	- National Primary Drinking Water Regulation
NPL	- National Priorities List
NTIS	- National Technical Information Service
NTNCWS	- Non-Transient Non-Community Water System
ppm	- part per million
PWS	- Public Water System
SARA Title III	- Superfund Amendments and Reauthorization Act
SDWA	- Safe Drinking Water Act
SDWIS	- Safe Drinking Water Information System
SDWIS FED	- the Federal Safe Drinking Water Information System
SOC	- synthetic organic compound
STORET	- Storage and Retrieval System
SW	- surface water
TRI	- Toxic Release Inventory
UCM	- Unregulated Contaminant Monitoring
UCMR	- Unregulated Contaminant Monitoring Regulation/Rule
URCIS	- Unregulated Contaminant Monitoring Information System
U.S. EPA	- United States Environmental Protection Agency
USGS	- United States Geological Survey
VOC	- volatile organic compound
µg/L	- micrograms per liter
mg/L	- milligrams per liter
>MCL	- percentage of systems with exceedances
>MRL	- percentage of systems with detections

APPENDIX B: Round 1 and Round 2 Occurrence Data Tables for Hexachlorobutadiene

Hexachlorobutadiene Occurrence in Public Water Systems in Round 1, UCM (1987) results

STATE	TOTAL UNIQUE PWS	# GW PWS	# SW PWS	% PWS with detections	% GW PWS with detections	% SW PWS with detections	% PWS > HRL	% GW PWS > HRL	% SW PWS > HRL	99% VALUE (µg/L)
AK	665	540	130	1.50%	1.48%	1.54%	0.00%	0.00%	0.00%	< 0.00
AL	131	93	42	3.05%	4.30%	0.00%	1.53%	2.15%	0.00%	0.50
AR										
AZ	448	407	47	0.89%	0.74%	2.13%	0.22%	0.00%	2.13%	< 2.00
CA	585	571	21	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 6.00
CO	6	3	4	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.64
DC	1	0	1	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
DE	10	8	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
FL	112	7	105	5.36%	0.00%	5.71%	5.36%	0.00%	5.71%	5.00
GA										
HI	127	112	16	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.30
IA										
IL	213	149	64	0.47%	0.67%	0.00%	0.00%	0.00%	0.00%	< 2.00
IN	357	321	37	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 2.00
KY	524	291	233	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 1.00
LA	13	9	4	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
MA										
MD	983	936	50	0.10%	0.11%	0.00%	0.00%	0.00%	0.00%	< 0.50
MI										
MN	1,553	1,529	28	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
MO	85	71	14	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 20.00
MS										
MT										
NC	297	254	44	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
NE										
NH										
NJ	801	790	11	0.75%	0.76%	0.00%	0.25%	0.25%	0.00%	< 1.20
NM	590	555	35	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 1.00
NV	8	7	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.20
NY	356	252	123	0.28%	0.40%	0.00%	0.28%	0.40%	0.00%	< 5.00
OH	2,655	2,493	166	0.11%	0.12%	0.00%	0.08%	0.08%	0.00%	< 2.00
SD	335	306	29	0.30%	0.33%	0.00%	0.00%	0.00%	0.00%	< 0.50
TN	303	156	147	0.33%	0.64%	0.00%	0.33%	0.64%	0.00%	< 0.50
TX	2	2	0	100.00%	100.00%	0.00%	100.00%	100.00%	0.00%	8.00
UT	411	391	34	1.22%	1.02%	2.94%	0.00%	0.00%	0.00%	< 5.00
VI	3	0	3	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 1.00
VT										
WA	992	937	77	0.10%	0.11%	0.00%	0.00%	0.00%	0.00%	< 0.50
WV	57	26	31	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 4.00
WY	145	116	38	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 2.00
TOTAL	12,768	11,332	1,538	0.36%	0.32%	0.65%	0.12%	0.07%	0.46%	< 5.00

Appendix B, Round 1 Data (continued)

Hexachlorobutadiene Occurrence in Public Water Systems in Round 1, UCM (1987) results										
STATE	TOTAL UNIQUE PWS	# GW PWS	# SW PWS	% PWS with detections	% GW PWS with detections	% SW PWS with detections	% PWS > HRL	% GW PWS > HRL	% SW PWS > HRL	99% VALUE (µµg/L)
24 states	12,284	10,980	1,385	0.35%	0.30%	0.72%	0.11%	0.06%	0.51%	< 5.00

PWS = Public Water Systems; GW = Ground Water; SW = Surface Water; MRL = Minimum Reporting Limit (for laboratory analyses);
Health Reference Level = Health Reference Level, an estimated health effect level used for preliminary assessment for this revie
The Health Reference Level used for hexachlorobutadiene is 0.9 µµg/L. This is a draft value for working review only.
Total Number of PWSs = the total number of public water systems with records for hexachlorobutadiene
% PWS with detections, > 1/2 Health Reference Level, > Health Reference Level = percent of the total number of public water systems with at least one analytic result that exceeded the MRL, 1/2 Health Reference Level, Health Reference Level, respectively
99th Percentile Concentration = the concentration value of the 99th percentile of all analytical results (in µµg/L)
Median Concentration of Detections = the median analytical value of all the detections (analytical results greater than the MRL) (in µµg/L)
The highlighted states are part of the URCIS (Round 1) 24 State Cross-Section.

Appendix B, Round 2 Data

Hexachlorobutadiene Occurrence in Public Water Systems in Round 2, UCM (1993) results

STATE	TOTAL UNIQUE PWS	# GW PWS	# SW PWS	% PWS with detections	% GW PWS with detections	% SW PWS with detections	% PWS > HRL	% GW PWS > HRL	% SW PWS > HRL	99% VALUE (µµg/L)
Tribes (06)	22	21	1	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 50.00
AK	625	481	144	3.36%	2.70%	5.56%	0.00%	0.00%	0.00%	< 0.00
AL										
AR	407	319	88	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.10
AZ	68	60	8	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 1.00
CA	14	11	3	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
CO	831	619	212	0.24%	0.00%	0.94%	0.00%	0.00%	0.00%	< 0.00
CT	84	43	41	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.00
IN	117	107	10	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 2.00
KY	121	50	71	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 2.50
LA	1,310	1,241	69	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
MA	418	344	74	0.24%	0.00%	1.35%	0.24%	0.29%	0.00%	< 0.50
MD	976	920	56	0.20%	0.11%	1.79%	0.00%	0.00%	0.00%	< 0.50
ME	744	676	68	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.00
MI	2,739	2,647	92	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.00
MN	1,558	1,528	30	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
MO	1,412	1,297	115	0.07%	0.08%	0.00%	0.00%	0.00%	0.00%	< 1.00
MS	1	1		100.00%	100.00%		0.00%	0.00%		0.60
NC	1,775	1,585	190	0.51%	0.44%	1.05%	0.00%	0.00%	0.00%	< 0.00
ND	296	258	38	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
NH										
NJ	7	7		0.00%	0.00%		0.00%	0.00%		< 1.00
NM	720	693	27	0.14%	0.14%	0.00%	0.00%	0.00%	0.00%	< 1.00
OH	2,232	2,050	182	0.04%	0.05%	0.00%	0.04%	0.00%	0.55%	< 0.50
OK	790	541	249	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.00
OR	17	15	2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.00
PA										
RI	115	103	12	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 1.00
SC	237	216	21	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
SD	27	19	8	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.50
TN										
TX	4,412	3,825	587	0.07%	0.08%	0.00%	0.05%	0.00%	0.34%	1.00
VT	1		1	0.00%		0.00%	0.00%		0.00%	< 0.50
WA	2,548	2,429	119	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.00
WI	191	188	3	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	< 0.30
TOTAL	24,815	22,294	2,521	0.17%	0.13%	0.56%	0.02%	0.00%	0.12%	< 1.00
20 STATES	22,736	20,380	2,356	0.18%	0.13%	0.59%	0.02%	0.00%	0.13%	< 1.00

PWS = Public Water Systems; GW = Ground Water; SW = Surface Water; MRL = Minimum Reporting Limit (for laboratory analyses);
Health Reference Level = Health Reference Level, an estimated health effect level used for preliminary assessment for this revie
The Health Reference Level used for hexachlorobutadiene is 0.9 µµg/L. This is a draft value for working review only.
Total Number of PWSs = the total number of public water systems with records for hexachlorobutadiene
% PWS with detections, > 1/2 Health Reference Level, > Health Reference Level = percent of the total number of public water systems with at least one analytical result that exceeded the MRL, 1/2 Health Reference Level, Health Reference Level, respectively
99th Percentile Concentration = the concentration value of the 99th percentile of all analytical results (in µµg/L)
Median Concentration of Detections = the median analytical value of all the detections (analytical results greater than the MRL) (in µµg/L)
The highlighted States are part of the SDWIS/FED (Round 2) 20 State Cross-Section.