DRAFT

For Review Only

Public Health Goal for
Hexachlorobenzene
In Drinking Water

NOT A REGULATORY STANDARD

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PREFACE

Drinking Water Public Health Goals

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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based upon currently available data and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.

8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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DRAFT

PUBLIC HEALTH GOAL FOR HEXACHLOROBENZENE IN DRINKING WATER

SUMMARY

A Public Health Goal (PHG) of 0.03 ppb (0.03 µg/L) is proposed for hexachlorobenzene (HCB) in drinking water. The proposed PHG is based on carcinogenic effects observed in experimental animals. Oral exposure to HCB is carcinogenic to rodents (mice, rats, and hamsters), producing multiple tumors in the liver, adrenal gland, thyroid gland, parathyroid gland, kidney, lymphoid tissue, and/or endothelial tissue.

For the calculation of the proposed PHG, cancer potency estimates were based on the recommended practices of the United States Environmental Protection Agency for carcinogenic risk assessment (U.S. EPA, 1996, 1999). According to these methods, a polynomial model is fit to the experimental data in order to establish the lower 95 percent confidence bound on the dose associated with a 10 percent increased risk of cancer (LED_{10}). Similar cancer potency estimates were obtained with the linear multi-stage (LMS) model. The cancer potency estimate (q_1*) for this model is the upper 95 percent confidence limit on the cancer potency slope. Both methods were used to calculate the potency estimates. The proposed PHG was calculated assuming a *de minimis* excess individual cancer risk level of 10^{-6} from exposure to HCB. Cancer potency estimates were derived using time-dependent models from the observed incidences of hepatocarcinomas (Lambrecht *et al*., 1983a,b) and adrenal pheochromocytomas (Arnold *et al.*, 1985, 1988; Lambrecht *et al*., 1983a,b) in female rats exposed to dietary HCB. The latter were the most sensitive endpoints.

An estimate of the concentration of HCB in drinking water protective against chronic toxicity other than cancer was derived based on hepatotoxicity following oral administration of HCB. Slight liver changes (centrilobular basophilic chromogenesis) in female rats in the 2-generation study were observed at a dose of 0.01 mg/kg-day (an apparent LOAEL) (Arnold *et al*., 1985). This dose and an uncertainty factor of 300 were used in the estimation of a health protective level of 0.23 ppb. OEHHA concludes that a health-protective concentration in drinking water for non-cancer effects is 0.23 ppb (0.23 µg/L).

The U.S. EPA has established a Maximum Contaminant Level Goal (MCLG) of zero for HCB, and a Maximum Contaminant Level (MCL) of 0.001 mg/L (U.S. EPA, 2000a) based upon analytical feasibility (U.S. EPA, 2002). The California Department of Health Services (CDHS) currently lists a Maximum Contaminant Level of 0.001 mg/L (1 ppb) for HCB.

INTRODUCTION

Hexachlorobenzene (HCB) is a chlorinated aromatic hydrocarbon (Figure 1) that was widely used as a seed dressing for prevention of fungal growth on crops, and is also a component of fireworks, ammunition, and synthetic rubbers. Restrictions initiated in the 1970s have caused a decline of HCB manufacturing. In the past few years, the production of HCB has been as an unintentional by-product in the synthesis of chlorinated solvents, aromatics, and pesticides. The primary source of HCB exposure is food. However, exposure to HCB can also occur from contaminated air, water, or soil.

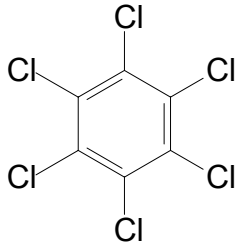
The purpose of this document is to establish a Public Health Goal (PHG) for HCB in drinking water. For a more comprehensive review of the occurrence and toxicity of HCB, the reader is referred to recent reviews by the International Programme for Chemical Safety (IPCS, 1997) and the Agency for Toxic Substances and Disease Registry (ATSDR, 1997) as well as some earlier reviews (Courtney, 1979; U.S. EPA, 1985). In this document, we provide an overview of the properties, occurrence and toxicity of HCB. In particular, we have evaluated the available data on the toxicity of HCB with a primary focus on effects in animals and humans that are likely to occur at relatively low exposure levels. The estimate health-protective levels and their derivation are described in the latter sections.

CHEMICAL PROFILE

Chemical Identity

Hexachlorobenzene (HCB) (CAS No. 118-74-1) is a chlorinated aromatic hydrocarbon that forms a white crystalline solid at ambient temperature. It has a molecular formula of C_6Cl_6 and a molecular weight of 284.8 g/mole (Brooks and Hunt, 1984; Howard, 1991). This compound should not be confused with the insecticide benzene hexachloride (Lindane), which is *gamma*-hexachlorocyclohexane ($C_6H_6Cl_6$). HCB is synthesized industrially through either chlorination of benzene at 150-200°C using ferric chloride as a catalyst or through distillation of residues from production of tetrachloroethylene (Brooks and Hunt, 1984). Synthesis of HCB can also be accomplished by refluxing hexachlorocyclohexane isomers with sulfuryl chloride or chlorosulfonic acid in the presence of ferric chloride or an aluminum catalyst (Brooks and Hunt, 1984). Technical grade HCB is available as a wettable powder, liquid or dust, and contains approximately 98 percent HCB, 1.8 percent pentachlorobenzene and 0.2 percent 1,2,4,5-tetrachlorobenzene (IARC, 1979; IPCS, 1997). Other known impurities include hepta- and octachlorodibenzofurans, octachlorodibenzo-p-dioxin and decachlorobiphenyl (Goldstein *et al*., 1978; IPCS, 1997).

Figure 1. Chemical Structure of Hexachlorobenzene



Physical and Chemical Properties

HCB has a melting point of 231°C, a boiling point of 323-326°C, and low flammability. It is relatively insoluble in water (0.0062 mg/L at 25°C), but is soluble in ether, benzene and chloroform. HCB has a high octanol/water partition coefficient (log K_{ow} = 5.31), a low vapor pressure (1.9 x 10^{-5} mm Hg at 25°C), and a moderate Henry's Law constant (0.0013 atm-m^3/mol at 23°C) (Brooks and Hunt, 1984; Howard, 1991).

Production and Uses

Historically HCB was widely used as a seed dressing for prevention of fungal growth on crops such as wheat, barley, oats and rye (IPCS, 1997). Concern for adverse affects to the environment and human health resulted in the discontinued use of HCB as a pesticide in many countries during the 1970s. However, additional uses for HCB continued beyond this period including its application in fireworks, ammunition, and synthetic rubbers. Recent data regarding production levels of HCB are limited. Worldwide production of pure HCB was estimated to be 10,000 tons/year from 1978-1981 (Rippen and Frank, 1986). An estimated 300 tons were produced by three manufacturers in the United States in 1973 (IARC, 1979), while approximately 1500 tons of HCB were manufactured annually in Germany for production of the rubber auxiliary PCTP (IPCS, 1997). This latter use was discontinued in 1993.

While restrictions initiated in the 1970s resulted in the decline of HCB manufacturing, it continues to be produced as an unintentional by-product in the manufacturing of chlorinated solvents, aromatics and pesticides (IPCS, 1997). In the 1980s, an estimated 4130 tons of HCB were generated annually as a by-product in the production of chlorinated organics and pesticides. In the United States, the majority of this is due to the manufacture of chlorinated solvents such as carbon tetrachloride, trichloroethylene and perchloroethylene (Brooks and Hunt, 1984; Jacoff *et al*., 1986). HCB is also inadvertently produced through incineration of HCB-containing materials, which constitutes a further source of entry into the environment. While emission levels from incinerators are considered site-specific, crude estimates of total HCB release from municipal incinerators in the United States have been estimated to be between 310 and 977 kg/year (Brooks and Hunt, 1984).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

With its resistance to environmental degradation and mobility, HCB is widely distributed throughout the environment. HCB undergoes limited atmospheric photolytic degradation [$t_{1/2}$ ~ 80 days; Mill and Haag, 1986]. As a result, long-distance atmospheric transport is possible and is considered to play a significant role in its wide environmental distribution. Wet deposition via rain and snowfall are believed to be the primary means of HCB transfer from the atmosphere to aquatic and terrestrial systems in Canada (IPCS, 1997). Major mechanisms of removal from water include volatilization to air and adsorption to sediments (IPCS, 1997). Once in the sediment, HCB tends to become trapped by overlying sediment. Desorption from soil and sediment although limited, does occur and is a potential continual source of HCB re-entry into the environment, even after other inputs have been discontinued (IPCS, 1997). In soil, volatilization is the primary means of removal at the surface interface (IPCS, 1997). Aerobic ($t_{1/2}$ of 2.7-5.7 years) and anaerobic ($t_{1/2}$ of 10.6-22.9 years) biodegradation are the major means of HCB removal at lower soil depths (Howard, 1991).

Air

While HCB is widely dispersed in air, concentrations are generally low. Reported HCB air levels are generally similar at urban, rural and remote sampling sites. Typical mean concentrations of HCB in air collected from point sources in Canada, Norway, Sweden, Germany, the United States, the Arctic, and the Antarctic range from 0.04 to 0.6 ng/m^3 (Table 1) (IPCS, 1997). Similar low levels were detected in the Nonoccupational Pesticide Exposure Study conducted by the U.S. EPA in the mid to late 1980s (U.S. EPA, 1990). HCB was detectable in 6 to 45 percent of the samples collected in Jacksonville Florida at concentrations ranging from below detection to 21 ng/m^3. The mean concentration for the sampled homes was 0.4-0.9 ng/m^3. In contrast, HCB was rarely detected in Springfield/Chicopee Massachusetts, the other area of study, and the levels were very low (<0.1 ng/m^3). Interestingly, the levels detected in indoor air were consistently higher than those measured in outdoor air. Occasionally other studies have reported higher values for HCB in the air. For example, Grimalt and associates reported an average airborne HCB concentration of 35 ng/m^3 in the vicinity of an organochlorine factory in Catalonia, Spain, compared with an average of 0.3 ng/m^3 for Barcelona, a nearby reference city (Grimalt *et al*., 1994). Spigarelli and associates detected HCB in the air at concentrations as high as 24 µg/m^3 at an on-site landfill of a manufacturer of perchloroethylene, carbon tetrachloride and chlorine (Spigarelli *et al*., 1986).

Soil

Limited data regarding HCB levels in soil have been reported. One of the most thorough data sets for HCB soil levels in the United States was developed from the 1972 US National Soils Monitoring Program in which 1,486 soil samples from 37 states were analyzed for organochlorines, organophosphates, PCBs, and elemental arsenic (Carey *et*

al., 1979). HCB was detected in samples from 11 sites at concentrations ranging from 10 to 440 µg/kg dry weight. Sampling sites located near industrial sources typically contain the highest levels of HCB. Measurements as high as 12.6 µg/g were reported at a Canadian landfill site, and 5700 µg/g in the loading and transfer area of a plant manufacturing chlorinated solvents that used offsite disposal methods (Spigarelli *et al*., 1986). Mean concentrations of HCB reported for uncontaminated soil in Europe ranged from 0.3 ng/g in Switzerland to 5.1 ng/g in a Swedish rural heartland soil (IPCS, 1997).

Table 1. Concentrations of Hexachlorobenzene in Ambient Air (ng/m^3)

Location	Year	Detection limit	Mean	Range[a]
Canada (Windsor, Ontario)	1987-1990	0.03	0.13	ND-0.44
Canada (Walpole Island)	1988-1989	0.02	0.15	ND-.34
Germany (Hamburg-residential, suburban and industrial site)	1986-1987		0.6	0.3-2.5
South Germany	1986-1990		0.21	0.058-.52
Sweden (Aspvreten)	1984		0.067	0.054->0.165
Sweden (Stockholm)	1983-85		0.07	0.054->0.13
Spain (near organochlorine compounds factory) (near hospital in Barcelona)	1989 and 1992			 35 0.3
Bear Island (Artic) Summer Winter		 0.001 0.001	 0.04 0.111	 0.029-0.045 0.059-0.188
Southern Ocean and Antarctica	1990		0.06	0.04-0.078
United States (Portland, Oregon)	1984		0.075	0.05-0.11
United States (chemical production plant)				ND-24000
United States (urban areas)	1975-1979	0.1	0.5	ND-4.4

[a]ND = not detected
Adapted from IPCS, 1997.

In surface waters, HCB strongly adsorbs to sediment and suspended matter, resulting in a wide range of water and sediment concentrations due to differences in water levels, sediment composition and suspended matter (IPCS, 1997).

Water

From 1987 to 1993, HCB releases to surface water were estimated at 1,286 pounds according to the U.S. EPA's Toxic Release Inventory (U.S. EPA, 1998). These releases were primarily from alkali, chlorine and agricultural chemical industries. The largest releases were reported from Louisiana and Texas. However, HCB levels in fresh water in Europe and North America were quite low, typically below 1 ng/L (Table 2). Higher

Table 2. Concentrations of Hexachlorobenzene in Surface Water (ng/L)

Country	Year	Detection Limit	Mean	Range
Canada	1986	0.07		
Lake Superior			0.026	0.018-0.040
Lake Huron			0.033	0.018-0.073
Georgian Bay			0.041	0.032-0.054
Lake Erie			0.078	0.025-0.260
Lake Ontario			0.063	0.020-0.113
Canada	1985			
St. Clair River				0.30-87
Tributaries to St. Clair River				0.08-0.79
Canada	1979-1989			
Atlantic Region; streams, lakes, reservoirs, estuaries		2.0		ND-2.2
Spain (Ebre Delta)	1985-86	0.0005	0.041	ND-1.0
Germany (Elbe)	1990		12	3-62
Italy (tributaries to Adriatic Sea)	1977-78	1.0		ND
Netherlands/Belgium	1993	10	<10	<10
Netherlands	1987		<10	ND-100
United States – Texas estuary	1980		0.24	<0.01-0.61
United States				
Coastal, surface microlayer		<0.1		<0.1-26

ND – Not Detected
Adapted from IPCS, 1997.

values have been reported for aquatic systems that receive direct industrial discharge and surface run-off. For example, the channels connecting the Great Lakes in Canada are often found to exceed HCB levels of 1.0 ng/L. Levels in the St. Clair River near the Dow Chemical outfall were as high as 87 ng/L in 1985 and 75 ng/L in 1986 (IPCS, 1997).

HCB is infrequently detected in drinking water, and when measurable, the levels are typically at very low concentrations (Table 3). Drinking water samples collected in 1980 from Canadian cities located near Lake Ontario ranged between 0.06 to 0.20 ng/L (Oliver and Nicol, 1982). In most other Canadian and United States surveys, HCB has not been detected. However, of the 17 public water supplies monitored and reported recently in National Drinking Water Contaminant Occurrence Database (NCOD), HCB was detected in one large system in Massachusetts at a reported concentration of 1.2 ng/L (U.S. EPA, 2000c). Slightly higher concentrations of HCB (median of 1-2 ng/L) were reported in Croatian drinking water obtained from a polluted river (Fingler *et al*., 1992).

Table 3. Concentrations of Hexachlorobenzene in Drinking Water (ng/L)

Country	Year	Detection Limit	Mean	Range
Canada (Ontario)	1980	0.01	0.1	0.06-0.2
Canada (Maritime Provinces)	1985-88	2.0		ND
Croatia	1988-89			
Sisak		0.5	1.0	<1-4
Zagreb			2.0	1-3
United States	1977-81	100	ND	

ND-Not detected
Adapted from IPCS, 1997.

Food

Because of its high octanol/water partition coefficient, HCB levels tend to be highest in foods with a high fat content. Foods treated with HCB-contaminated pesticides also tend to have higher levels of HCB. An extensive database regarding HCB concentrations in foods was compiled during the U.S. Food and Drug Administration's Total Diet Study. Surveys from 1981-1991 indicated that HCB was present at detectable levels (detection limit = 0.1 ng/g) in a small fraction of food items, mostly dairy products, meats, and peanuts/peanut butter (IPCS, 1997). In one recent survey (1990-91), mean levels were less than 1 ng/g for all products tested. Results from more recent but limited surveys of HCB levels in commercial foods from several countries in North America, Europe and Asia (Table 4) have been consistent with the results from the Food and Drug Administration's Total Diet Study, as reported by IPCS (1997).

Table 4. Concentrations of Hexachlorobenzene in Various Foods (μg/kg wet weight)

Country	Food	Mean Content	Range
United States	Processed cheese	0.2	ND-0.5
	Cheddar cheese	0.1	ND-0.5
	Ground (regular) beef	0.1	ND-0.4
	Chuck roast beef	0.3	ND-1.0
	Round steak beef	0.2	ND-1.0
	Loin/sirloin steak beef	0.2	ND-1.0
	Lamb chop	0.3	ND-1.0
	Frankfurters	0.1	ND-0.6
	Cod/haddock fillet	ND (0.1)	ND-0.2
	Scrambled eggs	0.1	ND-0.3
	Fried eggs	0.2	ND-0.7
	Peanut butter	0.2	ND-0.4
	Dry roasted peanuts	0.3	ND-1.0
	Watermelon	0.1	ND-0.5
	Butter	0.6	ND-1.0
	Cream	0.1	ND-0.4
Canada	Fresh meat and eggs	0.17	
	Root vegetables and potatoes	0.04	
	Fresh fruit	ND (<0.01)	
	Leafy/other above ground vegetables	0.02	
	2 percent milk	0.16	
Canada	Apples		ND (<0.2)-2.6
	Peaches	ND (<0.2)	
	Tomatoes	ND (<0.2)	
	Potatoes	ND (<0.2)	
	Wheat	ND (<0.2)	
	Eggs	ND (<0.2)	
	Hamburger	0.39	0.2-0.57
	Prime beef		ND (<0.2)-0.21
	Pork	ND (<0.2)	
	Chicken	ND (<0.2)	

ND-Not detected (detection limit provided in bracket).
Adapted from IPCS, 1997.

METABOLISM AND PHARMACOKINETICS

Absorption

Exposure to HCB is believed to occur chiefly by absorption across the gastrointestinal tract following the ingestion of contaminated food. The absorption of HCB from the intestine is generally very efficient but may be influenced by dietary and other factors.

For example, Koss and Koransky (1975) orally administered HCB to female Wistar rats using either an oil-based solution or an aqueous suspension. Approximately 80 percent of the dose was absorbed from the oil solution whereas only 6 percent was absorbed from the aqueous suspension. Similarly, Zabik and Schemmel (1980) found that HCB administered to female rats fed high fat diets enhanced HCB accumulation in the perirenal fat pad, gastrocnemius muscle and liver.

A limited number of studies have also evaluated the absorption of HCB in humans. By comparing intake and fecal elimination in breast-fed or formula-fed infants, Abraham *et al*. (1994) estimated that absorption of HCB was clearly higher in the breast-fed infant. Furthermore, absorption of HCB from breast-milk was nearly complete. Recently, seven individuals ranging in age from 24 to 81 years with differing HCB body burdens were compared for differences between ingested and excreted amounts of HCB as well as other persistent lipophilic organic pollutants (Schlummer *et al*., 1998). Net HCB absorption for four of the seven study participants ranging in age from 24 to 36 years was between 70 and 82 percent. The three older participants had reduced absorption including a 1 percent value in a 56-year old male and net excretions of 56 and 210 percent in the two elder participants aged 76 and 81 years respectively. The study authors concluded that the results were supportive of a diffusion model of absorption in which the contaminant concentration in the blood was the primary determinant of the degree of absorption. As age increases, HCB tends to accumulate in the tissues resulting in lower net absorption (Schlummer *et al*., 1998). Based upon this information, a high net absorption of HCB in infants might be expected when comparing their relatively low body burden of HCB to the high concentrations of HCB in the mother's milk.

Limited evidence for dermal absorption is provided by one study in which approximately 2.5 mg/4 cm^2 of ^{14}C-HCB was topically applied in tetrachloroethylene to Fisher 344 rats (Koizumi, 1991). The investigator observed an increase in dermal absorption from 1 percent at six hours to 9.7 percent at 72 hours after dosing. Washing the skin with soap and water at six hours after dosing removed 34 percent of the dose and decreased absorption by 50 percent over the next 66 hours.

A review of the literature failed to identify studies of HCB absorption through inhalation. HCB in the vapor phase would likely be very well absorbed through inhalation. Despite the low vapor pressure, inhalation has sometimes been considered to be a relevant HCB exposure route (To-Figueras *et al*., 1997).

Distribution

Tissue distribution of HCB is believed to correlate with lipophilicity as measured by its octanol/water partition coefficient. However, tissue distribution can also be influenced by differences in diffusion through cell membranes, steric effects, adsorption-desorption, blood flow and metabolism. HCB concentration has been measured in a number of tissues in humans. The highest levels of HCB in samples from the general population were found in a small autopsy study where tissue levels were found to be the highest in the adipose tissue with decreasing levels in the adrenals, bone marrow and liver (Schecter

et al., 1998). Robinson and associates (1990) collected samples as part of the National Human Adipose Tissue Survey and estimated that the average human adipose tissue concentration of HCB in the U.S. between 1973 and 1983 was 0.053 ppm (Table 5). The most recent data from this data set showed a decrease in 1983 to 0.037 ppm.

Table 5. Summary Statistics for the Unweighted U.S. National Human Adipose Tissue Survey Data

Category	Number of Samples	Average level (ppm)	Standard deviation (ppm)	Geometric mean (ppm)	90th percentile (ppm)	Maximum (ppm)
Overall	6115	0.05	0.11	0.04	0.09	4.33
Sex						
Male	3070	0.05	0.07	0.04	0.10	2.83
Female	3045	0.05	0.13	0.04	0.09	4.33
Age group						
0-14 years	1255	0.06	0.18	0.03	0.09	4.33
15-44 years	2240	0.05	0.09	0.04	0.08	3.35
45+ years	2618	0.06	0.07	0.05	0.10	2.63
Census division						
New England	287	0.05	0.03	0.04	0.07	0.23
Mid Atlantic	1095	0.07	0.19	0.05	0.11	4.33
East north central	1278	0.05	0.05	0.04	0.08	2.63
West north central	439	0.05	0.10	0.04	0.07	1.99
South Atlantic	897	0.04	0.04	0.03	0.06	0.61
East south central	546	0.04	0.03	0.03	0.07	0.26
West south central	782	0.06	0.09	0.04	0.09	1.81
Mountain	311	0.07	0.14	0.05	0.10	2.23
Pacific	480	0.10	0.13	0.08	0.16	2.83

Adapted from Robinson *et al*., 1990.

The observation of increased levels of HCB in tissues with high lipid content is also supported by laboratory studies in a number of animal species. In general, HCB levels have been found to be the highest in adipose tissue, adrenal cortex, bone marrow, skin and some endocrine tissues (including thyroid and ovary) following ingestion or injection of HCB (IPCS, 1997).

For example, Kuiper-Goodman and co-workers (1977) conducted a subacute toxicity study in which rats of the Charles River strain were dosed by incorporating HCB into the feed resulting in dose levels of 0, 0.5, 2, 8, and 32 mg/kg-day. Subgroups of four rats of each sex were killed after 3, 6, 9, 12, and 15 weeks of feeding. The remaining rats at 15 weeks were fed a HCB-free diet and subgroups were killed after an additional 1, 2, 4, 7, 16, and 33 weeks. Tissue levels of HCB reached a dose-related plateau before

15 weeks, with concentrations ranging from highest to lowest in the adipose tissue, liver, brain, and serum.

Yang and associates (1978) studied the distribution of HCB in male Sprague-Dawley rats and female rhesus monkeys following intravenous injection of ^{14}C-HCB in 1,2-propanediol:plasma (1:8). Rats were administered 0.1 mg ^{14}C-HCB and were then placed in metabolic cages for 48 hours before sacrifice. Over 20 tissues from the rats were analyzed and all were found to contain radioactivity. The highest levels were in fat (approximately 3 μg/g tissue). The adrenal glands also contained a relatively high level of radioactivity, whereas the other tissues contained much lower levels, generally 1/12 to 1/300 of those found in fat tissue. In the Yang study, the tissue distribution of ^{14}C-HCB in individual rhesus monkeys was also determined at 100 days, 6 months, and 1 year after intravenous injection of ^{14}C-HCB at 0.38, 0.32, and 0.22 mg/kg, respectively. Upon sacrifice, the highest levels of radioactivity were detected in the subcutaneous fat (3170 ng/g on day 100, 1830 ng/g on day 182 and 828 ng/g on day 365) and the bone marrow (1638 ng/g on day 182 and 373 ng/g on day 365) from among the >30 tissues analyzed in the three monkeys. HCB equivalents in the adrenal glands were 1/6 to 1/8 the levels present in fat (368 ng/g, 329 ng/g, and 73 ng/g at the three respective time points), whereas the other tissues contained radioactivity levels 1/10 to less than 1/800 of those in the fat.

Metabolism

The metabolism of HCB has been studied in a number of species using various routes of exposure, and has been the subject of several reviews (Renner, 1988; Rietjens *et al*., 1997). Metabolism is believed to occur primarily in the liver. Some types of metabolism such as dechlorination have been demonstrated to occur *in vitro* with enzyme preparations from the lung, kidney and small intestine (IPCS, 1997; Mehendale *et al*., 1975). In mammals, HCB is slowly metabolized into other less chlorinated benzenes, chlorinated phenols, and other minor metabolites. Koss *et al*. (1986) identified 21 different metabolites in the urine of rats exposed to HCB (Table 6). Three distinct pathways appear to operate in mammals including oxidation which gives rise to phenolic metabolites including pentachlorophenol, tetrachlorohydroquinone and tetrachlorobenzoquinone; glutathione-conjugation leading to pentachlorobenzenethiol, pentachlorothioanisole and other sulfur-containing metabolites; and a minor reductive dechlorination pathway that yields less chlorinated benzenes (IPCS, 1997). Historically the classical view of HCB metabolism, which is now believed to be overly simple, was an oxidative attack by the cytochrome P-450 monooxygenases resulting in the formation of an electrophilic intermediate, typically an epoxide that covalently interacts with tissue macromolecules (Rietjens *et al*., 1997). Alternative pathways that compete for interaction with the epoxide include spontaneous isomerization of the epoxide to a phenol, epoxide hydrolase-catalyzed conversion of the epoxide to a dihydrodiol, and either spontaneous or glutathione-S-transferase-mediated conjugation of epoxides with glutathione. These latter pathways have been considered as detoxification steps.

Alternatively, the phenol may undergo further oxidation to form the hydroquinone and quinone metabolites.

Table 6. Urinary Metabolites of Hexachlorobenzene in Rats

Metabolite
Pentachlorobenzene
2,3,4,6-Tetrachlorophenol
2,3,5-Trichloro-1,4-hydroquinone
2,3,4,5-Tetrachlorophenol
Pentachlorophenol
Tetrachloropyrocatechol
Tetrachloro-1,4-hydroquinone
2,3,4,5-Tetrachlorothioanisol
Tetrachloromethoxythioanisol
Pentachlorothioanisol
1,4-Methoxy-3-methylmercapto-2,5,6-trichlorobenzene
Tetrachloro-1,4-dithioanisol
1-Methyl-(4-methylmercapto-trichlorophenyl)sulfoxide
3,5,6-Trichloro-1,2,4-trithioanisol
1-Methyl-(methoxy-tetrachlorophenyl)sulfoxide
1-Methyl-(4-methoxymercapto-trichlorophenyl)sulfone
1-Methyl-(methoxy-tetrachlorophenyl)sulfone
1-Methyl-(4-methoxymercapto-2,3,5,6-tetrachlorophenyl)sulfoxide
1-Methyl-(4-methoxymercapto-2,3,5,6-tetrachlorophenyl)sulfone
1-Methyl-[*bis*(methylmercapto)-trichlorophenyl]sulfoxide
Dimethyl-[(methylmercapto)-trichlorophenyl]sulfoxide

Adapted from Koss *et al*. 1986.

Renner (1988) reported that various species differ greatly in their ability to metabolize HCB. In rodents, enzyme induction has been observed following short-term repeated dosing, including induction of isoforms of cytochrome P-450 (P-4501A1, P-4501A2, and P-4502B) and glutathione-S-transferases. These studies have indicated that HCB is a mixed-type cytochrome P-450-inducing compound exhibiting phenobarbital- and 3-methyl-cholanthrene-like inducing properties.

Various phenolic derivatives identified in the excreta of rats dosed with HCB (Koss *et al*., 1986) include pentachlorophenol (PCP), tetrachloro-1,4-hydroquinone (TCHQ), and an assortment of tetra- and trichlorophenols (Table 6).

Glutathione conjugation is also known to occur in mammals. The glutathione-conjugate is further transformed through cleavage of the glycine and glutamate residues to yield

pentachlorophenyl-N-acetyl-L-cysteine. The derived mercapturate is either eliminated unchanged via the urine or as pentachlorobenzenethiol (PCBT) after cleavage of the C-S bond.

In spite of knowledge of HCB metabolism generated from rodent studies, limited confirmation of these findings has been achieved in humans. In humans, PCP has been proposed to be a significant metabolite of HCB. Although several studies have reported the presence of PCP in human excreta (Gomez-Catalan *et al*., 1987; To-Figueras *et al*., 1997), the significance of these observations with regards to HCB exposure is difficult to assess as humans may be directly exposed to PCP through its use as a wood preservative and food contaminant (To-Figueras *et al*., 1997).

To obtain insights into HCB metabolism in humans, To-Figueras *et al*. (1997) evaluated the serum and urine of 100 subjects of a general population living in a region of Spain with relatively high HCB airborne levels. HCB was detected in all serum samples with levels ranging from 1.1 to 953 ng/ml. Pentachlorophenol (PCP), a major HCB metabolite in animal studies, was also found in all urine samples ranging from 0.58 to 13.9 µg excreted in 24 hrs. A sulfur derivative that yielded pentachlorobenzenethiol (PCBT) after hydrolysis, was also identified and quantified in all the urine specimens at levels ranging from 0.18 to 84.0 µg excreted in 24 hr. PCBT appeared to be the main metabolite, with urinary concentrations surpassing those of PCP in the subjects with higher HCB levels. A strong association was shown between PCBT concentration in urine collected over 24 hr and HCB concentration in serum. Furthermore, the association was stronger in males than in females. A weaker association was found between PCP in urine and HCB in serum. These results would appear to support the formation of the cysteine conjugate over that of PCP as being the dominant metabolic pathway in humans.

Excretion

Elimination of HCB from the body is generally slow, and is believed to occur primarily through the feces. The two principal mechanisms for eliminating chemicals from the body via the feces are through the bile and intestines. Based on findings from studies in rats given HCB via stomach intubation or intravenous injection and monitored for elimination, the intestinal pathway appears to be the predominant mechanism for clearing HCB via the feces (Rozman *et al*., 1982; Rozman, 1985). This finding is further supported by a study in rhesus monkeys in which complete biliary bypass did not alter HCB elimination via the feces (Rozman *et al*., 1983; Rozman, 1985).

HCB has also been detected at significant levels in the breast milk of several animal species including humans (Bailey *et al*., 1980; Czaja *et al*., 1999; Ejobi *et al*., 1996; Gladen *et al*., 1999; Hooper *et al*., 1997; Schecter *et al*., 1998). A number of studies suggest that a significant portion of the maternal HCB body burden may be eliminated through breast milk (Bailey *et al*., 1980; Bleavins *et al*., 1982, 1984a). Peters *et al*. (1966) found a marked elevation in HCB in the maternal milk of mothers that consumed HCB-contaminated wheat in Turkey during the late 1950s. More recently, Schecter and associates (1988) found that levels of HCB in the breast milk of a mother feeding twins

systematically declined from 10.7 ng/g lipid two months following initial breast feeding to a level below the limit of detection of 1.8 ng/g lipid 31 months later.

Elimination of HCB via breast milk is also a potentially significant route of exposure to nursing offspring (Bailey *et al*., 1980). Bleavins *et al*. (1982) studied the tissue distribution and placental transfer of HCB in female European ferrets. A single dose of 57.6 µg of ^{14}C-HCB was given to three bred and five non-bred ferrets. Dosed ferrets and offspring were then evaluated for tissue ^{14}C-HCB levels. Results from this study indicate that nursing mothers can transfer a significant portion of their body burden of HCB through transfer to their offspring via maternal milk. A similar transfer of HCB from mother to infant was seen in a study of Rhesus monkeys in which the HCB levels in the nursing infants were shown to be significantly higher than that in the mothers (Bailey *et al*., 1980).

In addition to defining routes of HCB elimination from the body, a number of studies have also been conducted to determine its biological elimination half-life. In female Wistar rats exposed every other day to HCB for 6 weeks, the half-life was determined to be approximately 8 days initially following cessation of the treatment. At 3 and 12 months, the $t_{1/2}$ was determined to be 10 weeks and 1.5 years, respectively (Koss *et al*., 1983). The International Program for Chemical Safety reported that the biological half-life for HCB in sheep, pigs and dogs was between 10 and 18 weeks (ICPS, 1997). For rhesus monkeys, the half-life was 2.5 to 3 years (IPCS, 1997).

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

HCB exhibits low acute toxicity in studies involving either oral or inhalation exposure to experimental animals (IARC, 1979; Lewis, 1992; Strik, 1986). Oral LD_{50} values reported for acute exposure to HCB in variety of species range from 1700 mg/kg in the cat to between 3,500 and 10,000 mg/kg for the rat. Values for the mouse, rabbit, and guinea-pig fall within those reported for the cat and rat. LC_{50} values for inhalation exposure range from 1,600 mg/m^3 for the cat to 4,000 mg/m^3 for the mouse, with intermediate values for the rat and rabbit. Acute lethal and near lethal doses of HCB elicit convulsions, tremors, weakness, ataxia, and paralysis. While limited details were included, Strik (1986) reported a low score for skin irritation, absence of eye irritation, and absence of sensitization in the guinea pig. Elevated liver enzyme activity was also observed in rats within 24 h of receiving oral doses from 100 to 1000 mg/kg (Strik, 1986).

Subchronic Toxicity

Primarily hepatotoxic and neurological effects have been observed in studies that involve short-term repeated dosing in animals. Enzyme induction has also been seen. Induction of isoforms of cytochrome P-450 (CYP1A1, CYP1A2, and CYP2B) and glutathione-S-transferases have typically been observed at the lower doses employed in these studies. For instance, den Tonkelaar and van Esch (1974) noted induction of microsomal liver enzymes in Wistar rats at a dosage of 1 mg HCB/kg-day for 14 days. Various signs of toxicity have been observed at higher doses (30-325 mg/kg-day) in rats including altered body weight, cutaneous lesions, hepatomegaly, liver damage, and tremors, as well as signs of early alterations in porphyrin or heme metabolism (Courtney, 1979; Strik, 1986; U.S. EPA, 1985).

Similarly, Kuiper-Goodman *et al*. (1977) conducted a subacute toxicity study in Charles River rats which were dosed by incorporating HCB into the feed to give dose levels of 0, 0.5, 2, 8, and 32 mg/kg-day. Subgroups of four rats of each sex were killed at 3, 6, 9, 12, and 15 weeks of feeding. The remaining rats at 15 weeks were fed an HCB-free diet and subgroups were killed after 1, 2, 4, 7, 16, and 33 weeks. An increased liver weight was observed in both sexes at the two highest dose levels. Histological evaluation revealed an enlargement of the liver centrilobular hepatocytes in rats of both sexes at the highest doses. Serum sorbitol dehydrogenase activity, an indicator of liver damage, was maximally elevated at 6 weeks at the highest dose in males. Female rats developed porphyria, with elevated porphyrin values persisting after the animals were placed on an HCB-free diet.

Effects observed in experimental animals following subchronic exposure to HCB were generally similar to those produced from short-term exposure, albeit with effects seen at relatively lower daily doses (ATSDR, 1997; Courtney, 1979; U.S. EPA, 1985). Effects reported at dosages of 32 mg/kg-day or greater for periods ranging from several weeks to 90 days included death, skin lesions, behavioral and neurological alterations, decreased body weight gain, increased organ weights, altered thyroid function, and changes in serum levels of thyroid hormones. Hepatotoxic effects including histological changes, induction of hepatic microsomal enzymes and porphyria have been commonly reported at lower exposure levels.

HCB-induced Porphyria

HCB-induced porphyria has been extensively studied since the documentation of an HCB-induced porphyria outbreak in Turkey during the second half of the 1950s (Cam and Nigogosyan, 1963; De Matteis *et al*., 1961; Schmid, 1960). During the incident, as many as 5,000 people acquired porphyria and other types of toxic effects following consumption of HCB-treated wheat. HCB-induced porphyria has been observed following both subchronic and chronic dosing studies in animals and is characterized by an elevated excretion of porphyrins and their metabolites. Under normal conditions, production and utilization of the end products of porphyrin metabolism, various

hemoproteins, are coordinated in order to maintain a metabolic balance (Fig. 1). HCB disrupts this balance by inhibiting the heme-synthetic enzyme uroporphyrinogen decarboxylase and this is believed to be the primary mechanism underlying the illness.

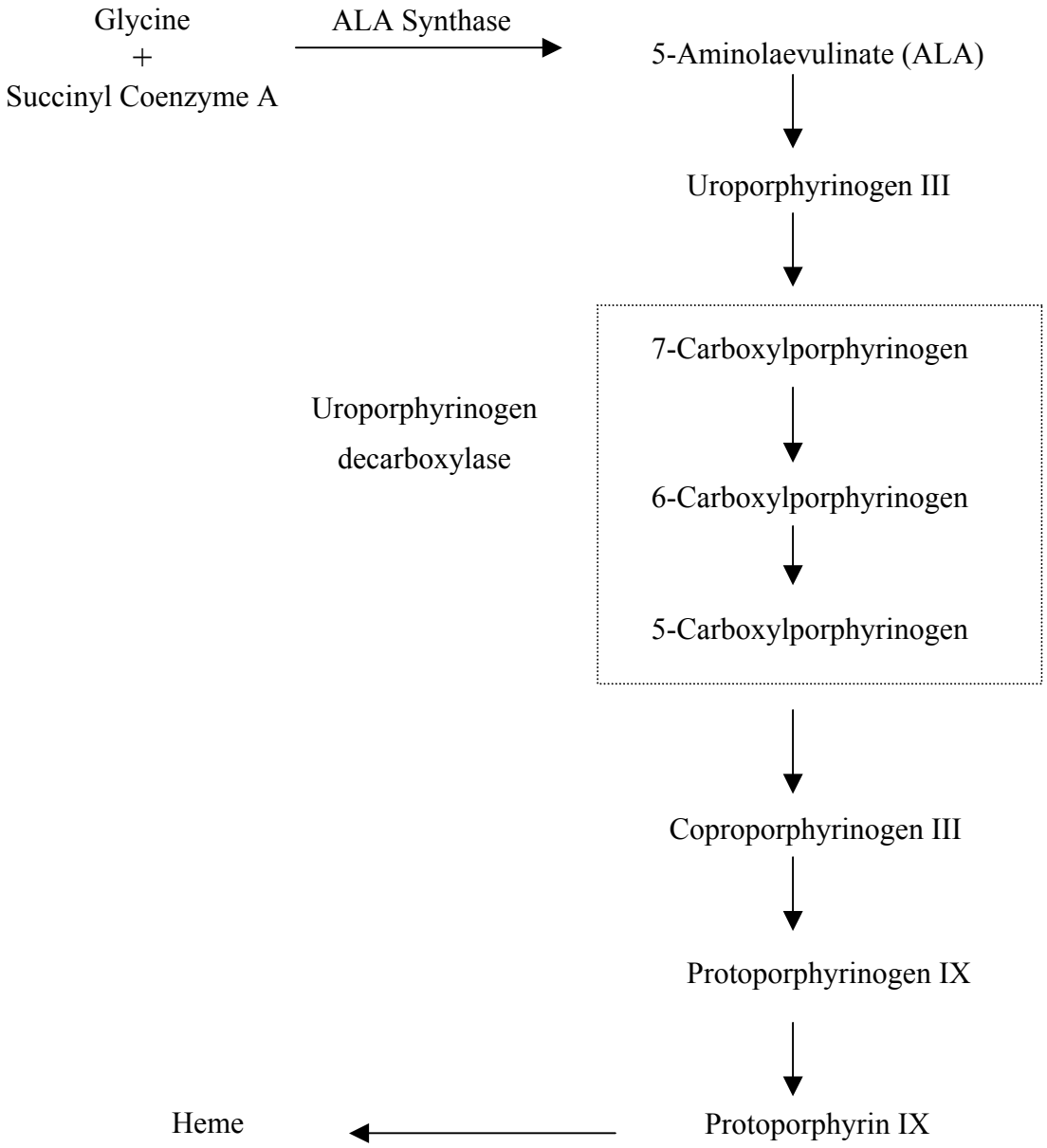


Figure 1. Pathway of heme biosynthesis (adapted from Jandl, 1996)
Note that HCB disrupts the balance of porphyrin metabolism by inhibiting the heme-synthetic enzyme uroporphyrinogen decarboxylase in the pathway above.

HCB-induced porphyria has been extensively studied in various strains of rats. Hepatic porphyria onset, and in some instances increased levels of kidney and spleen porphyrins has been observed following dietary and intragastric exposure to HCB at doses between 2.5 and 15 mg/kg-day for periods from 8 to 15 weeks (IPCS, 1997). While Kuiper-Goodman *et al*. (1977) observed a wide variation among individual rats as to the onset of porphyria, by 12 weeks of dosing with HCB all female rats examined from the 8 and 32 mg/kg-day groups had elevated total porphyrin concentrations. There was also a significant increase in kidney and spleen porphyrin concentrations in females in the 8 and 32 mg/kg-day test groups at 15 weeks of exposure as well as a significant increase in kidney porphyrin levels in males in the 32 mg/kg-day group. The induced porphyria can persist for extended periods after HCB exposure has ended. Koss *et al*. (1978) reported that female rats orally treated with 50 mg/kg every other day for 15 weeks had elevated levels of liver porphyrin 38 weeks following the end of dosing. Twenty years following the poisoning episode in Turkey, alterations in porphyrin metabolism continued to be seen in a portion of the previously affected patients (Cripps *et al*., 1980, 1984).

Studies of porphyria in rats also indicate that females are more sensitive than males. Various strains of female rats exposed to HCB for 3 months or longer at doses ranging from 5 and 10 mg/kg-day developed marked porphyria, which was absent or greatly reduced in males (Grant *et al*., 1975; Kuiper-Goodman *et al*., 1977; Rizzardini and Smith, 1982; Smith *et al*., 1985). The induction of porphyria appears to be at least indirectly under hormonal control as a reduction in liver porphyrin levels has been observed in ovariectomized female Sprague-Dawley rats given a subchronic dose of HCB while elevated liver porphyrin levels were observed in castrated male rats (Grant *et al*., 1975). Sex-related differences in urinary and hepatic porphyrin levels were also reported to parallel differences in the excretion of phenolic metabolites (Rizzardini and Smith, 1982). These findings were further investigated in a short-term study conducted by D'Amour and Charbonneau which indicated that sex-related differences in susceptibility to HCB-induced porphyria may be due in part to differences in hepatic conjugation of HCB with glutathione, where male rats appear to have greater glutathione-S-transferase activity (D'Amour and Charbonneau, 1992). Male rats receiving a porphyrinogenic dose of 100 mg HCB/kg-day by gavage for 5 days had significantly lower reduced glutathione concentrations and higher glutathione transferase activity than controls whereas significant differences were not seen in the females. Biliary excretion of the glutathione-derived metabolite of HCB, PCTP, as well as the rate of elimination of HCB from the liver was greater in males than females.

Although the mechanism remains to be clearly defined, results from mechanistic studies suggest that oxidative metabolism of HCB plays a role in the development of porphyria. Van Ommen *et al*. (1989) studied the relationship between hepatic porphyria and oxidative biotransformation of HCB. Female rats were fed diets containing either HCB alone or were co-treated with HCB and triacetyloleandomycin (TAO), a selective inhibitor of the cytochrome P450 isoforms (CYP3A1, CYP3A2) involved in both the hydroxylation of HCB and its primary oxidative metabolite, pentachlorophenol (PCP). The urinary excretion of the major oxidative metabolites, pentachlorophenol and

tetrachloro-1,4-hydroquinone as well as the extent of hepatic accumulation and urinary excretion of porphyrins, were greatly reduced in the female rats co-treated with HCB and TAO as compared to the animals given HCB alone.

However, the induction of porphyria does not seem to be simply due to the formation of PCP or tetrachloro-1,4-hydroquinone. Den Besten *et al*. (1993) conducted a 13-week feeding study on female Wistar rats that were given 300 ppm HCB in their diet in either presence or absence of TAO. The biotransformation of HCB was also compared with that of pentachlorobenzene (PCB), since this chemical is oxidized to the same products as HCB. Both urinary porphyrin excretion and hepatic porphyrin accumulation were greatly reduced in rats receiving co-treatment with HCB and TAO. Diminished formation of the oxidative metabolites alone could not account for the findings since rats treated with PCB had a higher urinary excretion of PCP and TCHQ, but did not develop porphyria. Since both HCB and pentachlorobenzene were oxidized to PCP and tetrachlorohydroquinone (TCHQ), it was postulated that a different mechanism of oxidation of HCB to PCP and PCB to PCP might exist and be responsible for the different results.

Several studies reported increased hepatic and/or urinary porphyrin levels in various strains of mice following exposure to HCB (Rizzardini *et al*., 1988; Smith and Francis, 1983; Vincent *et al*., 1989). Recently, it has been shown using mice lacking the CYP1A2 gene [Cyp1a2(-/-) mice] that CYP1A2 is required for hepatic uroporphyria induced by HCB (Sinclair *et al*., 2000). Mice lacking the Cyp1a2 gene treated with HCB (and iron or iron + 5-aminolevulinate to stimulate uroporphyrin formation) failed to accumulate uroporphyrin in the liver, whereas large increases were seen in wild type mice. HCB induced small increases in CYP2B and CYP3A in the Cyp1a2(-/-) mice. These results indicate that in the mouse CYP1A2 is essential in the process leading to HCB-induced porphyria.

There is also limited evidence from studies in mice to suggest that the aryl hydrocarbon receptor (Ah receptor) may be involved in the accumulation of hepatic porphyrins (Hahn *et al*., 1988; Linko *et al*., 1986). Ah-responsive strains of inbred mice were more susceptible to HCB-induced hepatic accumulation of porphyrins than non-responsive mice (Hahn *et al*., 1988; Smith and Francis, 1983). However, HCB has been shown to be only a weak agonist for the Ah receptor (Hahn *et al*., 1989) and mechanistic studies for delineating the pathway are lacking.

Limited data regarding porphyria are available from other species (IPCS, 1997). The lowest doses producing porphyria and related effects were reported in a subchronic study reported by den Tonkelaar and associates (1978). These investigators exposed groups of five SPF pigs to doses of 0, 0.05, 0.5, 5.0, and 50 mg HCB/kg-day for 90 days by incorporating HCB into the feed. Animals in the highest dose group showed clinical signs associated with porphyria and died during the experiment. An increased excretion of coproporphyrin was measured in the 0.5 and 5.0 mg HCB/kg-day groups, as well as microsomal liver enzyme induction and increased liver weight at the 5.0 mg HCB/kg-day level.

In contrast, some species seem to be resistant to the porphyrinogenic effects of HCB. Male and female beagle dogs treated daily with 0, 1, 10, 100 or 1000 mg/dog/day for 1 year did not exhibit hepatic fluorescence during necropsy indicating the absence of porphyria (Gralla *et al*., 1977). Porphyria was also not seen in minks and ferrets chronically treated with HCB (Bleavins *et al*., 1984a).

Non-Hepatic Effects

Additional non-hepatic effects have been observed in studies involving repeated subchronic exposure to HCB. Andrews *et al*. (1989, 1990) observed changes in calcium homeostasis and bone morphometry following subchronic exposure of rats to HCB at low doses. Male Fischer-344 rats were dosed intragastrically with 0, 0.1, 1, 10, or 25 mg HCB/kg in 0.4 ml of corn oil for 5 days per week for 5, 10, or 15 weeks. Serum alkaline phosphatase was significantly decreased at the two highest dose levels after both 10 and 15 weeks of exposure while parathyroid hormone concentration was significantly elevated at the two highest doses after 5 and 15 weeks of exposure. 1,25-dihydroxy-vitamin-D3 was measured in the 5-week exposure group only, and was significantly elevated in the three highest dose groups (Andrews *et al*., 1989). Femur weight was significantly increased in rats dosed at levels of 0.1, 1, and 25 mg HCB/kg, whereas bone density was significantly increased at 1, 10, or 25 mg HCB/kg dose levels (Andrews *et al*., 1990).

The observation of changes in calcium homeostasis and bone morphometry may be of particular relevance to humans in light of the reports of osteoporosis and resorption of digits observed during the HCB-induced epidemic in southeast Turkey during the 1950s. Cripps *et al*. (1984) conducted a follow-up study 20 to 30 years after the inadvertent HCB poisoning episode in Turkey and noted that 64.7 percent of the 204 patients evaluated had distinctively small hands. The shape of the hands with severe shortening of the digits was attributed to osteoporosis and resorption of the terminal phalanx of the digits.

Genetic Toxicity

In general, studies investigating the genotoxicity of HCB have indicated that it exhibits weak or no genotoxic activity (Brusick, 1986; Gorski *et al*., 1986; IARC, 1979; Jackson *et al*., 1993). For example, Haworth and associates (1983) evaluated the mutagenicity of HCB using *Salmonella typhimurium* strains TA98, TA1535, TA1537 and TA98, at five dose levels up to a maximum of 10 mg/plate both with and without metabolic activation with rat and hamster liver post-mitochondrial supernatant. HCB exhibited no detectable mutagenic activity in any of the *Salmonella* strains used either with or without microsomal activation. Gorski and associates (1986) using TA1538, TA98 and TA100 also saw negative results with and without metabolic activation.

In contrast, a modest positive response was reported for HCB in *S. typhimurium* TA 98 in the presence of rat liver S-9 at concentrations of 50 or 100 μg per plate (Gopalaswamy and Aiyar, 1986; Gopalaswamy and Nair, 1992). However, it should be noted that the

number of revertants induced varied considerably from experiment to experiment (Gopalaswamy and Aiyar, 1986).

Ishidate and associates (1988) reported that in the absence of metabolic activation no increase in structural chromosome aberrations was seen in Chinese hamster lung cells at a 2000 µg/ml HCB concentration. Canonero *et al*. (1997) evaluated the genotoxicity of HCB in primary cultures of rat and human hepatocytes. DNA fragmentation was measured using the alkaline elution method and by an increase in micronucleus formation. HCB concentrations ranging from 0.1 to 0.56 mM did not induce any significant increase in the frequency of micronucleated cells in rat hepatocytes. Under the same experimental conditions using human hepatocytes, HCB produced a weak but significant increase in the frequency of both DNA breaks and micronuclei (Canonero *et al*., 1997).

Several investigators have evaluated the *in vivo* genotoxicity of HCB. A low degree of binding of HCB to DNA from livers of rats exposed to 25 mg HCB/kg was reported by Gopalaswamy and Nair (1992). Moreover, HCB did not increase the frequency of sister chromatid exchanges in the bone marrow of male mice administered HCB at doses up to 400 mg/kg (Gorski *et al*., 1986). No significant increase in DNA fragmentation as measured by the alkaline elution assay was observed in the liver of rats administered HCB at doses up to 300 mg/kg (Gorski *et al*., 1986). In other studies, HCB failed to induce dominant lethal mutations in male rats administered HCB at 0, 70 or 221 mg HCB/kg-day for 5 consecutive days (Simon *et al*., 1979).

Although HCB was not mutagenic in microbial test systems and was negative in dominant lethal mutation tests, it did cause terata in mice (renal and palate malformations) and in rats (increased incidence of the 14th rib). HCB was also found to be particularly toxic to the developing perinatal animal, transplacentally and via the milk, causing enlarged kidneys, hydronephrosis, hepatomegaly, and possible effects on the immune system (Ecobichon, 1996).

Developmental and Reproductive Toxicity

Studies of reproductive toxicity have indicated that the ovary is a target site for HCB toxicity. Altered steroidogenesis in absence of changes in circulating levels of pituitary gonadotrophs was seen in rhesus monkeys (Muller *et al*., 1978). Altered ovarian steroid levels in the absence of systemic toxicity were also seen in both the cynomolgus monkey (Foster *et al*., 1992a) and in the rat (Foster *et al*., 1992b). In a recent study of rats dosed with a high dose of HCB (1000 mg/kg), altered ovarian steroid levels as well as alterations in the pituitary hormones, FSH and prolactin were observed (Alvarez *et al*., 2000). Changes in the estrus cycle and uterine E2 levels were also seen in the HCB-treated rats. Similarly, female ferrets fed a 125 ppm HCB-containing diet failed to come to estrus at any time during their normal breeding season (Bleavins *et al*., 1984a).

In addition to altering function, HCB-induced structural changes have also been observed in the ovary (Alvarez *et al*., 2000; IPCS, 1997). For instance, oral dosages as low as

0.1 mg/kg-day administered for 90 days to cynomolgus monkeys resulted in degenerative changes in the primordial follicles as well as the ovarian germinal epithelium (Babineau *et al*., 1991; Jarrell *et al*., 1993). These investigators also observed an increased number of lysosomal elements in germ cells of the ovarian follicles at the low dose. Furthermore, the basal lamina was thickened. At higher dosages, greater degenerative changes were observed (IPCS, 1997).

Male reproduction is also altered in a number of mammalian species following repeated exposure to HCB at doses typically higher than those affecting female reproduction. Histological changes in the testes were observed by den Tonkelaar *et al*. (1978) in pigs administered HCB in their diet at 50 mg HCB/kg -day for 90 days. Elissalde and Clark (1979) noted reduced serum testosterone levels in male mice fed diets containing 250 ppm of HCB (approximately 30 mg HCB/kg-day) for 21 days. Simon *et al*. (1979) observed a reduction in the mating index for male rats given five consecutive daily dosages of HCB via gavage at 221 mg/kg in corn oil in comparison with rats receiving either 0 or 70 mg HCB/kg. Fertility was not affected as measured by a smear test (Simon *et al*., 1979).

HCB has been shown to cause significant toxicity in the offspring of the exposed animals. Arnold and associates (Arnold and Krewski, 1988; Arnold *et al*., 1985) evaluated the offspring of Sprague-Dawley rats exposed to HCB in their feed at 0, 0.32, 1.6, 8 and 40 ppm. The study design included *in utero* and lactational exposure prior to weaning. The test animals (50 per group) were fed the same diet as their parents for the remainder of their lives or up to 2 years of age. HCB had no effect on fertility but pup viability was significantly reduced in the 40 ppm group (approximately 1.39 and 1.72 mg/kg-day for males and females, respectively). According to the report, decreases in pup viability had also been seen in preliminary studies at higher dietary levels. Other endpoints in this study are discussed in the chronic toxicity section. Decreases in pup viability were also seen at a 100 ppm dietary concentration by Vos and associates (1983) and at dietary concentrations of 40 ppm and higher in a multi-generation study in rats (Grant *et al*., 1977).

Adverse reproductive effects of HCB have also been observed in species other than rats (Bailey *et al*., 1980; Bleavins *et al*., 1984a). In particular, mink appear to be highly sensitive to the effects of HCB. Effects were assessed in the offspring of standard dark minks exposed to HCB (Bleavins *et al*., 1984a; Rush *et al*., 1983). Parental mink were given HCB incorporated into their feed at 0, 1, 5, 25, 125 or 625 ppm for 331 days prior to mating and throughout gestation and nursing. All of the mink fed the 625 ppm diet failed to survive to the breeding season and none of the mink receiving the 125 ppm diet whelped. Effects observed in the offspring included decreased litter size, increased percentage of stillbirths, increased kit mortality and decreased early kit growth (Table 7). Increased kit mortality was seen at the 1, 5 and 25 ppm dietary concentrations. The 1 ppm dose is approximately 0.16 mg HCB/kg-day based upon standard mink consumption levels (IPCS, 1997; Moore *et al*., 1997), which we will accept as a LOAEL from this study. By modeling the mink data, Moore and associates (1997) estimated that an HCB dose of 59.6 µg/kg-day would result in a 20 percent decline in the reproductive

fecundity of the mink. Both exposure *in utero* and exposure during lactation appear to contribute to the lethality of the young kits. In a cross-fostering study in mink exposed to 2.5 ppm HCB (Bleavins *et al*., 1984a), lethality was seen both in the offspring exposed only during gestation and in those exposed only via the milk. Earlier cross-fostering studies in rats and mice have also shown that lethal amounts of HCB can be readily transmitted through the milk (Courtney, 1979).

Goldey and Taylor (1992) observed altered neurobehavioral development in the offspring of rats exposed to 2.5 or 25 mg HCB/kg-day by gavage for 2 weeks prior to mating (1992). Pups in both dosing groups were hyperactive (based on tests of negative geotaxic reflex, olfactory discrimination, and exploratory locomotor activity) at 6-20 days of age. Pups from the highest treatment group showed a reduced acoustic startle response at 90 days. These doses did not affect learning as evaluated using a swim T-maze or motor activity in older offspring, nor maternal or fetal body weights, length of gestation, number of pups/liter at birth, or the number of days to eye opening.

Table 7. Effect of Chronic Dietary HCB on Mink Reproduction

Dietary treatment	At birth	Three weeks	Six weeks
Mink	Average number of kits alive at birth	Kit Mortality (%)	Kit Mortality (%)
0 ppm	4.9 ± 0.72	8.2	8.2
1 ppm	4.3 ± 0.81	32.4	44.1
5 ppm	5.3 ± 0.72	77.4	77.4
25 ppm	1.9 ± 0.81	86.7	86.7
Mink	Kit body weight (g)	Kit body weight (g)	Kit body weight (g)
0 ppm	9.0 ± 0.21	97 ± 3.4	245 ± 6.9
1 ppm	7.9 ± 0.25	87 ± 4.7	229 ± 17.1
5 ppm	8.1 ± 0.20	68 ± 6.2	173 ± 20.0
25 ppm	7.6 ± 0.38	36 ± 1.5	71 ± 4.0

Adapted from Bleavins *et al*., 1984a.

Lilienthal *et al*. (1996) reported HCB-induced effects on the neurobehavioral development of rat pups exposed both maternally and through the diet (1996). The dams were exposed to 0, 8, or 16 ppm HCB in the diet for 90 days prior to mating and throughout gestation and nursing, after which the offspring were fed the same corresponding diet as the parent for 150 days. Except for males at 150 days of age, exposure to HCB did not alter the mean body weight of the pups, or the number of pups/liter, but did increase the mean body weight of the dams, and their liver-to-body weight ratios. Schedule-controlled behavior in the pups was affected at 8 and 16 ppm

HCB, as indicated by a dose-related decrease in post-reinforcement pause at the end of the experiment. Other endpoints such as exploratory locomotor activity, open field behavior at 21 days of age, and active avoidance learning at 90 days of age were unaffected.

In summary, HCB has altered reproductive function in male pigs and female monkeys, ferrets, and rats with high dose exposures. Developmental effects (i.e., altered neurobehavioral development) have also been observed in exposed rats. Reproductive toxicity was also seen in the rat and mink. For both species, viability of the offspring was decreased with exposure to HCB. The lethality in pups reported by Bleavins *et al*. (1984) were noted to occur in groups exposed only during gestation as well as in groups exposed only via the milk. The effects reported in this cross-fostering study appear to be related to the lipophilicity or high octanol/water partition coefficient of HCB.

Immunotoxicity

A number of studies in experimental animals have indicated that HCB affects the immune system. For a review, see Michielsen *et al*. (1999b) and Vos (1986). In general, HCB exhibits immunosuppressive effects in mice and immunostimulatory effects in rats. Several examples of the immunomodulatory effects of HCB are presented below.

Loose and colleagues (Loose, 1982; Loose, *et al*., 1981) observed various alterations to the immune system of Balb/C mice given 5 ppm HCB in the diet (approximately 0.6 mg HCB/kg-day) daily for 3 to 18 weeks. The observed alterations included a reduction in resistance to challenge with tumor cells, a depression of cytotoxic macrophage activity of the spleen (Loose *et al*., 1981), and an increased susceptibility to *Leishmania* infection (Loose *et al*., 1981). Barnett *et al*. (1987) also observed a depression of the delayed-type hypersensitivity response to a contact allergen (oxazolone) in the offspring of Balb/C mice exposed to HCB *in utero* and through nursing at maternal doses of either 0.5 or 5 mg HCB/kg-day.

In the rat, immunostimulatory effects have been observed following exposure to HCB in the diet at concentrations of 500, 1000 and 2000 ppm for 3 weeks (Vos *et al*., 1983, 1979a). Effects observed were consistent with a stimulatory action of HCB on the immune system and included elevated serum levels of IgM, and increased spleen, and popliteal and mesenteric lymph node weights. Also observed was an elevation in the number of peripheral neutrophilic, basophilic and mononuclear leukocytes. Microscopic examination revealed an enlargement of the splenic white pulp due to lymphocyte hyperplasia of both the marginal zones and follicles spleen (Vos *et al*., 1983, 1979b).

Since HCB is known to cross the placenta in rats (Villeneuve and Hierlihy, 1975) and is excreted through breast milk, studies have also been conducted in order to investigate the potential for HCB to induce alterations to the developing lymphoid system in young animals. Vos *et al*. (1979b) exposed pregnant rats to HCB at concentrations of 0, 50 or 150 mg/kg and continued the exposure through lactation until weaning when the pups were 5 weeks of age. The investigators observed elevated IgM and IgG serum levels as

well as an increase in the number of blood basophilic and eosinophilic granulocytes in the blood of the pups from dams exposed at the highest dose. Testing of immune function showed a decrease in resistance to *Trichinella spiralis* and to *Listeria monocytogenes* infection for pups in the highest dose group.

In a follow-up study (Vos *et al*., 1983), pups were exposed to HCB during pre- and postnatal development through maternal exposure at doses of 0, 4, 20 or 100 mg/kg. Pups in the highest dose group had elevated serum IgM levels and increased numbers of basophilic peripheral granulocytes while animals in the 20 and 100 mg/kg dose group had elevated popliteal lymph node weights. Primary and secondary IgM and IgG antibody responses to tetanus toxoid were increased in animals from the 4 and 20 mg/kg groups.

Inflammation of the skin as well as alterations to the lung have also been observed in Wistar rats exposed to HCB (Michielsen *et al*., 1997, 1999a). Several studies were conducted in order to determine whether the parent compound itself or one of its reactive metabolites is involved in these effects. Because skin lesions have been attributed to the dermal accumulation and subsequent photochemical activation of porphyrins, several studies investigated the relationship between porphyria and the formation of skin lesions. Wistar rats co-administered with the P450IIIA1/2 inhibitor triacetyloleandomycin (TAO) and HCB showed a strong reduction of hepatic porphyria and diminished formation of the oxidative metabolites, PCP and TCHQ (den Besten *et al*., 1993; Schielen *et al*., 1993). However, the immunomodulating effects of HCB including the formation of antibodies and the induction of skin lesions remained unaffected. This line of evidence suggests that the skin lesions in the rat are not solely due to the dermal accumulation and subsequent photochemical activation of porphyrins. This was confirmed by the absence of porphyrin fluorescence in the skin of HCB-treated rats with and without lesions (Michielsen *et al*., 1997). As a result, it has been speculated that juvenile rats develop the porphyria-independent form of HCB-induced skin lesions similar to those observed in young children during the Turkish poisoning episode (Michielsen *et al*., 1997).

The induction of skin lesions in rats appears to be strain dependent and correlates with several parameters of immunomodulation (Michielsen *et al*., 1997, 1999a). Furthermore, thymus-dependent T cells appear not to be required for the induction of skin lesions but may enhance the rate of induction and progression of the lesions in the Brown Norway rat. In their review of HCB-induced immunotoxicology, Michielsen and associates (1999b) concluded that the immunotoxicity of HCB in the rat did not occur through the mechanisms by which most allergic or autoimmunogenic chemicals work (i.e. by binding to macromolecules with subsequent T- and B-cell activation). They found the thymus-independent immunopathology to be remarkable, as HCB strongly modulated T-cell-mediated immune parameters. This suggested that HCB acts through a very complex mechanism possibly involving multiple factors.

HCB-induced alterations to the immune system have been reported in other test animals as well. Microscopic examination of the thymus from rhesus monkeys dosed with HCB via gastric intubation at 0, 8, 32 or 128 mg/kg-day daily for 60 days showed a reduction or absence of individual lobules (Iatropoulos *et al*., 1976). In beagle dogs given 0, 1, 10, 100 or 1,000 mg HCB/day in gelatin capsules (equivalent to approximately 0.12, 1.2, 12,

and 120 mg/kg-day) for one year, Gralla *et al*. (1977) observed nodular hyperplasia of the gastric lymphoid tissue in dogs of all dose groups.

Neurotoxicity

HCB has been reported to cause neurological effects in adult animals with or without evidence of histopathological alterations of neurological structures. At low doses, HCB produces little central nervous system toxicity. However, central nervous system disorders can be the major symptom in the clinical profile at high doses. Some of the effects include electrophysiological changes, tremors, seizures, muscular weakness, lethargy, and unsteady gait. The extent of effect or severity was dose and time dependent.

Sundlof *et al*. (1981) reported electrophysiological changes (dysrhythmic electroencephalogram) in the central nervous system in dogs receiving doses of 50 mg/kg-day or greater for 21 days. In adult female Rhesus monkeys receiving 64 mg/kg-day for 60 days, severe tremors and muscular weakness were observed (Knauf and Hobson, 1979). Marked lethargy and weakness were observed in animals receiving the higher dose of 128 mg/kg-day. Similar findings were reported in adult rats exposed to 100 mg/kg-day for 60 days (Okner and Schmid, 1961). In another study, rats fed 50 mg/kg-day HCB for 56 days were also lethargic and had constant tremors (Kennedy and Wigfield, 1990). Muscle fasciculations and tremors were also noted in female Wistar rats exposed to 50 mg/kg-day HCB by gavage after the fourth week and during the remaining 15 weeks study or 38-week observation period (Koss *et al*., 1978). Lower doses of HCB (40 mg/kg-day) caused fibrillations, repetitive or pseudomyotonic discharges, and mild slowing of conduction velocities in the sciatic nerve of rats fed HCB (Sufit *et al*., 1986). Sufit *et al*. (1986) also found similar effects in rats exposed to 3.75 mg/kg-day or greater during the 2-year study. However, no tremors were observed in Agus or Wistar rats fed diets containing 100 ppm (5 mg/kg-day) HCB for 75-90 weeks (Smith and Cabral, 1980). Neurotoxic effects have also been observed in mice and pigs treated with HCB. Mice orally exposed to 26 mg/kg-day HCB for up to 17 weeks exhibited severe tremors prior to death (Hahn *et al*., 1988). Male SPF pigs fed 50 mg/kg-day HCB for days also exhibited tremors, panting, and unsteady gait without histopathology (Den Tonkelaar *et al*., 1978).

Chronic Toxicity

A variety of non-neoplastic effects have been observed in experimental animals resulting from chronic exposure to HCB. The effects primarily observed at the lowest dose levels are hepatic in nature, although effects to the kidney, spleen, and thyroid have been observed (IPCS, 1997).

Arnold and co-workers (Arnold and Krewski, 1988; Arnold *et al*., 1985) conducted a two-generation study in which Sprague-Dawley rats were dosed by incorporating HCB into the feed at 0.32, 1.6, 8 and 40 ppm. Using the body weight and food consumption

data supplied by the authors, U.S. EPA estimated the time weighted average dose levels received by male rats as 0.01, 0.05, 0.27 and 1.39 mg/kg-day, and by female rats as 0.01, 0.07, 0.35 and 1.72 mg/kg-day (U.S. EPA, 1985). The study design included in utero and lactational exposure prior to weaning by dosing both parents of the test animals. The test animals (50 per group) were subsequently dosed and observed for their whole lives. A control group received feed without HCB.

There were no treatment-related effects on growth, feed consumption, hematological parameters or survival in either generation. Increased heart and liver weights were observed in the 8 and 40 ppm F_0 males treated with HCB for 3 months. HCB had no effect on fertility but pup viability was significantly reduced in the 40 ppm group. Histopathological changes observed in the F_1 generation rats included linear trends in the incidence of centrilobular basophilic chromogenesis of the liver, of nephrosis in both sexes, and of peliosis of the liver in females. Hepatic peribiliary lymphocytosis and chronic fibrosis appear to be increased in males of all dose groups, but without a dose-related trend. Table 8 shows the incidence of these non-cancer effects in this study. Incidence of tumors in several organs, including the liver, is reported in the carcinogenesis section.

Table 8. Liver Histopathologic Changes in F_1 Rats, from Arnold *et al.*, 1985

Effects	PPM in the diet				
	0	0.32	1.6	8.0	40
Females					
peliosis	3/49	7/49	3/50	5/49	11/49[b,c]
chromogenesis					
slight	2/49	5/49[c]	7/50[c]	17/49[a,c]	13/49[a,c]
moderate	--	--	--	-	26/49[a,c]
extensive	--	--	--	--	3/49[c]
nephrosis					
moderate	--	1/49[c]	-[c]	1/49[c]	4/49[c]
severe	1/49	-	1/50[d]	1/49[d]	4/49[d]
Males					
chromogenesis					
slight	3/48	4/48[c]	3/48[c]	14/49[a,c]	16/49[a,c]
moderate	–	–	1/48[c]	3/49[c]	21/49[a,c]
lymphocytosis	16/48	27/48[b,d]	26/48[b,d]	21/49[d]	32/49[a,d]
fibrosis	13/48	23/48[b]	21/48	21/49	23/49[b]
nephrosis - severe	14/48	19/49[c]	12/48[c]	17/49[c]	27/49[a,c]

[a-d] – Superscripts ([a]) through ([d]) indicate values of statistical significance: [a] and [b] – significantly from controls by Fisher's exact test, $p < 0.01$ ([a]) and $p < 0.05$ ([b]); [c] and [d] – exact test for linear dose response was significant, $p < 0.01$ ([c]) or $p < 0.05$ ([d]).

The dose-related increases in centrilobular basophilic chromogenesis observed in both male and female rats are significant at 8 and 40 ppm. Given the clear trend, the increases seen in the females at the 0.32 ppm (5/49) and 1.6 ppm concentrations (7/50), compared to controls (2/49), while not statistically significant, may be biologically significant (i.e., representing a toxic effect at these doses). The increases in hepatic lymphocytosis and fibrosis in males are of uncertain physiological significance because of the absence of any dose-related trend. HCB has been clearly shown to be hepatotoxic in humans and experimental animals, and the Arnold *et al*. results indicate that modest hepatotoxic effects can be seen at low HCB exposure concentrations. The LOAEL for this study will be considered to be 0.32 ppm (0.01 mg/kg-day). A NOAEL was not identified.

Other hepatic and non-hepatic effects have been reported with chronic HCB exposure. Similar to that seen in short-term studies, induction of mixed-function oxidase activity has also been reported in male Sprague-Dawley rats given HCB in their diet at a concentration of 10 ppm [approximately 0.5-0.6 mg/kg-day (IPCS, 1997)] for 9-10 months (Grant *et al*., 1974). Mollenhauer *et al*. (1975) observed ultrastructural changes in the liver following exposure to HCB in the diet at 5, 10, and 25 ppm for 3, 6, and 12 months. This included alterations to cellular regions normally containing smooth endoplasmic reticulum (SER). Porphyria was observed in female but not male Sprague-Dawley rats given 80 or 160 ppm (equivalent to approximately 4 or 8 mg HCB/kg-day) for 9-10 months (Grant *et al*., 1974). In addition, Bleavins and associates (1984b) reported altered concentrations of neurotransmitters in the hypothalamus of female minks following dietary exposure to approximately 1 ppm in the diet (approximately 0.16 mg/kg-day) for 47 weeks.

Carcinogenicity

Oral Exposure

A considerable number of studies have been conducted to evaluate the carcinogenicity of HCB in animals. Significant increases in tumors have been seen in various tissues in mice, rats, and hamsters. Based upon these studies, IARC and U.S. EPA have determined that there was sufficient evidence to conclude that HCB induces cancer in laboratory animals (IARC, 1987; U.S. EPA, 1985). Early studies were conducted with HCB alone (see below). More recently, HCB has been tested in initiation-promotion assays and in combination with iron overload (Cabral *et al*., 1996; Gustafson *et al*., 2000; Shirai *et al*., 1978; Smith *et al*., 1985, 1989, 1993). These studies have clearly shown that HCB can act as a tumor promoter and that iron overload greatly increases the yield of liver tumors in animals administered lower doses of HCB. The present report expands upon the IARC and U.S. EPA reviews of the animal data and has been updated with additional data that have been published since these last evaluations were performed.

Cabral *et al*. (1977) dosed groups of 30 to 60 Syrian golden hamsters by incorporation of HCB into the feed at 50, 100 and 200 ppm for the lifetime of the animals (101-120 weeks). The study authors calculated that these levels were equivalent to

dosages of 4, 8 and 16 mg/kg-day. A control group receiving an HCB-free diet was included in the analysis. The mean survival in the treated groups was reported to be 71 percent at 50 weeks of age and was comparable with that of the controls. At 70 weeks, a reduced lifespan was reported for male and female animals treated at the highest dose level. Tissues in which increases in tumors were seen are reported in Table 9. A significant dose-related increase in hepatomas was seen in animals of both sexes in all treated groups. Dose-related increases in liver hemangioendotheliomas were also seen with significant increases occurring in the males in the 100 and 200 ppm treatment groups, and in the females at the highest dose level.

Thyroid alveolar adenomas, absent in the control group, were significantly elevated in male hamsters in the highest dose group (Table 9). A modest, although not significant elevation in thyroid tumors was observed in female hamsters in all of the treated groups. The appearance of thyroid tumors in the animals studies is noteworthy considering that a very high incidence of enlarged thyroids was reported among victims examined 25 years after accidentally being exposed to HCB in Turkey (Peters *et al*., 1982).

Table 9. Tumor Incidence in Hamsters Given Hexachlorobenzene

Animals with Tumors	Control	50 ppm	100 ppm	200 ppm
Hepatoma				
Males	0/40	14/30	26/30	49/57
Females	0/39	14/30	17/30	51/60
Thyroid alveolar adenomas				
Males	0/40	0/30	1/30	8/57
Females	0/39	2/30	1/30	3/60
Liver haemangioendotheliomas				
Males	0/40	1/30	6/30	20/57
Females	0/39	0/30	2/30	7/60

Adapted from Cabral *et al*., 1977

Limited evidence for HCB-induced tumor formation in hamsters is also provided by a one-year bioassay conducted by Lambrecht and co-workers (1982). Syrian golden hamsters were given HCB in their feed at 200 and 400 ppm for 90 days, and observed for the remainder of the 1-year study period. Slight increases in hepatoma were reported in 7.7 percent (1/13) males and 6.7 percent (1/15) females at the 200 ppm level and in 5 percent (1/20) males and 14.3 percent (1/7) females at the 400 ppm level. Given the short dosing period with only a one-year follow-up, these results are suggestive of an association between HCB exposure in hamsters and hepatomas. The incidence data was

published only in abstract form (Lambrecht *et al*., 1982) and briefly described in a later publication (Erturk *et al*., 1986).

HCB-induced carcinogenicity has also been observed in mice. Cabral and co-workers (1979) dosed groups of 30 to 50 outbred Swiss mice by incorporation of HCB into the feed at 50, 100 and 200 ppm for most of the lifetime of the animals (101-120 weeks). The study authors calculated that these dose levels were equivalent to dosages of 6, 12, and 24 mg/kg-day. An additional group of mice was dosed at 300 ppm in feed (36 mg/kg-day) for 15 weeks, then held without further dosing for 105 weeks. A control group was fed an HCB-free diet.

Compared to controls, a shortening of life spans occurred after 30 weeks of age in mice exposed to 200 ppm and 300 ppm. Tremors and convulsions were associated with the reduced survival in these animals. By 70 to 90 weeks, survival was modestly decreased in the two lower dose groups and severely decreased in the 200 ppm dose group as compared with the controls.

The study findings are summarized in Table 10. There was a statistically significant increase in the incidence of liver cell tumors in females in the 200 ppm group along with a moderate elevation in the males in the 200 ppm group. In both cases, there was also a positive dose-related trend. There was also a dose-dependent decrease in the latency and a dose-dependent increase in the size and multiplicity of liver cell tumors.

Table 10. Tumor Incidence in Mice Fed HCB

Animals with Tumors	Control	50 ppm	100 ppm	200 ppm
Liver				
Males	0/47	0/30	3/29	7/44
Females	0/49	0/30	3/30	14/41

Adapted from Cabral *et al*., 1979.

Other studies have evaluated the potential for HCB to induce tumors in rats. Smith and Cabral (1980) administered 100 ppm HCB in feed to 14 female Agus and six Wistar rats for 90 days. According to the authors, this was equivalent to a dosage between 6 and 8 mg/kg-day. The study included control groups of 12 female Agus and four Wistar rats. Interim sacrifices were made at 52, 63, and 75 weeks. All of the exposed Agus (14/14) and 67 percent (4/6) of the exposed Wistar rats developed liver tumors (histological type not reported) (Table 11). None of the control animals exhibited liver tumors. All but one of the treated Agus (1/14) rats and all of the controls survived until sacrifice. The earliest time-to-onset of a liver tumor was 52 weeks in an Agus rat.

Table 11. Incidence of Tumors in Rats Fed HCB

Animals with hepatomas	Control	100 ppm
Female Agus Rats	0/12	14/14
Female Wistar Rats	0/4	4/6

Adapted from Smith and Cabral, 1980.

Lambrecht and co-workers (Erturk *et al*., 1986; Lambrecht *et al*., 1983a,b; U.S. EPA, 1985) also evaluated HCB-induced tumor formation in rats. Groups of 94 Sprague-Dawley rats were given HCB in their feed at 75 or 150 ppm. A group of animals serving as controls were fed an HCB-free diet. The study design also included interim sacrifices of four rats from each dose group at 1, 2, 3, 4, 16, 32, 48, and 64 weeks. The remaining 54 animals in each group were permitted to continue on the diet until they died, or reached 2 years of age. Based on food consumption and average body weight data provided by the authors, estimated dosages of 4-5 or 8-9.5 mg/kg-day have been calculated for the 75 and 150 ppm dose levels, respectively (U.S. EPA, 1985).

For animals surviving beyond 12 months, a statistically significant elevation in the incidence of hepatomas/hemangiomas was observed in males and females in both treatment groups. Study findings are summarized in Table 12. A statistically significant increase in the incidence of hepatocellular carcinoma was also noted in female rats at both dose levels. In addition, in both male and female rats, a significant elevation of renal cell adenomas was seen at both dose levels. The U.S. EPA (1985) reported that liver pathology had occurred prior to the appearance of hepatoma or hepatocellular carcinoma. Observed pathology included parenchymal degeneration, preneoplastic foci and adenoma. At 48 and 64 weeks, HCB-exposed female rats had gross liver tumors that measured between 1 and 2 mm^3. Porphyria was also reported. None of the liver cell tumors metastasized.

An additional report on this study (U.S. EPA, 1985) stated that there was also a statistically significant increase in adrenal pheochromocytoma in female rats at both 75 and 150 ppm. The incidence of pheochromocytoma increased from 14.3 percent (5/35) in the controls to 66 percent (31/47) and 90.6 percent (29/32) for rats treated at 75 and 150 ppm, respectively (Table 13). A modest non-significant increase in adrenal cortical adenomas was also reported for the female rats in the treated groups.

Smith and co-workers (1985) fed male and female F344 rats diets containing 200 ppm HCB for 90 weeks (10 mg/kg-day by U.S. EPA default conversion). After 90 weeks of treatment, 100 percent (10/10) of surviving female rats had multiple liver tumors, which were histologically classified as neoplastic nodules and hepatocellular carcinomas. Only 16 percent (2/12) of the male rats developed liver tumors, which were smaller and less frequent per liver compared to the female animals. Liver tumors were absent in the male and female controls (0/10 and 0/10).

Table 12. Liver and Kidney Tumors in Sprague-Dawley Rats Given HCB in the Diet for up to 2 years

Animals with Tumors	Control	75 ppm	150 ppm
Hepatoma/hemangioma			
Males	0/54	10/52	11/56
Females	0/52	23/56	35/55
Hepatocarcinoma			
Males	0/54	3/52	4/56
Females	0/52	36/56	48/55
Bile-duct adenoma or carcinoma			
Males	0/54	2/52	2/56
Females	1/52	19/56	29/55
Renal cell adenoma			
Males	7/54	41/52	42/56
Females	1/52	7/56	15/54

Adapted from Erturk *et al*., 1986; Lambrecht *et al*., 1983a,b.

Table 13. Histopathology of Neoplasms in F_1 Generation Rats Fed 0-40 ppm HCB

Animals with Tumors	Control	0.32 ppm	1.6 ppm	8 ppm	40 ppm
Adrenal pheochromocytomas					
Males	10/48	12/48	7/48	13/49	17/49
Females	2/49	4/49	4/50	5/49	17/49
Parathyroid adenoma					
Males	2/48	4/48	2/48	1/49	12/49
Females	0/49	0/49	0/50	1/49	2/49
Neoplastic liver nodules					
Females	0/49	0/49	2/50	2/49	10/49
Adrenal cortical adenomas					
Females	7/49	8/49	7/50	5/49	13/49

Adapted from Arnold and Krewski, 1988 and Arnold *et al*., 1985.

Arnold and colleagues (Arnold and Krewski, 1988; Arnold *et al*., 1985) provide one of the most detailed accounts of HCB-induced tumor formation available in the literature. They conducted a two-generation study in which parental Sprague-Dawley rats were treated by incorporating HCB in feed at 0.32, 1.6, 8, and 40 ppm for three months. These animals were then mated and the females continued to receive their HCB-based diets throughout pregnancy and lactation. At weaning, 50 pups of each sex were separated and

fed identically to their parents for the remainder of their lifetime or up to 2 years of age. Controls were fed an HCB-free diet. Based on the body weight and food consumption data, time-weighted-average dose levels received by the male rats were calculated to be 0.01, 0.05, 0.27 and 1.39 mg/kg-day, and those for the female rats were 0.01, 0.07, 0.35 and 1.72 mg/kg-day (OEHHA, 1988; U.S. EPA, 1985).

The findings for tissues exhibiting dose-related increases in tumors are provided in Table 13. Significant increases in neoplastic liver nodules (10/49) and adrenal pheochromocytomas (17/49) were observed in the F_1 females of the 40 ppm group compared to the controls (0/49 and 2/49, respectively). Significant dose-related trends were also observed for both of these tumors in the F_1 females. A significant elevation in the incidence of adrenal pheochromocytomas was also observed in the 40 ppm males.

A significant increase in the incidence of parathyroid tumors was observed for the F_1 males in the highest dose group (12/49) compared with the controls (2/48). A slight but statistically significant dose-related increase in parathyroid tumors was also seen in the F_1 female rats (Arnold and Krewski, 1988). The incidences were 1/49 at 8.0 ppm and 2/49 at 40 ppm as compared to 0/49 in the controls**.** In a similar fashion, an apparent increase in cortical adenomas was seen in the F_1 females.

Survival at 104 weeks was less than 50 percent in some low-dose groups. Other than a decreased viability index in the pups of the 40 ppm dose group from which the test (F_1) animals were selected, survival was reported to be unaffected by treatment.

A single generation study was also conducted by Arnold and co-workers (1985) in which male Sprague-Dawley rats were exposed to HCB in their feed at 40 ppm for 119 weeks. The HCB was given in combination with reduced (0.1 times normal), normal or increased (10 times normal) levels of dietary Vitamin A. Control groups receiving reduced, normal and increased dietary Vitamin A were included. No significant increase in tumors was seen in the HCB-treated rats although modest elevations were seen in tumors of the liver and thyroid.

A few recent studies have been conducted to identify possible mechanisms by which HCB induces tumors in animals. Carthew and Smith (1994) have hypothesized that some HCB-induced hepatic tumors may occur through a non-genotoxic mechanism (IPCS, 1997). They observed that the hepatotoxicity of HCB in rodents produces peliosis and necrosis with hemosiderosis, indicating that vascular damage had occurred. They confirmed the presence of these types of damage in the livers of rats chronically exposed to HCB by identifying widespread fibrin deposits. The deposits occurred in association with extensive hemosiderosis in hepatocytes and areas of widened hepatic sinusoids. Based on the results, they suggested that the formation of hepatomas and hemangiomas exhibiting elements of peliosis could be explained by a compensatory hyperplastic response to hepatocellular necrosis combined with a simultaneous loss of hepatocellular cords. The authors concluded that non-genotoxic compounds to which humans are exposed in very small non-cumulative amounts with no significant hepatotoxicity, or compounds to which the liver was not chronically exposed, could be considered as

lacking a significant carcinogenic risk to humans in spite of the occurrence of hemangiomas in rodents.

In studies of male rats treated with HCB, Bouthillier and co-workers (1991) reported that kidneys of the rats treated with 100 mg HCB/kg exhibited many of the histopathological features of protein droplet nephropathy (IPCS, 1997). Cytosolic α2u-globulin was increased 11-fold in the treated rats as compared to controls, and HCB was found to bind reversibly to the α2u protein. The authors proposed that HCB induced a male rat-specific nephropathy and that this could be the basis for the higher kidney tumor incidence seen in male rats as compared to female rats. The mechanism by which structurally diverse hydrocarbons induce hyaline droplets has been well documented and involves accumulation of α2u-globulin, leading to necrosis, regeneration, and, in some cases, tumor formation. These effects are sex- and species-specific, and are generally accepted as not being relevant to humans. It should be noted that this mechanism would not explain the dose-related increase in renal tumors that was seen in female rats in the same study (Erturk *et al*., 1986).

Lastly, because the adrenal, parathyroid and thyroid glands are all endocrine organs, the tumors observed in these organs may be due to chronic endocrine disruption with compensatory proliferation. However, mechanistic studies that address the relevance of these tumor types were not identified.

Other Routes

Theiss *et al*. (1977) gave 24 i.p. injections of 190, 480, or 960 mg/kg HCB to groups of 20 Strain A mice over an 8 week period. Animals were observed for an additional 24 weeks. The study was designed only to detect pulmonary carcinogens, and the lungs were the only tissues examined. No dose-related increases in the incidence of lung tumors were reported in either gender.

A review of the literature failed to produce studies assessing the carcinogenicity potential of HCB via other routes.

Toxicological Effects in Humans

Acute Toxicity

A search of the available literature failed to identify reports of acute toxic effects. However, several reviews have been published of an accidental poisoning incident in Turkey that occurred in 1955-1959 as a result of HCB-treated wheat grain being ground into flour and made into bread (Cam and Nigogosyan, 1963; De Matteis *et al*., 1961; Schmid, 1960). The affected individuals consumed HCB-treated wheat seed originally intended for agricultural usage that was improperly diverted for food. During the incident, an estimated 3,000 to 5,000 people acquired a mixed porphyria resembling porphyria cutanea tarda (PCT) (sometimes referred to as porphyria turcica) following

consumption of an estimated 0.05 to 0.2 g of HCB per day per person (Cam and Nigogosyan, 1963). The period between the initial ingestion of the treated wheat and the onset of disease was estimated to have been 6 months.

Subchronic Toxicity

Porphyria

HCB-induced toxic effects in humans have been extensively studied since the documentation of an HCB-induced porphyria outbreak in Turkey from 1955 to 1959 (Cam and Nigogosyan, 1963; De Matteis *et al*., 1961; Schmid, 1960). HCB-induced porphyria has been observed following both subchronic and chronic dosing studies in animals and is characterized by an elevated excretion of porphyrins and their metabolites. Disruption of the balance between the production and utilization of various hemoproteins through an inhibition of porphyrin metabolism, specifically uroporphyrinogen decarboxylase, appears to be the underlying cause of the illness.

Clinical symptoms reported in porphyric patients from the epidemic in Turkey include weakness, inability to handle eating utensils, inability to rise from a squat or climb stairs, loss of appetite, photosensitivity and development of erythema on the sun-exposed areas of the skin, and porphyrinuria with red or brown urine. Arthritis was common in the younger victims, with swelling and spindling of the fingers. Osteoporosis (primarily in the phalanges) developed in some of the patients with some individuals showing partial resorption of the digits. In some people these effects persisted for many years, whereas in others a reversal of the bone thinning and a return to normal was seen. Many of the patients had hepatomegaly early during the course of the disease with the incidence decreasing with time. Enlargement of the thyroid was reported as well as a high incidence of goiter (Gocmen *et al*., 1986). In about 10 percent of the cases, the porphyria led to the patients' death (Peters, 1976; Schmid, 1960).

Most of the victims of the epidemic were children and adolescents, with males being somewhat more susceptible than females (Cam and Nigogosyan, 1963; Cripps *et al*., 1984). Dogramaci (1964) conducted an extensive survey from 1958-1963 and reported that approximately 5 percent of the cases occurred in children less than 5 years of age while most other patients were between the ages of 6 and 15 years (1964). Adults above the age of 20 years accounted for less than 6 percent of the total cases reported.

Cam and Nigogosyan (1963) diagnosed a second distinctive disease in children under the age of 5 years. The clinical symptoms of the disease were evident in children of mothers who had shown either symptoms of PCT or had ingested contaminated food during gestation or lactation or both. The disease was described as pembe yara or "pink sore". The disease was so prevalent that virtually all of the children (reported as approximately 95 percent) below 2 years of age died in villages where HCB-contaminated food had been consumed (Peters, 1976). Accurate records are not available but it is estimated that between 1000 and 2000 of the breast-fed infants died during their first year of life (Cripps

et al., 1984). Porphyria was absent in these infants and toddlers who exhibited weakness, convulsions and localized cutaneous annular erythema (Cripps *et al*., 1980). Cases of pembe yara occurred more frequently in the years immediately following the outbreak of porphyria (1959-1962) (Cripps *et al*., 1984). Peters and associates (1966) were able to demonstrate elevated HCB levels in the breast milk from mothers in the region and it is believed that exposure by this route played an important role in the infants' exposure. HCB levels in breast milk continued to be elevated, albeit at lower levels, more than 20 years after the poisoning episode (Cripps *et al*., 1984).

Cripps *et al*. (1980) conducted a follow-up study in which 32 of the HCB-poisoned patients were examined 20 years later. Approximately half of the patients had clinical symptoms of the disease including hyperpigmentation, scarring, pinched facies, hypertrichosis, enlarged thyroid and arthritis. Cripps and co-workers (Cripps *et al*., 1984; Gocmen *et al*., 1986) conducted an additional follow-up study in 204 patients 20 to 30 years after the epidemic in Turkey. They examined 132 males and 72 females with an average age of 32.1 years at the time of the examination. The patients continued to show signs of neurological, dermatological, and orthopedic abnormalities. Small stature (44 percent), small hands (64 percent) and painless arthritis (64 percent) were evident in many of the individuals affected prior to puberty. Other symptoms observed in the patients include severe residual scarring (85 percent), pinched facies (42 percent), hirsutism (47 percent) and hyperpigmentation (71 percent). Excretion of porphyrins was significantly elevated in seventeen of the examined patients, particularly uroporphyrins in the urine and stools. Although there was no evidence of an increased incidence of tumors in this population, enlargement of the thyroid was observed in 37 percent of the patients (59 percent of the females), which was well above the average of 5 percent in that area of Turkey.

Gocmen *et al*. (1989) published an additional follow-up study 20 to 30 years after exposure in which 252 patients were evaluated. Clinical findings from the study are summarized in Table 14 and are similar to those reported previously (Cripps *et al*., 1984).

Milk samples from porphyric mothers had an average HCB concentration of 0.51 ppm which was above the controls which averaged 0.07 ppm. There was not a statistically significant difference between the mean urine and stool porphyrin values measured in 84 children of porphyric mothers and those from 24 control children.

Cutaneous porphyria has also been evaluated in a number of others studies following unintentional exposure to HCB. Burns *et al*. (1974) evaluated approximately 20 vegetable pesticide applicators spraying HCB-contaminated dimethyl-1,2,3,5,6-tetrachloroterephthalate (DCPA) working in the lower Rio Grande Valley. Nineteen of 20 workers had measurable plasma levels of HCB, ranging from less than 1 to 310 ppb and averaging 40 ± 63 ppb. Physical examination or medical history of the workers failed to detect any evidence of cutaneous porphyria. There was also no correlation between plasma HCB levels and urinary uroporphyrins or coproporphyrins regardless of whether porphyrins were based on 24-hour excretion amounts or per gram of creatinine.

Table 14. Clinical Findings of Porphyria Turcica 30 Years After Onset

Symptom	No. of patients with symptoms / total patients examined	Percent patients with symptom
Severe scarring	211/252	83.7
Hyperpigmentation	164/252	65.0
Hypertrichosis	113/252	44.8
Pinched facies	101/252	40.1
Fragile Skin	85/252	33.7
Arthritis	177/252	70.2
Small hands	168/252	66.6
Short stature	106/252	42.1
Weakness	157/252	62.3
Paresthesia	135/252	53.6
Sensory shading	131/216	60.6
Myotonia	82/216	37.9
Cogwheeling	70/167	41.9
Enlarged liver	12/252	4.8
Enlarged thyroid	88/252	34.9

Adapted from Gocmen *et al*., 1986.

No correlation was seen between HCB levels and the levels of three serum enzymes, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, or lactate dehydrogenase.

Burns and Miller (1975) evaluated the plasma HCB levels of 86 residents living and/or working in an area of Louisiana where transportation and disposal of chemical waste containing HCB took place. Plasma levels of HCB were also correlated with demographics, occupation, food analysis and analysis of house dust. Control samples were obtained from an area 16 km to the north-northwest of the study region. Average plasma HCB levels in the exposed subjects ranged from 2.4 to 3.6 ppb as compared with 0.5 ppb in controls. Higher residue levels were found in (4.71 ppb) males than (2.79 ppb) females, but there were no age differences. There was also no correlation with ethnicity or exposure through consumption of homegrown vegetables or animals. Approximately 68 percent of the homes evaluated in the test region had detectable levels of HCB averaging 380 ppb compared to 30 percent of the homes in the control area with an average of 20 ppb. A significant correlation was found between HCB levels in house dust and plasma levels in residents of the corresponding home. There was no evidence of cutaneous porphyria by history and skin examination.

Genotoxicity

A search of the available scientific literature failed to identify studies regarding the genotoxic effects of HCB in humans. Based on the limited bacterial data available, HCB is suggested not to be genotoxic.

Developmental and Reproductive Toxicity

A follow-up study of the Turkish women who were exposed to HCB in the 1950s was conducted approximately 40 years later (Jarrell *et al*., 1998) to determine adverse reproduction outcome. Three groups were compared in this retrospective cohort study; one group of women previously exhibiting PCT, a control group from the region and a control from the Turkish capital of Ankara. The group of women previously exhibiting PCT reported a small decrease in the percentage of live births as compared to the other comparison groups. The other reproductive outcomes measured did not differ consistently between the three groups. The authors concluded that the induction of porphyria was not associated with adverse reproductive outcomes when measured 40 years after exposure. As part of the study, serum levels of HCB were measured in all three groups. The previously exposed women in this study did not report evidence of premature ovarian failure nor were associations seen between serum FSH, estradiol or inhibin levels and serum HCB. Interestingly, a significant association between the concentration of HCB in the serum and the risk for spontaneous abortion was seen when the analysis was conducted using the combined values from all three groups.

In a recent study in Germany, HCB concentration in neonatal serum correlated with maternal age ($r = 0.249$; $p < 0.01$), with 2.7-fold higher serum levels in offspring of 40-year-old as compared with 20-year-old women. The authors concluded that the neonatal burden depends on maternal age and duration of pregnancy. This reflected the increase in body accumulation with these substances during human life as well as continuous transplacental transfer from mother to fetus during pregnancy (Lackmann *et al*., 1999). In a followup, Lackmann (2002) reported that neonatal blood levels of hexachlorobenzene (and PCBs) have greatly decreased over the last 15 years. This is presumably due to decreased use of PCBs, and decreasing maternal exposures.

Neurotoxicity

HCB has also been reported to cause neurological effects in man. Two studies reported neurological effects from HCB exposure of humans in Turkey. The consumption of HCB-contaminated bread resulted in muscle weakness (66 percent), paraesthesia (54 percent), neuritis (63 percent), myotonia (49 percent), and occasional 'cogwheeling' (29 percent) (Cripps *et al*., 1984; Gocmen *et al*., 1986). A dose or duration of exposure was not established for these effects. However, Cam and Nigogosyam (1963) estimated a dose of 0.05-0.2 g/day (0.7-2.9 mg/kg-day for a 70-kg person) for exposure, which has been considered to be reliable.

Immunotoxicity

Limited evidence for HCB-induced alterations to the human immune system exists from occupational and environmental studies of human populations. Queiroz and associates (Queiroz *et al*., 1997, 1998a,b, 1999) examined 51-66 workers exposed to HCB while working in a chemical plant producing carbon tetrachloride and perchloroethylene. The study participants in the exposed group showed impaired functions of neutrophilic granulocytes compared to those of a control group of 48 non-exposed age- and sex-related individuals. Neutrophils from the HCB-exposed individuals also exhibited a significant reduction in chemotaxis along with a significantly reduced respiratory burst activity (measured by nitroblue tetrazolium dye reduction). There was, however, no correlation between the length of exposure or HCB concentrations in the blood and alterations in neutrophil functions (Queiroz *et al*., 1997). Increased serum IgM and IgG levels were also observed in these workers although IgA levels were normal (Queiroz *et al*., 1998a). In this case, a correlation was seen between IgM levels and length of HCB exposure.

In a subsequent study, phagocytosis and killing of *Candida albicans* and *Candida pseudotropicalis* by polymorphonuclear granulocytes from the HCB-exposed workers and non-exposed workers were compared (Queiroz *et al*., 1998b). There was no HCB-related effect on phagocytosis, whereas lysis of *C. albicans* and *C. pseudotropicalis* was significantly decreased in the HCB-exposed group as compared to controls. There was, however, no correlation between the length of exposure or HCB blood levels and the changes in polymorphonuclear cell function.

In a recent study of the susceptibility of Inuit infants to infections and their immune status, an association between breast milk concentration of HCB (as well as other persistent organochlorine compounds) and risk for otitis media was seen during the first year of life (Dewailly *et al*., 2000). Both the risk of experiencing one episode of acute otitis media and the risk of experiencing three or more episodes increased with HCB exposure levels among breast-fed Inuit infants during the first year of life. Although the association was the most consistent with HCB and p,p'-DDE, it is not possible to determine with confidence which organochlorine compounds are responsible because all of the organochlorine compounds originate from the same food sources and are highly inter-correlated. None of the immunological parameters measured showed an association with prenatal organochlorine exposures.

Carcinogenicity

Grimalt and associates (1994) reported a small study of cancer incidence (129 cases in all) among the inhabitants of Flix, Spain; a small village located near an organochlorine compound manufacturing plant. Air levels of HCB were higher in Flix (35 ng/m^3) than in nearby Barcelona (0.3 ng/m^3). Serum HCB levels were obtained from a non-random sample of the general population and were recruited from among those who carried out routine clinical blood analysis. Samples collected from a hospital in Barcelona, Spain

served as controls. The mean HCB level in serum obtained from village inhabitants was 26 µg/L whereas the mean level from the reference population was 4.8 µg/L. Compared with the cancer incidence rates for the province as a whole, there was a significant excess of thyroid neoplasms and soft-tissue sarcoma in men. It should be noted that the reported increases were based on only two and three cases, respectively and all of the cases for whom occupational histories were available (~50 percent) had worked in the factory.

Subsequent to the previous report, a cross-sectional study was performed in Flix, Spain (Herrero *et al*., 1999). Residents that participated in the study were those above the age of 14 years. High air levels of HCBs had previously been found in the city and these were more than 100 times those of nearby Barcelona (Grimalt *et al*., 1994). Biological samples were obtained from a random sample of the total population (n =777), from which 324 inhabitants provided blood and 24-hour urine samples. An additional 280 inhabitants responding to a questionnaire also provided blood and 24-hour urine samples. One hundred eighty five of the participants were employed in the chemical plant. HCB was detected in all serum samples ranging from 1.1-1616.0 ng/ml with a mean of 39.8 ng/ml. HCB levels were higher in males (mean = 72.8 ng/ml) than females (mean = 17.7 ng/ml). Higher levels of HCB were found in factory workers (mean = 93.4 ng/ml), most of whom were males. There was no association between HCB serum levels and porphyrin levels for the total population of 604 study participants. One of the chemical plant workers in the study with a porphyrin excretion of 1009 nmol/L had an increase of uroporphyrin and hepatocarboxylporphyrins. The authors concluded that serum HCB levels measured in the study population, although much higher than those found in other studies to date, are probably not as high as those from the incident in Turkey during the late 1950s. Furthermore, the elevated body burden of HCB as measured in the study was not sufficient to trigger a significant alteration of the heme biosynthesis pathway.

In recent years there has been substantial interest in the possible association between exposure to persistent organochlorine compounds as measured by their tissue levels and increased risks of various cancers, such as breast cancer. Because of its persistent nature and ability to accumulate in body fat, HCB is frequently detected in these studies. In some cases, increased levels of HCB have been detected in the tissues of cancer patients as compared to control tissues (Guttes *et al*., 1998; Liljegren *et al*., 1998; Dorgan *et al*., 1999). However, in others, no association has been seen (Mendonca *et al*., 1999; Moysich *et al*., 1998; Scheele *et al*., 1996; Zheng *et al*., 1999). In cases where the positive associations have been seen, frequently there was no clear dose response, or the effects were seen within a subset of the study group. At this point, there is no consensus within the scientific community whether there is a significant link between organochlorine tissue levels and human cancer risk.

DOSE-RESPONSE ASSESSMENT

As described in the previous sections, HCB exposure is associated with a wide variety of toxic effects in humans and experimental animals. Serious hepatotoxic, neurotoxic,

reproductive, developmental and carcinogenic effects have been seen. Although considerable information exists about the adverse effects of HCB for humans, there is much less certainty about the doses at which these effects were seen. Cam and associates (1963) estimated that the affected individuals in Turkey consumed approximately 50 to 200 mg HCB per day. However, it is not clear how these dose estimates were obtained or even the body weights of the affected individuals. Since most of those affected in the Turkish epidemic were children and adolescents, one can estimate that their doses were approximately 0.9 to 10 mg/kg-day assuming a body weight of 20 to 58 kg. The critical doses for the infants who died of pembe yara would be those of the mothers. Assuming a maternal body weight of 60 kg, an estimate of the maternal dose would be 0.8 to 3.3 mg/kg-day. At this estimated maternal dose, 95 percent of the newly born and breast-fed infants were reported to have died.

On the other end of the spectrum, porphyria and related effects were not seen in the adult inhabitants of Flix, Spain who reportedly had chronic exposure to air concentrations of 35 ng HCB/m^3. If one assumes that this is the only source of exposure and that a 70-kg person inhales 20 m^3 per day, these individuals would be exposed to approximately 10 ng/kg-day.

Although valuable for providing reference points, we do not believe that these doses can be confidently used for quantitative risk assessment, particularly for estimates in the low dose region.

Noncarcinogenic Effects

A large number of animal studies have been conducted to assess the adverse effects of HCB at relatively low doses. A summary of the key non-cancer studies, the estimated lowest observable exposure levels, and the critical effects induced by HCB are shown in Table 15. As can be seen in the table, upon subchronic and chronic exposure, effects were frequently observed at daily doses below 1 mg/kg-day.

Two studies in which adverse effects were seen at low exposure levels appear to be particularly appropriate for deriving a PHG for non-cancer effects – the two-generation study in rats (Arnold and Krewski, 1988; Arnold *et al*., 1985) and the reproductive study in mink (Bleavins *et al*., 1984a). In the two-generation study conducted by Arnold *et al*. (1985), significant increases in both hepatic peribiliary lymphocytosis (27 animals affected out of 48 total animals) and hepatic fibrosis (23/48) were seen at the 0.32 ppm (0.01 mg/kg-day) exposure level. However, there was no dose-related trend in these parameters through 40 ppm and these changes were fairly common in the control animals (16/48 and 13/48, respectively). Clear dose-related increases in centrilobular basophilic chromogenesis were seen in both male and female rats. Given the clear trend, the increases seen in the females at the 0.32 ppm (5/49) and 1.6 ppm concentrations (7/50), while not statistically significant, may be biologically significant when compared to the controls (2/49). HCB has been clearly shown to be hepatotoxic in humans and experimental animals, and the Arnold *et al*. results indicate that modest hepatotoxic

effects can be seen at low HCB exposure concentrations. Therefore the LOAEL for this study appears to be 0.32 ppm (0.01 mg/kg-day). A NOAEL was not identified.

Table 15. Summary of Key Non-Cancer Toxicity Studies on Hexachlorobenzene

Species	Route	Duration	LOAEL	Effect	Reference
Rat	Oral (diet)	Up to 130 wks in utero, through gestation, then via diet	0.01 mg/kg-day	Peribiliary (liver) lymphocytosis and fibrosis; centrilobular chromogenesis. Decreased pup survival at 1.5 mg/kg (males) and 1.90 mg/kg (females)	Arnold *et al*., 1985
Rat	Oral (diet)	Exposed 15 wks and held to 48 wks	2.0 mg/kg-day	Increased liver porphyrin levels in females Increased centrilobular size	Kuiper-Goodman *et al*., 1977
Rat	Oral (gavage)	5 to 15 wks	1 mg/kg-day	Altered calcium homeostasis and bone morphometry	Andrews *et al*., 1989, 1990
Rat	Oral (diet)	Chronic	0.2 mg/kg-day	Liver enzyme induction	Grant 1974
Rat	Oral (diet)	Through gestation and nursing to 5 wks of age	0.2 mg/kg-day (maternal)	Increase humoral and cell-mediated immune response; accumulation of macrophages in lung	Vos *et al*., 1983
Rat	Oral (diet)	3, 6 or 12 mon	0.25-0.3 mg/kg-day	Liver ultrastructural changes (proliferation of SER, altered mitochondria, increased number of storage vesicles)	Mollenhauer *et al*., 1975, 1976
Rat	Oral (diet)	75-90 wks	6-8 mg/kg-day	Decrease in body weights, enlarged livers and porphyria	Smith and Cabral, 1980
Beagle Dog	Oral (capsule)	1 yr	0.12 mg/kg-day	Nodular (dose related) hyperplasia of gastric lymphoid tissue in all treated animals	Gralla *et al*., 1976
Pig	Oral (diet)	90 days	0.5 mg/kg-day	Increased coproporphyrin	Den Tonkelaar *et al*., 1978
Mice	Oral (diet)	3, 6 or 18 wks	0.6 mg/kg-day	More susceptible to Leishmania infection, decreased resistance to tumor cell challenge, decreased spleen cytotoxic macrophage activity	Loose *et al*., 1981, 1982

Table 15. Summary of Key Non-Cancer Toxicity Studies on Hexachlorobenzene (Continued)

Species	Route	Duration	LOAEL	Effect	Reference
Mice	Oral (diet)	Exposed through gestation and nursing to 45 days of age	0.5 mg/kg-day	Depressed delayed hyper-sensitivity response to oxazolone	Barnett *et al*., 1987
Mink	Oral (diet)	47 weeks	0.16 mg/kg-day (maternal dose)*	Decreased birth weights and increased mortality of mink kits with *in utero* and lactational exposure Altered neurotransmitter concentration in hypothalamus of adult dams	Bleavins *et al*., 1984a,b
Monkey (infants)	Oral (nursing)	60 days	64 mg/kg-day (maternal dose) 7.51-186 ppm milk	2 of 3 infants died resulting from exposure to HCB in breast milk	Bailey *et al*., 1980
Monkey	Oral (gavage)	60 days	8 mg/kg-day	Dose-related pathology in liver, kidney, ovaries, and thymus	Iatropoulos *et al*., 1976
Cynomol gus monkey	Oral (capsule)	90 days	0.1 mg/kg-day	Degenerative ultra-structural changes in ovarian surface epithelium	Babineau *et al*., 1991

*Dose calculated based upon Moore, 1997.

Bleavins and associates studied the effects of HCB on the reproductive fitness of dark minks (Bleavins *et al*., 1984a). Following the administration of HCB in the diet, a number of adverse effects were seen including decreased birth weights, increased mortality of the young and altered neurotransmitter levels in the brain of the adult dams. Mortality of the young mink increased in a dose-related fashion increasing from 8.2 percent in the controls to 44.1 percent, 77.4 percent and 86.7 percent in animals when the mothers were fed diets containing 1 ppm, 5 ppm and 25 ppm HCB, respectively.

A NOAEL was not seen in the Bleavins *et al*. (1984a) study, with 1 ppm (0.16 mg/kg-day) being a LOAEL. Benchmark modeling has been utilized to define a 20 percent response level for this study of 0.06 mg/kg-day (Moore *et al*., 1997). However, for our purposes, we will estimate a NOAEL utilizing a 10-fold uncertainty factor from the LOAEL of 0.16 mg/kg-day.

Similar perinatal mortality effects were seen in other species at somewhat higher doses (Arnold *et al*., 1985; Bailey *et al*., 1980; Bleavins *et al*., 1984a; Courtney, 1979; Grant *et al*., 1977; Vos *et al*., 1983). Due to the high levels of infant mortality observed in the

Turkish epidemic, these results would seem particularly relevant. In addition, the 77 to 87 percent mortality seen in the mink offspring of mothers exposed to 5 and 25 ppm HCB (0.8 and 4 mg/kg-day) is quite close to the 95 percent mortality figure reported for the Turkish infants that occurred at estimated maternal doses of 0.8 to 3.3 mg/kg-day (Cam and Nigogosyan, 1963; Peters, 1976). This suggests that the mink is likely to be a relevant and appropriate model for estimating the reproductive effects of HCB in humans.

Carcinogenic Effects

HCB has not been shown to be carcinogenic in humans. However, the epidemiological studies to date have not been designed to measure increases in cancer incidence and are therefore inadequate for risk assessment. HCB has been clearly shown to be tumorigenic in rats, hamsters and mice following chronic administration in the diet. Increases in tumors were seen in the liver and kidney as well as the adrenal, parathyroid and thyroid glands. To date, the mechanisms underlying carcinogenesis in these organs remain unclear.

As described above, several theories have been proposed to explain the basis for certain tumors induced by HCB. While these theories merit further study, none of them has been sufficiently well tested or established at this time for us to recommend that a non-linear dose-response approach be used to estimate risks at low exposure levels. Given the current mechanistic uncertainty, we have used a linear approach to extrapolate carcinogenic risks from high to low doses.

To estimate these risks, cancer slope factors (CSFs) were derived from key rodent studies in which significant increases in tumors were seen. A listing of the CSFs by study and species is shown in Table 16. The LED_{10} values were generated using the U.S. EPA benchmark dose software. To derive the animal CSF, 0.1 was divided by the LED_{10}. The animal CSF was then converted into a human CSF by using the body surface area conversion [CSF (human) = CSF (animal) x $\{(BW_{human})/(BW_{animal})\}^{1/4}$].

Earlier guidelines for cancer risk assessment, including those formerly used by OEHHA in establishing other PHGs have required the use of the linearized multistage (LMS) model to estimate an upper bound on the low-dose potency (q_1^*). This was used, for example, in the review of tetrachloroethylene (Alexeeff *et al*., 1992). The animal cancer potency estimates obtained with LMS (MSTAGE v. 1.1) ranged from 0.009 to 0.321 $(mg/kg\text{-}day)^{-1}$. The values are listed in Table 16. These values were then converted into a human q_1* by using the body surface area conversion [CSF (human) = CSF (animal) x $\{(BW_{human})/(BW_{animal})\}^{1/4}$]. Both of the human potency estimates, the CSF and q_1*, were used in calculating the PHG because a substantial part of our current experience-base is with the LMS model.

Table 16. HCB Cancer Potency Estimates for Critical Endpoints from Key Studies

Tumor Type	q_1* (animal) $(mg/kg\text{-}d)^{-1}$	q_1* (human) (mg/kg-d)	CSF (animal) $(mg/kg\text{-}d)^{-1}$	CSF (human) $(mg/kg\text{-}d)^{-1}$
Arnold *et al.* (1985); Sprague-Dawley rat (BW 0.353 kg female and 0.653 kg male)				
Females				
Adrenal cortical adenoma	0.156	0.585	0.148	0.56
Liver neoplastic nodule	0.218	0.818	0.207	0.78
Pheochromocytoma	0.307	1.152	0.291	1.09
Parathyroid adenoma	0.155	0.582	0.155	0.58
Males				
Pheochromocytoma	0.296	0.952	0.281	0.90
Parathyroid adenoma	0.066	0.212	0.063	0.20
Lambrecht *et al.* (1983a, b) and Erturk *et al.* (1986); Sprague-Dawley rat (BW 0.265 kg female and 0.4 kg male)[a]				
Females				
Pheochromocytoma	0.321	1.294	0.261	1.05
Hepatoma/hemangioma	0.160	0.645	0.130	0.52
Hepatocarcinoma	0.314	1.262	0.255	1.03
Bile-duct adenoma or carcinoma	0.120	0.484	0.098	0.39
Renal cell adenoma	0.050	0.202	0.040	0.16
Males				
Hepatoma/hemangioma	0.050	0.182	0.045	0.1
Hepatocarcinoma	0.019	0.069	0.017	0.06
Bile-duct adenoma or carcinoma	0.012	0.044	0.012	0.04
Renal cell adenoma	0.267	0.971	0.239	0.87
Cabral *et al.* (1979); Swiss mice (BW 0.035 kg for both female and male)[a]				
Females - Liver	0.010	0.067	0.011	0.07
Males - Liver	0.010	0.067	0.010	0.07
Cabral *et al.* (1977); Syrian golden hamster (BW 0.1 kg for both female and male)[a]				
Females				
Hepatoma	0.147	0.756	0.140	0.72
Liver hemangioendothelioma	0.011	0.057	0.011	0.06
Males				
Hepatoma	0.188	0.967	0.178	0.92
Thyroid alveolar adenoma	0.009	0.046	0.009	0.05
Liver hemangioendothelioma	0.032	0.165	0.031	0.16

[a]The body weight values used were obtained from U.S. EPA (1985).

The derived human CSFs and q_1* range from 0.04 to 1.09 and 0.044 to 1.294 (mg/kg-day)$^{-1}$. Of the three species, the mouse was the least sensitive to the tumorigenic effects of HCB. Adrenal pheochromocytomas and hepatocarcinomas in female rats exhibited the highest potency values derived from the Arnold *et al*. (1985) and Lambrecht *et al*. (1983a,b) studies.

For estimating risks in humans, the human CSF of 1.09 and q_1* of 1.294 derived from the adrenal pheochromocytoma data in female rats from the two-generation study by Arnold and associates (1985) and Lambrecht *et al*. (1983a,b), respectively, will be used. These were the most sensitive endpoints. The unique design of the Arnold study involving exposure to relatively low concentrations of HCB in the diet (including *in utero* and lactational exposure) seems particularly relevant for estimating risks due to low level ambient exposure. In addition, the overall report and presentation of the pathology data were much more comprehensive in this study than for the other studies.

Carcinogen risk assessment guidelines used by OEHHA normally recommend selection of human cancer potency estimates based on the most sensitive study, site and species. This applies unless there is evidence to indicate that the most sensitive site(s) are not relevant to human cancer induction, or represent data sets with unusually wide error bounds. However, the selection of a potency value may take into account the appropriateness of the route of exposure in the various studies, and a geometric mean of several estimates may be chosen where several similar values are available. In this case, the values from the Arnold *et al*. (1985) and Lambrecht *et al*. (1983a,b) studies are preferred both because of appropriateness of the route to a proposed public health goal for drinking water, and by the most sensitive study/site/species criterion. The values for hepatocarcinoma [1.262 (mg-metabolized/kg-day)$^{-1}$] and adrenal pheochromocytomas [1.09 and 1.294 mg-metabolized/kg-day)$^{-1}$] in female rats are not regarded as significantly different. The preferred value for the oral cancer potency is a CSF_{human} of 1.09 (mg-metabolized/kg-day)$^{-1}$ for adrenal pheochromocytomas in female rats in the gavage studies by Arnold *et al*. (1985) and for the cancer potency estimate $(q_1*)_{human}$ of 1.294 (mg-metabolized/kg-day)$^{-1}$ for the same endpoint from the gavage studies of Lambrecht *et al*. (1983a,b).

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or non-carcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water and for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets and other household uses that may result in dermal and inhalation exposures.

Noncarcinogenic Effects

The primary hepatotoxic, developmental toxicity and neurological effects in rodents were observed in the subchronic and chronic studies. The more sensitive effects reported were the developmental toxicity and hepatotoxic effects. The most sensitive developmental toxicity effect consisted of increased kit mortality in minks following the administration of 0.16 mg/kg-day over the study period (Bleavins *et al*., 1984a). This dose was used to calculate a noncarcinogenic health-protective level below. An uncertainty factor (UF) of 1,000 was used in the calculation, consisting of 10 for use of a LOAEL rather than a NOAEL, 10 for interspecies extrapolation, and 10 to account for interindividual variability.

A health-protective level was also calculated for the other sensitive noncancer endpoint, slight liver changes (centrilobular basophilic chromogenesis) in female rats during a 2-generation study (Arnold *et al*., 1985) at a dose of 0.01 mg/kg-day. For this endpoint the uncertainty factors were 3 for the use of a LOAEL of probable biological significance, but no statistical significance; 10 for interspecies extrapolation; and 10 for human variability, for a total UF of 300.

Calculation of a public health-protective concentration (C, in mg/L) for HCB in drinking water for non-carcinogenic endpoints follows the equation:

$$C = \frac{\text{LOAEL (mg/kg-d)} \times \text{BW} \times \text{RSC}}{\text{UF} \times \text{L/day}} = \text{mg/L}$$

where,

LOAEL	=	lowest-observed-adverse-effect-level;
BW	=	adult body weight (default 70 kg);
RSC	=	relative source contribution (default 0.2, or 20 percent);
UF	=	uncertainty factors (common default values include 10 for use of a LOAEL rather than a NOAEL, 10 for interspecies extrapolation, and 10 for potentially sensitive human subpopulations);
L/day	=	adult daily water consumption rate (2 L/day).

For the developmental endpoint of Bleavins *et al*. (1984a), the estimate of the health-protective concentration of HCB in drinking water from the LOAEL of 0.16 mg/kg-day and combined uncertainty factors of 1,000 as discussed above is:

$$C = \frac{0.16 \text{ mg/kg-d} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ L/day}} = 1.12 \times 10^{-3} \text{ mg/L} = 1.1 \text{ ppb}$$

Using the LOAEL for mild liver changes from the Arnold *et al*. (1985) study of 0.01 mg/kg-day, and combined uncertainty factors of 300, the estimate of the health-protective concentration of HCB in drinking water is:

$$C = \frac{0.01 \text{ mg/kg-d} \times 70 \text{ kg} \times 0.2}{300 \times 2 \text{ L/day}} = 2.3 \times 10^{-4} \text{ mg/L} = 0.23 \text{ ppb}$$

OEHHA concludes that a health-protective concentration for non-cancer effects is 0.23 ppb based on the mild liver changes reported by Arnold *et al*. (1985). The protective concentration derived below for cancer is lower, and therefore the drinking water concentration proposed below to protect against carcinogenic effects is also protective against non-cancer chronic toxicity.

Carcinogenic Effects

For carcinogens, the following general equation can be used to calculate the public health-protective concentration (C) for HCB in drinking water (in mg/L):

$$C = \frac{R \times BW}{q_1^* \text{ or CSF} \times \text{L/day}} = \text{mg/L}$$

where,

R	=	*de minimis* level for lifetime excess individual cancer risk (a default of 10^{-6});
BW	=	adult body weight (a default of 70 kg);
q_1* or CSF	=	cancer slope factor, where q_1* is the upper 95 percent confidence limit on the cancer potency slope calculated by the LMS model, and CSF is a potency derived from the lower 95 percent confidence limit on the 10 percent tumor dose (LED_{10}); CSF = 10 percent/LED_{10}; both potency estimates are converted to human equivalent [in $(\text{mg/kg-day})^{-1}$] using $BW^{3/4}$ scaling;
L/day	=	daily volume of water consumed by an adult (a default of 2 L/day is used for direct oral consumption).

The potency estimates for carcinogens are calculated by both methods because a substantial part of our current experience-base is with the LMS model. The new methodology, which is based on the LED_{10} and is similar to that proposed by U.S. EPA (1999) in its proposed guidelines for carcinogen risk assessment, has been in regular use for only a few years. It may therefore present problems of interpretation, particularly

when comparisons with earlier risk estimates are necessary. The LMS model focuses on the linear low dose extrapolation, and analysts (e.g., U.S. EPA) have often accepted relatively poor fits to the observed tumor incidence data. The new method places a higher premium on fitting the observed data to estimate the ED_{10} and the 95 percent lower bound (LED_{10}), the point from which the low dose extrapolation is made (U.S. EPA, 1999). In the specific case of HCB, the two methods show no major divergences in results, reinforcing the confidence in use of the LED_{10} methodology.

For the carcinogenic endpoint and the linear approach based on the oral CSF_{human} of 1.09 $(mg/kg\text{-}d)^{-1}$ for pheochromocytomas in female rats from Arnold *et al*. (1985), the water concentration equivalent to a negligible lifetime theoretical cancer risk of 10^{-6} can be calculated as follows:

$$C = \frac{1\text{x}10^{-6} \text{ x } 70 \text{ kg}}{(1.09 \text{ (mg/kg-d)}^{-1}) \text{ x } 2 \text{ L/day}} = 3.2 \text{ x } 10^{-5} \text{ mg/L} = 0.03 \text{ ppb}$$

For the LMS model using a cancer potency estimate $(q_1^*)_{human}$ of 1.294 $(mg/kg\text{-}d)^{-1}$ for pheochromocytomas in female rats from Lambrecht *et al*. (1983a,b), the water concentration equivalent to a negligible lifetime theoretical cancer risk of 10^{-6} can be calculated as follows:

$$C = \frac{1\text{x}10^{-6} \text{ x } 70 \text{ kg}}{(1.294 \text{ (mg/kg-d)}^{-1}) \text{ x } 2 \text{ L/day}} = 2.7 \text{ x } 10^{-5} \text{ mg/L} = 0.027 \text{ ppb}$$

The average of the results of these two dietary exposure studies is $2.95\text{x}10^{-5}$ mg/L or 0.03 µg/L (ppb). A PHG of 0.03 ppb is therefore proposed, based on the induction of pheochromocytoma in female rats exposed orally to HCB in both studies.

RISK CHARACTERIZATION

Hexachlorobenzene (HCB) is a chlorinated aromatic hydrocarbon that was widely used as a seed dressing for prevention of fungal growth on crops, and as a component of fireworks, ammunition, and synthetic rubbers. Restrictions in the 1970's resulted in the decline of HCB manufacturing and subsequent human exposures (Lackmann, 2002). Currently, HCB continues to be produced as an unintentional by-product in the manufacturing of chlorinated solvents, aromatics and pesticides (IPCS, 1997). With its resistance to environmental degradation and mobility, HCB is widely distributed throughout the environment. From 1987 to 1993, HCB releases to the water were estimated at 1,286 pounds according to the U.S. EPA's Toxic Release Inventory (U.S. EPA, 1998). These releases were primarily from alkali, chlorine, and agricultural

chemical industries. The public health risks of exposure to HCB can be characterized as follows:

Acute and Chronic Health Effects

Typical exposures to HCB in drinking water are not expected to result in any acute health effects, due to the low levels involved. This includes household airborne exposures from showering, flushing toilets, etc. Various health complaints, including liver and neurological changes, have been reported as a result of ingestion of HCB contaminated foodstuff. An increased incidence of infant mortality has also been reported among mothers exposed to HCB contaminated food. However, the effective levels for these changes do not indicate that humans exposed to typical background levels in the general environment are at significant risk of experiencing such effects. Levels protective against carcinogenicity should also be adequate to protect against all non-cancer effects in the general population, and in sensitive subpopulations including infants, children, and the elderly. The widespread detection of HCB in the environment and in the diet justifies the use of the default 0.2 for relative source contribution from drinking water.

Carcinogenic Effects

In animal studies, oral exposure to HCB produced increased incidences of adrenal pheochromocytomas in male and female rats, kidney tumors in male and female rats, and liver tumors in mice, rats, and hamsters. A summary of our evaluation is given below.

- OEHHA considers HCB to be an animal carcinogen and a possible human carcinogen.
- Four separate cancer bioassays have shown HCB to induce tumors at several sites, in three species (rats, mice, and hamsters), in both sexes, by the oral route of exposure.
- The cancer study results in these four studies show consistency, in that liver tumors and a high incidence of adrenal pheochromocytomas were induced by HCB exposures.
- The oral studies in rats were considered adequate for risk assessment of drinking water exposures to HCB.
- In general, studies investigating the genotoxicity of HCB are suggestive that HCB is not genotoxic.
- The mechanisms by which exposure to HCB leads to the observed tumors in mice, hamsters, and rats are unknown. For liver tumors, it is possible that non-genotoxic mechanisms (such as compensatory hyperplastic response to hepatocellular necrosis combined with a simultaneous loss of hepatocellular cords) may contribute to the effects observed, but this is unproven. Chronic endocrine disruption by HCB with compensatory proliferation has also been proposed as a potential mechanism for the tumors observed in the adrenal, parathyroid, and thyroid glands, but mechanistic studies that address the relevance of these tumor types were not identified.

- Lack of knowledge of the mode(s) of action of HCB in causing cancer in rodents and the implication of these processes for human disease is a limitation of this risk assessment.
- The proposed PHG of 0.03 ppb is based on the potency estimates for adrenal pheochromocytomas in two studies in female rats exposed to HCB by the oral route (diet).
- A CSF is an upper-bound estimate defined by the 95 percent confidence limit on the ED_{10}. It is theoretically possible that the true value of the cancer potency of HCB in humans could exceed this value, but that is considered unlikely. It is plausible that the true value of the human cancer potency for HCB has a lower bound of zero, based on statistical and biological uncertainties including interspecies extrapolation and mode of action.

According to this analysis, the health protective concentration of HCB in water, associated with a *de minimis* extra lifetime cancer risk, is 0.03 ppb (µg/L). The primary sources of uncertainty in the development of the proposed PHG for HCB in drinking water are also the general issues of uncertainty in any risk assessment, particularly inter- and intra-species extrapolation and issues relating to possible human exposure. We acknowledge that a more formal analysis of the uncertainties used in our approach would provide a more rigorous quantitative evaluation and description of the risks. However, such an analysis would add much more complexity to this already complicated analysis with little likelihood of any benefit.

The proposed PHG of 0.03 ppb was calculated based on the carcinogenic potency of HCB. In calculating this value, a *de minimis* theoretical excess individual cancer risk level of 10^{-6} was assumed. The corresponding levels for cancer risks of 10^{-5} or 10^{-4} are 0.3 and 3 ppb, respectively.

For PHGs, OEHHA's use of the relative source contribution (RSC) has generally followed U.S. EPA drinking water risk assessment methodology. The typical RSC range is 20 to 80 percent (0.2 to 0.8), depending on the scientific evidence. A more rigorous examination of relative exposure to environmental sources of HCB might justify an RSC for non-cancer effects other than the default value of 0.2, but this is judged unnecessary because the proposed PHG value is based on a cancer endpoint, which yields a lower health-protective value. For approaches that use low-dose extrapolation based on quantitative risk assessment, U.S. EPA does not factor in an RSC. The use of low-dose extrapolation is considered to be adequately health-protective without the additional source contributions, and is also estimated as extra risk (risk above background levels).

OTHER REGULATORY STANDARDS

The U.S. EPA has established a Maximum Contaminant Level Goal (MCLG) of zero and a Maximum Contaminant Level (MCL) of 0.001 mg/L for HCB (U.S. EPA, 2000a). These standards were based upon liver or kidney effects, reproductive effects, and

increased risk of cancer (U.S. EPA, 2000a), subject to analytical feasibility and a practical quantitation limit of 0.001 mg/L (U.S. EPA, 2002). In addition, the U.S. EPA has set Health Advisories for HCB (U.S. EPA, 1996). The 1-day, 10-day, and longer-term Health Advisories for a 10 kg child are all 0.05 mg/L. The longer-term Health Advisory for a 70 kg adult is 0.2 mg/L.

The U.S. EPA has established a reference dose of 0.0008 mg/kg-day and a Drinking Water Equivalent Level (DWEL) of 0.03 mg/L (U.S. EPA, 1996). The reference dose derived by the U.S. EPA was based upon the Arnold *et al*. studies (Arnold and Krewski, 1988; Arnold *et al*., 1985). In their judgment, no HCB-induced adverse effects were observed in the 0.32 and 1.6 ppm HCB F_1 groups and they considered these doses to be NOAELs. Although they recognized that significant ($p<0.05$) increases were observed in the incidences of periportal glycogen depletion at 1.6 ppm, peribiliary lymphocytosis at 0.32, 1.6 and 40 ppm, and peribiliary fibrosis at 0.32 and 40 ppm in the F_1 male rat groups, they did not consider these effects to be HCB-induced adverse effects because they were also observed in a large number of F_1 control males. They considered the increase in hepatic centrilobular basophilic chromogenesis observed in the 8.0 ppm groups to be the lowest biologically significant effect. Thus the U.S. EPA judged the NOAEL for this study to be 1.6 ppm (0.08 mg/kg-day). They applied a 100X uncertainty factor (10 for interspecies and 10 for intraspecies variability) to derive the reference dose of 0.0008 mg/kg-day.

In contrast, the Agency for Toxic Substances and Disease Registry, which also used the Arnold *et al*. study as the basis for establishing their Minimal Risk Level (MRL) for chronic exposure, had a different interpretation of the effects seen in the rat liver (ATSDR, 1997). They considered the peribiliary lymphocytosis and fibrosis to be biologically significant and used these as the basis for setting an MRL for chronic exposure at 0.00002 mg/kg-day. According to their calculation, the lowest exposure, 1 ppm, was equivalent to 0.016 mg/kg-day. They used uncertainty factors of 10 for using a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability. The MRL for acute exposure was set at 0.008 mg/kg-day and that for intermediate exposures was set at 0.0003 mg/kg-day. The OEHHA non-cancer chronic toxicity estimate for HCB falls between the U.S. EPA and the ATSDR values, being based upon an estimated LOAEL of 0.01 mg/kg from the Arnold *et al.* studies with a combined UF of 300.

The International Agency for Research on Cancer has evaluated HCB and listed it as a Group 2B carcinogen (IARC, 1987). They concluded that there was sufficient evidence that HCB is an animal carcinogen but that the evidence in humans was inadequate to make a determination.

The U.S. EPA determined HCB to be a probable human carcinogen (B2) and derived an oral slope factor of 1.6 $(\text{mg/kg-day})^{-1}$ based primarily upon the increase in hepatocellular carcinomas seen in the Lambrecht *et al*. study (Erturk *et al*., 1986; U.S. EPA, 2000b). The drinking water equivalent risk was calculated by the U.S. EPA to be $4.6\text{x}10^{-5}$ per (µg/L) (U.S. EPA, 2000b), which corresponds to a 1 in 1 million risk level of 0.02 µg/L.

HCB has also been listed by the National Toxicology Program in the 9th Annual Report on Carcinogens as an agent reasonably anticipated to be a human carcinogen (NTP, 2000).

The American Conference of Governmental Industrial Hygienists has listed HCB as a confirmed animal carcinogen with unknown relevance to humans (A3 classification) (ACGIH, 1993). They have also established a threshold limit value of 0.002 mg/m^3 as an 8-hr time-weighted-average for inhalation exposure.

HCB is also listed as a chemical known to cause cancer and reproductive toxicity by the California Environmental Protection Agency under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) (OEHHA, 2000).

For additional standards and regulatory levels at both the state and federal level, the reader is referred to the extensive listing in the ATSDR monograph (ATSDR, 1997).

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