EVIDENCE ON THE CARCINOGENICITY OF

1,3-HEXACHLOROBUTADIENE

FINAL

December 2000

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq*.) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity...if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of carcinogenicity are identified as the members of the Carcinogen Identification Committee of the OEHHA Science Advisory Board (22 CCR 12301).

1,3-Hexachlorobutadiene was assigned a final priority of 'high' carcinogenicity concern and placed on the Final Candidate list of chemicals for Committee review on August 6, 1999. A public request for information relevant to the assessment of the evidence on the carcinogenicity of this chemical was announced in the *California Regulatory Notice Register* on August 6, 1999. This document reviews the available scientific evidence on the carcinogenic potential of 1,3-hexachlorobutadiene. It was released as the draft document *Evidence on the Carcinogenicity of 1,3-Hexachlorobutadiene* in August 2000.

At their November 16, 2000 meeting the Committee, by a vote of none in favor and six against, did not find that 1,3-hexachlorobutadiene had been "clearly shown through scientifically valid testing according to generally accepted principles to cause cancer."

The following document is the final version of the document that was discussed by the Committee at their November 2000 meeting.

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1 EXECUTIVE SUMMARY

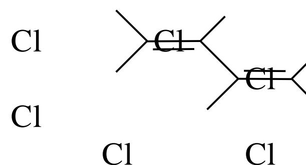
1,3-Hexachlorobutadiene (HCBD) is a chlorinated alkene. It is found predominantly as a by-product from the manufacture of chlorinated solvents and related products and is also used in some industrial processes. HCBD has been detected in air and water samples collected near chemical manufacturing facilities. It has been detected at low levels in human adipose tissue, fish, and cow milk.

HCBD induced renal tubular neoplasms (undifferentiated carcinoma, adenocarcinoma and adenoma) in female and male rats administered the chemical in the diet for 24 and 22 months, respectively. Metastases to the lung were observed in two cases. HCBD also has tumor promoting activity, promoting the development of renal cell tumors in male rats initiated with N-ethyl-N-hydroxyethylnitrosamine.

HCBD and its metabolites were genotoxic in bacteria and mammalian cell cultures. Exposure of rats or mice to HCBD led to the formation of renal DNA adducts. Although the mechanism of HCBD carcinogenesis has not been determined, there is evidence for a role for genotoxicity. The major metabolic pathway for HCBD, and the one postulated to result in formation of the ultimate DNA reactive metabolite, is the GSH/mercapturate/β-lyase pathway. However, more than one mechanism may be operative, as HCBD has also been shown to act as a renal tumor promoter and to increase the incidence of hyperplasia of the renal tubular epithelium in rats. Structural analogs that are known carcinogens and that produce renal tumors in rodents and exhibit similar metabolic pathways for the formation of genotoxic metabolites further contribute to the weight of evidence.

2 INTRODUCTION

2.1 Identity of 1,3-Hexachlorobutadiene (HCBD)



Molecular Formula:	C_4Cl_6
Molecular Weight:	260.76
CAS Registry No.:	87-68-3
Chemical Class:	Chlorinated alkene
Melting Point:	- 21°C
Boiling Point:	215°C
Vapor Pressure:	0.15 mm Hg (25°C)
Density:	1.55 gm/ml (20°C)
Partition coefficient:	log K_{ow} = 4.78
	Log K_{oc} = 3.67
Synonyms:	Perchlorobutadiene; HCBD; 1,1,2,3,4,4-Hexachloro-1, 3-butadiene; hexachlorobutadiene; Dolen-Pur; GP-40-66:120 (ATSDR, 1994).

2.2 Occurrence and Use

HCBD is found predominantly as a by-product formed during the manufacture of chlorinated solvents and related products (IARC, 1999b). It is also used directly as a solvent, heat transfer liquid, hydraulic fluid and lubricant and as a chemical intermediate in the manufacture of rubber compounds, chlorofluorocarbons and lubricants (ATSDR, 1994). Although HCBD is used outside the U.S. as a fumigant, such use does not occur in the U.S. (ATSDR, 1994).

HCBD has been detected in air samples collected near chemical manufacturing facilities, in effluent water from U.S. chemical manufacturing facilities, in Mississippi River water, and in U.S. drinking water (IARC, 1979; IARC, 1999b). NTP (1991) estimated between 3.3 to 6.6 million kilograms were produced annually in the U.S. The 1997 Toxics Release Inventory (TRI, 1999) reported 8.4 million pounds of HCBD as total production-related waste in the U.S. In California, between one and ten thousand pounds of HCBD, produced as a manufacturing by-product of chlorinated solvent manufacturing, was reported on-site at one plant (data from Toxics Release Inventory (TRI, 1992) for 1990, as described in ATSDR, 1994). In 1998 in California, 4071 pounds of HCBD were under waste management and five pounds were released to the air (TRI, 2000). The projected amounts of HCBD under waste management in 1999 and 2000 were given as 4274 and 4488 pounds respectively (TRI, 2000).

HCBD, at levels of 0.001 to 0.008 μg/gm wet weight adipose tissue (average, 0.004±0.000 μg/gm), was detected in 92/99 (93 percent) autopsied Canadian tissue samples taken from accident victims (Mes *et al.*, 1982). Among 29 Canadians whose cause of death was nonaccidental, the average level of HCBD in adipose tissue was 0.002 ± 0.000 μg/gm wet weight tissue (Mes *et al.*, 1985). No HCBD was detected in whole blood in any of 36 residents of Love Canal, New York or in any of 12 laboratory volunteers (Bristol *et al.*, 1982).

A survey of food products collected from farms and streams within 25 miles of ten perchloroethylene (PERC) or trichloroethylene (TCE) manufacturing plants revealed residue levels in fish fat ranging from 0.01 to 1.2 ppm, no residues in eggs or vegetables and a residue level of 1.32 ppm in the butterfat of one out of five milk samples (Yip, 1976).

3 DATA ON 1,3-HEXACHLOROBUTADIENE CARCINOGENICITY

Long-term rodent carcinogenicity studies have been conducted in male and female rats administered HCBD in the diet, and in male rats of a different strain administered HCBD by gavage. The ability of HCBD to induce lung tumors was investigated in one study in the male A/St mouse, and the ability of HCBD to act as a tumor promoter has been investigated in one study in the male Wistar rat. Other relevant data include studies on genotoxicity, metabolism, and comparisons with structurally similar compounds.

3.1 Epidemiological Studies of Carcinogenicity

No data on long-term effects of human exposure to HCBD were found in the literature by ATSDR (1994), by IARC (1999b) or in a recent search by OEHHA.

3.2 Carcinogenicity Studies in Animals

In one series of two-year bioassays, HCBD was administered in the diet to male and female Sprague-Dawley rats (Kociba *et al.*, 1977a; 1977b). In another bioassay, HCBD was administered by gavage to male Wistar (CPJ) rats (Chudin *et al.*, 1985). HCBD was also tested in a 24-week intraperitoneal (ip) injection study in male A/St mice (Theiss *et al.*, 1977).

3.2.1. Oral Exposure Studies

Rat Dietary Exposure: Kociba et al., 1977a; 1977b

Kociba *et al*. (1977a) describe the details of two-year carcinogenesis studies in female and male rats. Kociba *et al*. (1977b) is a review article that reports results from the studies in qualitative form.

Groups of 39-40 Sprague-Dawley rats (specific pathogen free) per sex per dose group were fed 0.2, 2 or 20 mg HCBD/kg_{bw}-day for 22 (males) or 24 months (females). Ninety animals per sex were fed control diets. The HCBD was dissolved in acetone prior to the preparation of the food mixture. At the end of the experiment, all rats were evaluated for pathologic changes. Statistical significance was defined by the authors as $p<0.05$. Where statistically significant results are noted, OEHHA also provides Fisher Exact Test results for pairwise comparisons between treated and control animals.

Kociba *et al*. (1977a) stated that mortality was significantly increased among the high-dose males (incidences not given). Mortality data presented in Figure 1 of Kociba *et al*. (1977a) indicate that males of all dose groups, including the controls, experienced early mortality, reaching approximately 25 percent mortality during months 14 to 16 and about 50 percent mortality during months 17 to 19 across all dose groups. By 22 months the mortality among the high-dose male rats was about 90 percent and among the remaining groups, including controls it was about 65 to 75 percent. Among female rats, mortality was less severe in all dose groups, reaching approximately 55 percent in the high-dose group and about 60 to 70 percent in the remaining dose groups, including controls, at 24 months.

Decreased body weight gain was observed among high-dose male rats, with significant ($p<0.05$) body weight decreases of 11 to 12 percent reported during weeks 18 to 35 and decreases of 13 to 20 percent during weeks 48 to 96. Among the high-dose females, body weight decreases of five to 12 percent were observed during weeks 27 to 73. Sporadic decreases or increases in food consumption that occurred among all dose groups, were considered by the authors to be of no toxicologic significance. Increased kidney weight (absolute and relative) was reported among high-dose males at the end of the study. An increase in the relative, but not absolute, testicular weight was noted among high-dose males, and was not considered of toxicologic significance by the authors due to the decreased body weight. Among the high-dose females at 24 months, there was an approximate three-fold increase ($p<0.05$, compared to controls) in urinary coproporphyrin, based on absolute levels or when expressed on the basis of weight of urinary creatinine. An approximate two-fold increase in urinary coproporphyrin ($p<0.05$, compared to controls) was also observed among high-dose males at 12 months exposure and among mid-dose females at 15 months exposure. Coproporphyrin levels greater than twice that of normal levels could be indicative of decreased heme synthesis or increased erythropoiesis, or alternatively they could be a sign of toxicity to renal tubules which are a source of coproporphyrin (Daniell *et al*., 1997). Renal tubular epithelial hyperplasia was observed in some of the high- and mid-dose female and male rats between 13 and 24 months; however, neither histologic nor quantitative details were presented.

Statistically significant ($p<0.05$ compared to controls) increased incidences of total renal tubular neoplasms were observed in high-dose males and females (Table 1). The total incidence of all renal tubular neoplasms in high-dose males was 9/39 compared to 1/90 in controls, and in high-dose females the incidence was 6/40 compared to 0/90 among controls. The neoplasms consisted of renal tubular adenomas, adenocarcinomas and undifferentiated carcinomas. The incidence of renal tubular adenocarcinoma was 7/39 in high-dose males ($p<0.05$, compared to 0/90 among controls) and 2/40 in high-dose

females. Renal tubular undifferentiated carcinoma was observed in one high-dose male and one high-dose female rat. Metastasis to the lungs of renal tubular adenocarcinoma was observed in one high-dose male and one high-dose female rat. Increased hyperplasia of the renal tubular epithelium and increased adenomatous proliferation was observed in mid- and low-dose males and females; however, no renal tubular neoplasms were observed in the mid- or low-dose rats. The authors concluded the renal neoplasms in high-dose rats of both sexes were related to treatment with HCBD.

Kociba *et al*. (1977a; 1977b) did not present historical laboratory control data on the incidence of kidney tumors in Sprague-Dawley rats. An analysis of historical control incidence data for renal tubular tumors in Sprague-Dawley (Crl:CDBr) rats, obtained from nine lifetime carcinogenicity studies carried out between 1984 and 1991 in a different laboratory was presented by McMartin *et al*. (1992). The average incidence of adenoma was 0.7 percent (range, zero to 2.9 percent) among a total of 585 males and 0.0 percent among a total of 584 females. The average incidence of adenocarcinoma was 0.5 percent (range, zero to 1.7 percent) in males and zero percent in females.

A statistically significant (p=0.004) increase was noted in the incidence of uterine adenomatous polyps among the high-dose female rats (13/40) compared to controls (10/90) (Kociba *et al.*, 1977a). The increase was not considered by the authors to be treatment related because of an "abnormally low incidence of uterine polyps in this group of controls (11% incidence versus 24% incidence in historical control female rats.)." The source of the historical data was not presented by the authors.

Table 1. Tumor Incidence of Kidney tumors in Sprague-Dawley rats that received HCBD in the diet for two years (Kociba *et al.*, 1977a).

Tumor Site and Type		Dose (mg HCBD/kg_{bw}-day) 0	0.2	2	20
Males					
Renal tubular tumors	Adenoma	1/90[a] (1%)	0/40 (0%)	0/40 (0%)	3/39 (8%)
	Adenocarcinoma	0/90 (0%)	0/40 (0%)	0/40 (0%)	7/39 (18%) $p^b < 0.001$
	Undifferentiated carcinoma	0/90 (0%)	0/40 (0%)	0/40 (0%)	1/39 (2.6%)
	Renal tubular neoplasms (total)	1/90 (1%)	0/40 (0%)	0/40 (0%)	9/39 (23%) $p < 0.0001$
Females					
Renal tubular tumors	Adenoma	0/90 (0%)	0/40 (0%)	0/40 (0%)	3/40 (8%) $p = 0.03$
	Adenocarcinoma	0/90 (0%)	0/40 (0%)	0/40 (0%)	2/40 (5%)
	Undifferentiated carcinoma	0/90 (0%)	0/40 (0%)	0/40 (0%)	1/40 (2.5%)
	Renal tubular neoplasms (total)	0/90 (0%)	0/40 (0%)	0/40 (0%)	6/40 (15%) $p < 0.001$

[a] Tumor incidence is the number of rats with tumor / total number of examined rats. The numbers in parentheses are the percent incidences.

[b] Where significant pairwise comparisons between controls and treated groups occur, Fisher Exact Test values, calculated by OEHHA, are given.

Rat Gavage Exposure: Chudin et al., 1985 (translated from the original in Russian)

Male Wistar (CPJ) rats were administered HCBD by gavage for about two years. The doses of HCBD, administered in sunflower oil, were 0.6, 5.8 or 37 mg/kg_{bw}-day (n=43, 43, and 41, respectively). Control rats were either untreated (n=90) or received only the sunflower oil vehicle (n=46). Detailed evaluation of the study is difficult due to incomplete reporting, in particular, the authors' use of poorly defined terminology in the tabular presentation of tumor incidence data. In the text, the authors reported that detailed

histopathologic analysis of liver and kidney tissues indicated the presence of two hepatomas, one benign bile duct tumor, and one kidney adenoma, in three animals.

3.2.2. Dermal Application Studies

Mouse Skin Application Study: Van Duuren et al., 1979

Female non-inbred Ha:ICR Swiss mice (n=30) (ARS Sprague-Dawley) received skin applications of HCBD in acetone (0.2 ml) at two or six mg/mouse, three times/week for 440 to 594 days. Control mice received only acetone (0.1 ml, n=30) or were untreated (n=100). Abnormal appearing tissues and organs underwent histopathologic examination; in addition, skin, liver, stomach and kidney sections were examined histopathologically from each animal. Statistical significance was set by the authors at $p<0.05$. Survival of HCBD treated mice was stated by the authors to be excellent.

No skin tumors were observed among either control or HCBD-treated mice. Tumors of the lung and forestomach were observed in treated and control mice. The authors analyzed the tumor data as number of mice with tumors/total tumors, and concluded that HCBD was not carcinogenic in dermally exposed mice. Analyses of the data were also performed by OEHHA, comparing tumor incidence (i.e., number of tumor-bearing animals/total number of animals) in treated versus control groups using Fisher's Exact Test. The incidence of forestomach papilloma and carcinoma (combined) was 5/100, 2/30, 3/30, and 1/30 in the untreated control, acetone control, low-dose and high-dose groups, respectively. Statistically significant increases in lung papillomas were observed in low-dose ($p=0.04$) and high-dose ($p=0.02$) groups when compared to the untreated control group, but not when compared to the acetone control group (30/100, 11/30, 15/30, and 16/30 for untreated control, acetone control, low-dose and high-dose groups, respectively).

3.2.3. Intraperitoneal Injection Studies

Mouse i.p. Injection Study: Theiss et al., 1977

Theiss *et al.* (1977) conducted an experiment in male A/St mice, a strain that is sensitive to the development of lung tumors. Twenty mice received a total of 13 or 12 i.p. injections of 4 or 8 mg HCBD/kg_{bw} in tricaprylin (each dose delivered three times per week). Control mice received tricaprylin. Positive control mice received urethane in 0.9 percent NaCl. Twenty-four weeks after the first injection, lungs of treated and vehicle control mice were evaluated for tumors. Other organs were not evaluated. Among the surviving HCBD treated mice (19 and 14 animals in the low- and high-dose groups, respectively), the number of lung tumors per mouse was not different from that observed among the vehicle control mice. Among the positive control mice, the number of lung tumors per mouse was 19.6 compared to 0.19 among the NaCl controls. IARC (1979) noted the limitations (short-term exposure and study duration) of negative results associated with this test system. The insensitivity of the strain A mouse bioassay, which detects lung tumors, for aliphatic chlorides was noted by Stoner (1991).

3.2.4. Summary of Studies

In summary, renal tubular neoplasms (undifferentiated carcinoma, adenocarcinoma, and adenoma) were induced in female and male Sprague-Dawley rats chronically administered HCBD in the diet (Kociba *et al*., 1977a; 1977b). No treatment-related tumors were observed in another bioassay of male rats of a different strain, Wistar rats, exposed by gavage to HCBD in sunflower oil for a comparable period (Chudin *et al*., 1985). While the range of doses tested and the duration of treatment were similar, differences in study design between the two series of bioassays include the strain of rat tested (Sprague-Dawley versus Wistar), the means by which HCBD was administered (diet versus gavage), and the vehicle in which HCBD was dissolved (acetone versus sunflower oil). Wistar rats, however, were susceptible to the promotion of kidney tumors by HCBD (Nakagawa *et al.*, 1998; described in more detail in Section 3.3.1). A statistically significant increase in benign lung tumors was observed in female Ha:ICR Swiss mice that received HCBD in acetone by dermal application three times/week for 440 to 594 days, as compared with untreated, but not acetone controls. The non-positive findings in the short-term bioassay of HCBD in A/St male mice (Theiss *et al*., 1977) are consistent with the reported insensitivity of this animal model to aliphatic chlorides (Stoner, 1991).

3.3 Other Relevant Data

3.3.1 Tumor initiation/promotion studies

Van Duuren *et al*. (1979) studied the action of HCBD as a tumor initiator in mice. Female non-inbred Ha:ICR Swiss mice (n=30) (ARS Sprague-Dawley) were initiated with a single topical application of HCBD (15 mg/mouse) in acetone, followed 14 days later by the promoter phorbol myristate acetate (PMA) at 5 µg/mouse, three times/week for 428 to 576 days. A control group (n= 90) received only PMA. Survival was considered excellent by the authors. Complete autopsy was performed at death or the end of the experiment. Skin was the only site for which the authors presented tumor findings. The incidence of skin papilloma, defined as the number of tumor-bearing animals/total number of animals, was not significantly different (p=0.1) between HCBD-initiated mice (5/30) and PMA controls (6/90), although there was a notable decrease in the time to first tumor in the group initiated with HCBD, compared to the PMA control group (63 versus 449 days).

Nakagawa *et al*. (1998) studied the action of HCBD as a tumor promoter in rats. Male Wistar rats (6-8 weeks old at start of study) were initiated with N-ethyl-N-hydroxyethylnitrosamine (EHEN) and then exposed to 0.1 percent HCBD in the feed for 30 weeks and necropsied at 32 weeks. Renal cell tumor incidence (15/21 versus 5/21, p=0.002) and preneoplastic adenomatous hyperplasia of the kidney (21/21 versus 4/21, $p<10^{-7}$) were increased in rats receiving EHEN+HCBD relative to those receiving only EHEN. Neither kidney tumors nor adenomatous hyperplasia was observed in rats receiving only HCBD or basal diet. Assuming an average body weight of 329 g (Nakagawa *et al.*, 1998) and a daily food consumption rate for rats of five percent of the body weight (Anderson and Carcinogen Assessment Group of the U. S. Environmental

Protection Agency, 1983), OEHHA estimates a daily dose of HCBD during the 30-week exposure duration of 50 mg HCBD/kg_{bw}-day. This value is 2.5-fold greater than the high dose in the Kociba *et al.* (1977a 1977b) studies. The results of Nakagawa *et al*. (1998) indicate that under the conditions of the study, HCBD acted as a tumor promoter.

3.3.2 Genetic Toxicology

<u>Bacterial and non-mammalian systems</u>

HCBD was tested for its ability to induce reverse mutations in *Salmonella typhimurium* in several experiments under conditions of preincubation or plate incorporation. The role of metabolism in the mutagenicity of HCBD was investigated under conditions that favored either NADPH-dependent cytochrome P450 (CYP)-mediated oxidative metabolism or a reduced glutathione (GSH)/mercapturate/β-lyase pathway. HCBD was also tested in a forward mutation assay in *Salmonella typhimurium* and in *Drosophila melanogaster*.

In a series of experiments carried out under conditions of preincubation, HCBD was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537, in the presence or absence of metabolic activation provided by either an Aroclor 1254-induced male Sprague-Dawley rat liver or a Syrian hamster liver postmitochondrial (S9) fraction and an NADPH generation system (Haworth *et al.*, 1983). The lack of mutagenicity in TA100 under preincubation conditions, in the presence of the S9 fraction and NADPH was also reported by Vamvakas *et al*. (1988) and under plate incorporation conditions in the presence or absence of metabolic activation by Stott *et al*. (1981). Negative results were reported by Rapson *et al*. (1980) in TA100 when HCBD was tested at levels of 0.1 to 1000 μg per plate.

In contrast, Reichert *et al*. (1984) reported HCBD was mutagenic toward TA 100 under preincubation conditions in the presence of metabolic activation, but only in the presence of higher than usual concentrations of rat S9 protein. The authors also showed that the chemically synthesized HCBD oxidation products, perchloro-3-butenoic acid chloride (PCBAC) and perchloro-3-butenoic acid (PCBA), were each mutagenic under these conditions. They hypothesized that PCBAC could form from a short-lived monoepoxide intermediate that spontaneously rearranges to the acid chloride and thence hydrolyzes to the free acid.

Other reverse mutation studies indicate a role for GSH in the mutagenicity of HCBD. Mutagenicity of HCBD towards TA100 was observed in the presence of rat liver or combined rat liver and kidney microsomal fractions, each supplemented with GSH, but not with NADPH (Vamvakas *et al.*, 1988). The authors also observed decreased mutagenic activity when an inhibitor to β-lyase activity was present. Green and Odum (1985) reported the mutagenicity of two HCBD metabolites, S-(1,2,3,4,4-pentachlorobutadienyl)glutathione (PCBG) and S-(1,2,3,4,4-pentachlorobutadienyl)cysteine (PCBC), towards TA100 in the presence of rat kidney microsomal fractions that did not contain NADPH. Vamvakas *et al*. (1988) reported the mutagenicity of PCBG in the presence of rat kidney microsomal, mitochondrial or

cytosolic fractions and the decreased mutagenicity of this metabolite in the presence of a β-lyase inhibitor. The N-acetylated metabolite of PCBC, which is the mercapturate of HCBD, was also shown to be mutagenic towards TA100 in the presence of rat liver cytosol (Wild *et al.*, 1986). Taken together, these results suggest that the mutagenic activity of HCBD requires metabolic activation that proceeds through a GSH-mediated pathway. The pathway leads to the formation of a GSH conjugate and thence to the corresponding mercapturate. A metabolite of the mercapturate is then metabolized through the β-lyase pathway to one or more reactive products that are responsible for HCBD mutagenesis. Details of this pathway are discussed in the section on Metabolism (Section 3.3.3).

Using the Ara test, which detects forward mutations in *Salmonella typhimurium* strain BA13, Roldan-Arjona *et al*. (1991) reported HCBD was positive under conditions of preincubation in the absence of metabolic activation provided by a rat liver S9 fraction. Mutagenicity was also observed in the presence of the S9 fraction, but such activity was less than that in its absence.

No sex-linked recessive lethal mutations were observed in *Drosophila melanogaster* exposed as adult flies to HCBD by diet or injection (Woodruff *et al.*, 1985).

Mammalian systems, in vitro

HCBD induced sister chromatid exchange (SCE) but not chromosome aberrations in Chinese hamster ovary (CHO) cells in the presence or absence of a rat liver extract supplemented with an NADPH generating system (Galloway *et al.*, 1987).

The HCBD metabolite PCBC induced DNA cross links, but not DNA single-strand breaks in isolated rabbit renal tubule preparations (Jaffe *et al.*, 1983). Unscheduled DNA synthesis (UDS) was induced by the HCBD metabolite, PCBG, in cultured porcine kidney cells (LLC-PK1) under conditions where cytotoxicity was not evident (Vamvakas *et al.*, 1989). The authors also reported that the induction of UDS was abolished in the presence of inhibitors of γ-glutamyltransferase or β-lyase activities. These results suggest an important role for the GSH/mercapturate/β-lyase pathway in the metabolic activation of HCBD to a genotoxic species. However, in another experimental system, HCBD and a suggested metabolite of oxidative metabolism, PCBA, each induced UDS and morphologic transformation in Syrian hamster embryo (SHE) cells (Schiffmann *et al.*, 1984).

Mammalian systems, in vivo

Stott *et al*. (1981) exposed male Sprague-Dawley rats by gavage to 20 mg HCBD /kg_{bw}-day (seven days/week for three weeks) and found a 40 percent increase in the repair of renal DNA. No increases in dominant lethal mutations or frequency of bone marrow chromosomal aberrations were observed in rats exposed by inhalation to 10 or 50 ppm HCBD for up to five days (NIOSH, 1981, as reported in ATSDR, 1994). No increases in chromosomal aberrations were observed in the bone marrow cells of rats that were fed 0.2, 2.0 or 20 mg HCBD/kg_{bw} for approximately five months (Schwetz *et al.*, 1977).

DNA adducts

In the study discussed above by Stott *et al.* (1981), the level of DNA alkylation was 0.78 alkylations/10^6 nucleotides. In another study, Schrenk and Dekant (1989) observed *in vivo* covalent binding of HCBD to renal and hepatic mitochondrial DNA and to renal nuclear DNA in female NMRI mice exposed by gavage to HCBD. The covalent binding index (CBI, a measure of DNA adducts per dose of chemical developed by Lutz (1979)) for each DNA fraction was: kidney mitochondria – 7506, kidney nuclei – 27, liver mitochondria – 513, liver nuclei – 11 ($p < 0.02$, < 0.05, < 0.02, > 0.05 respectively, compared to corn oil treated control mice.) Isolation and analysis of the renal mitochondrial DNA revealed radioactivity associated with altered bases but insufficient material was available for structural analysis. Schrenk and Dekant (1989) noted that higher levels of binding to mitochondrial DNA as compared to nuclear DNA, has also been observed for other chemical carcinogens such as benzene, polycyclic aromatic compounds and aflatoxin B1.

Summary of Genotoxicity Studies

HCBD has been shown to be mutagenic in *Salmonella typhimurium*, inducing reverse and forward mutations under conditions that favor GSH-mediated metabolism. HCBD has also been shown to be genotoxic in multiple mammalian test systems, inducing SCEs in CHO cells, UDS and morphological transformation in SHE cells, and increased repair of renal DNA in rats. Known or predicted metabolites of HCBD have also been shown to induce reverse mutations in *Salmonella*, DNA cross-links in isolated rabbit renal tubule preparations, UDS in porcine kidney cells, and UDS and morphological transformation in SHE cells. HCBD has also been show to bind covalently to rat and mouse DNA, *in vivo*.

HCBD did not induce mutations in *Salmonella typhimurium* under conditions that did not favor GSH-mediated metabolism, nor did HCBD induce sex-linked recessive lethal mutations in *Drosophila*, chromosomal aberrations in CHO cells, DNA single-strand breaks in isolated rabbit renal tubular preparations, dominant lethal mutations in rats, or bone marrow chromosomal aberrations in rats.

Studies conducted in *Salmonella typhimurium* and mammalian cells *in vitro* indicate that the genotoxicity of HCBD requires metabolism to one or more DNA reactive metabolites. The mutagenicity findings in *Salmonella typhimurium* were mostly negative under conditions favoring CYP-dependent oxidative metabolism and mostly positive under conditions favoring GSH- and β-lyase-mediated metabolism. The available data thus suggest the GSH/mercapturate/β-lyase pathway is likely to be the predominant means of bioactivation of HCBD to a DNA reactive species. CYP-dependent oxidative metabolism to one or more DNA reactive metabolites may also occur, but to a lesser extent. Additional support for this conclusion is provided by positive genotoxicity studies of GSH metabolites of HCBD in *Salmonella typhimurium* and mammalian cells *in vitro*, and by observations that two postulated oxidative metabolites of HCBD were mutagenic in *Salmonella typhimurium* and genotoxic to mammalian cells *in vitro*.

3.3.3 Pharmacokinetics and Metabolism

Pharmacokinetics

A log K_{ow} of 4.78 and a log K_{oc} of 3.67 (ATSDR, 1994) indicate HCBD is easily absorbed across the lipid rich membranes of biologic tissue. For example, complete dermal absorption occurred in eight hours when HCBD was administered as the neat solvent to rabbits (0.4 or 1.6 gm/kg_{bw}) (Duprat and Gradiski, 1978).

Excretion occurs in feces and urine. As the HCBD dose increases, the fractional amount excreted in the urine decreases while that in the feces increases. Reichert *et al*. (1985) reported the fractional urinary recovery absorption of one or 50 mg [^{14}C]HCBD/kg_{bw} in tricaprylin, administered to female Wistar rats by gavage, after 72 hours was 30 and 11 percent, respectively. Fecal recovery of the radioactive label was 42 and 69 percent, respectively. Birner *et al*. (1995) exposed female and male Wistar rats by gavage to 200 mg [^{14}C]HCBD/kg_{bw} and measured radioactivity after 48 hours. The percent total recoveries were similar between the male and female rats (20 and 18 percent, respectively). Among the male rats, the recovery of radioactivity in the feces, urine and exhaled breath was 16, 3, and 1 percent, respectively. Among the female rats the recoveries were 11, 4, and 2 percent, respectively. Female rats receiving 100 mg [^{14}C]HCBD/kg_{bw} by gavage excreted 5.4 percent of the radioactivity into the urine within 24 hours (Reichert and Schutz, 1986). Payan *et al*. (1991) reported male Sprague-Dawley rats receiving a single dose of one mg [^{14}C]HCBD/kg_{bw} in aqueous polyethylene glycol by gavage excreted 18 percent into urine within 72 hours.

Birner *et al*. (1995) observed a sex difference in the urinary excretion of unmetabolized HCBD among rats. Male Wistar rats excreted unmetabolized HCBD into urine, whereas female Wistar rats excreted one or more metabolites and no unmetabolized HCBD. In male Sprague-Dawley rats but not male NCI Black-Reiter rats, a strain deficient in the male rat specific protein α_{2u}-globulin, unmetabolized HCBD was also excreted into the urine (Pahler *et al.*, 1997). In the urine and the renal cytosol of the HCBD exposed male Sprague-Dawley rats, the unmetabolized HCBD was bound to α_{2u}-globulin (Pahler *et al.*, 1997).

Biliary excretion plays an important role in the disposition of HCBD and its metabolites. In one study, Nash *et al*. (1984) fed male Alderly Park (Wistar derived) rats 200 mg [^{14}C]HCBD/kg_{bw} and found 39 percent of the radioactivity was associated with the bile in the first two days. Maximal biliary excretion occurred at about two days post-exposure, while increased fecal was evident at about four days post-exposure.

The role of biliary excretion in the disposition of HCBD was also investigated in a series of experiments by Payan *et al*. (1991). Male Sprague-Dawley rats receiving a single dose of one mg [^{14}C]HCBD/kg_{bw} in aqueous polyethylene glycol by gavage excreted 62 percent of the label into feces+gastrointestinal tract and 18 percent into urine after 72 hours. When the dose was increased to 100 mg/kg_{bw}, the feces+gastrointestinal tract contained 72 percent of the label whereas the urine contained nine percent ($p<0.05$ compared to lower dose group). In bile-duct cannulated rats fed one and 100 mg

[^{14}C]HCBD/kg_{bw}, the biliary fractional excretion of label decreased with dose from 67 to 58 percent whereas the fecal excretion increased from three to 16 percent (each at $p<0.05$). Payan *et al*. (1991) also studied the distribution of the radioactive label from HCBD in donor and recipient rats that were joined surgically by crossover bile cannulation. The donor rats were fed 100 mg [^{14}C]HCBD/kg_{bw} and fractional excretion and tissue concentrations were determined after 30 hours. The authors calculated that the biliary contribution to the urinary label was about 40 percent and that about 80 percent of the biliary metabolites in the recipient rats were reabsorbed. They also determined that the radioactivity in the urine of the recipient rats was not extractable into organic solvent. Thus following oral exposure to HCBD, biliary excretion significantly contributes to renal excretion.

Although biliary excretion plays a major role in the disposition of HCBD and its metabolites to the kidney, evidence for a minor hepatic contribution has been presented by Koob and Dekant (1992). Using a perfused male Wistar rat liver system, the authors showed that one of the biliary HCBD metabolites can be efficiently absorbed from the perfusion medium and metabolized within the liver to one or more compounds that are excreted in the bile.

With respect to the disposition of HCBD, in a whole body autoradiographic experiment, the label from orally administered [^{14}C]HCBD (200 mg/kg_{bw}) was observed in the stomach, small intestine and outer medulla of the kidney in rats four to 16 hours post-exposure (Nash *et al.*, 1984). The radioactivity of HCBD in the gut at four hours post-exposure was 85 percent organic solvent soluble and 15 percent water soluble, while at 16 hours post-exposure it was mainly water soluble. In the Payan *et al*. (1991) study, the radioactivity in the urine of the donor male Sprague-Dawley rats was not extractable by organic solvent. Two HCBD metabolites were identified in the bile of male Wistar rats as PCBG and PCBcysteinylglycine (Nash *et al.*, 1984). Two HCBD metabolites were identified in the urine of the HCBD exposed female Wistar rats as pentachloro-1-methylthio-1,3-butadiene (PCMTB) and pentachloro-carboxymethylthio-1,3-butadiene (PCCMTB) (Reichert *et al.*, 1985) These studies indicate that HCBD undergoes metabolism before it is distributed to the kidney. Details of the metabolism of HCBD by animal tissues are discussed in the next section.

Metabolism

The major metabolic pathway for HCBD, and the one postulated to result in the ultimate DNA reactive metabolite, is the GSH/mercapturate/β-lyase pathway (Figure 1). The first step in the pathway is the glutathione-S-transferase (GST)-mediated conjugation of HCBD with GSH to form PCBG in the liver (Nash *et al.*, 1984; Dekant *et al.*, 1988b; Koob and Dekant, 1992). PCBG is then transported to the bile where it may be converted to PCBC by the actions of γ-glutamyltransferase (GGT) and dipeptidase (Nash *et al.*, 1984; Gietl and Anders, 1991). PCBC may undergo enterohepatic circulation and N-acetylation to the mercapturate, N-Ac-PCBC, in the liver or it may be translocated to the kidney where it can also be converted to N-Ac-PCBC (Dekant *et al.*, 1988b). Results obtained with a perfused liver system suggest that PCBG may also be transported intact from the bile to the kidney, where it is converted to PCBC (Koob and Dekant, 1992). A

sex-specific step in the formation of the mercapturate, N-Ac-PCBC, was observed in Wistar rats that were administered HCBD by gavage. Among the female rats, N-Ac-PCBC was a major urinary metabolite whereas it was a minor metabolite among the male rats (Birner *et al.*, 1995).

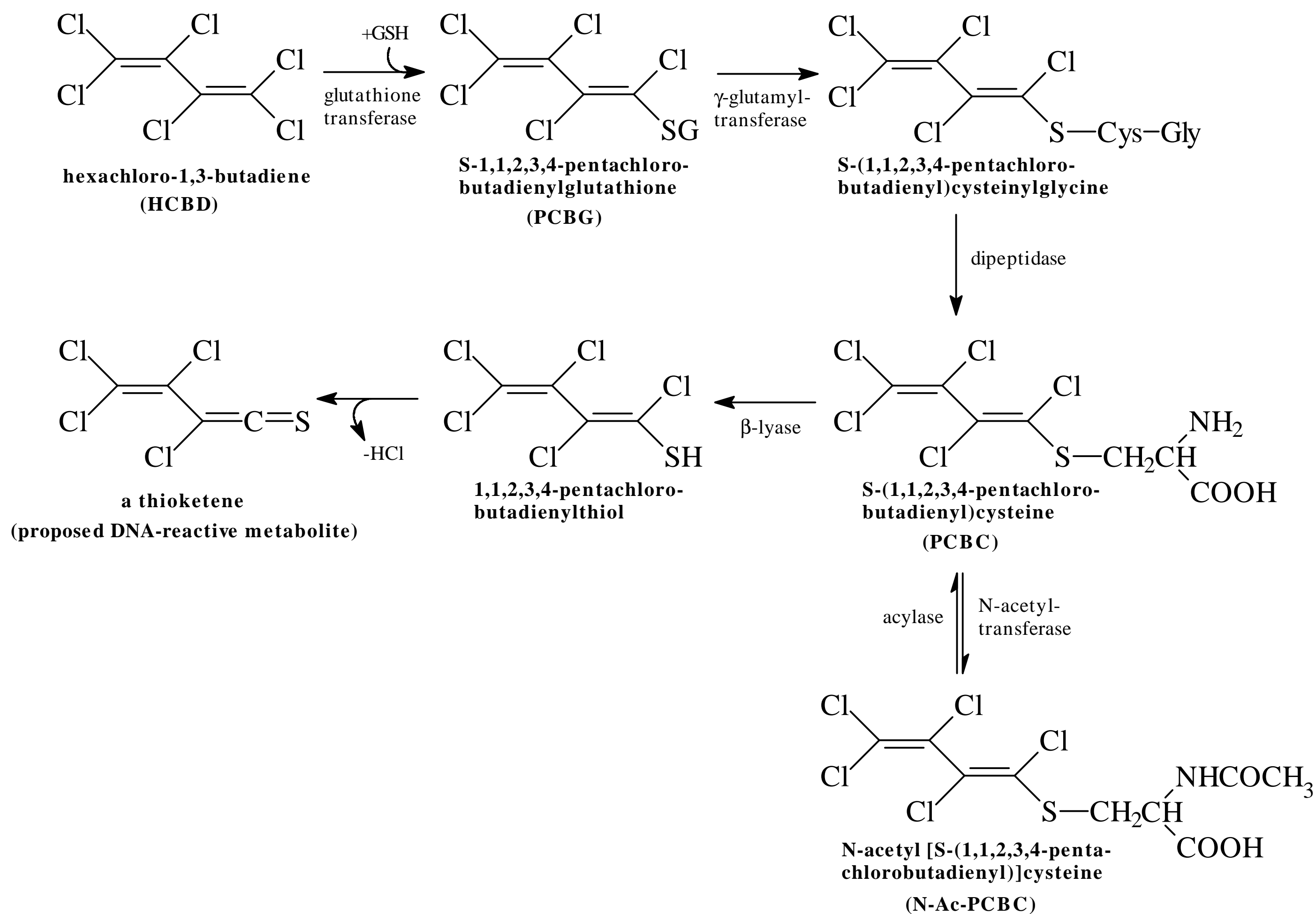
In the kidney, the site of HCBD tumorigenesis, PCBC, the cysteine conjugate of HCBD, is converted via β-lyase to a reactive thioketene (or a thiono acyl chloride). This reactive metabolite is hypothesized to be responsible for DNA alkylation (Dekant *et al.*, 1988a). The source of the PCBC in the kidney is deacylation of the mercapturate, N-Ac-PCBC (Pratt and Lock, 1988; Uttamsingh and Anders, 1999), or translocation from the small intestine (Dekant *et al.*, 1988b).

The metabolism of HCBD by the GSH/mercapturate/β-lyase pathway in the rat leads to the formation of non-toxic GSH conjugates of PCBC following its resorption from the bile into the liver (Koob and Dekant, 1992). Stable hydrolysis and oxidation products, considered to be metabolites of the reactive β-lyase-formed thioketene have also been described (Reichert *et al.*, 1985; Birner *et al.*, 1995).

Many of the enzymes required for HCBD metabolism via the GSH/mercapturate/β-lyase pathway have been observed in human tissue. GST activity towards HCBD has been demonstrated in human liver and is associated primarily with the microsomal fraction (Oesch and Wolf, 1989; McLellan *et al.*, 1989). The occurrence of other human enzymes that act on HCBD metabolites is suggested by the presence of the N-acetylated derivative (mercapturate) of the structurally related haloalkene, perchloroethylene (PERC) (Volkel *et al.*, 1998). Similarly, β-lyase activity towards the cysteinyl conjugates of PERC and trichloroethylene (TCE), another chlorinated alkene, has been demonstrated in human kidney (IARC, 1999b).

In addition to GSH-dependent metabolism, some oxidative metabolism of HCBD has been observed. One metabolic pathway leading to the formation of a mercapturate sulfoxide, N-acetyl-S-(1,2,3,4,4-pentachlorobutadienyl)-cysteine sulfoxide (N-Ac-PCBC-sulfoxide), has been described (Birner *et al.*, 1995). N-Ac-PCBC-sulfoxide is formed from N-Ac-PCBC by male rat liver microsomal fractions, but not in the presence of female rat liver microsomal fractions or rat kidney microsomal fractions from either sex (Birner *et al.*, 1995). Sulfoxidation of the HCBD mercapturate was also observed in human male and female liver (Werner *et al.*, 1995b). The authors also reported no sulfoxide was formed from PCBG or PCBC, and flavin monooxygenase exhibited no activity towards N-Ac-PCBC. The enzyme responsible for the formation of N-Ac-PCBC-sulfoxide in humans and rats was identified as a member of the cytochrome P450-3A (CYP3A) monooxygenase family, although in rats CYP1B1/2 may also play a role (Werner *et al.*, 1995b; Werner *et al.*, 1995a). N-Ac-PCBC-sulfoxide cytotoxicity towards rat renal proximal tubular cells, measured as cell viability, was not inhibited by a β-lyase inhibitor as was the cytotoxicity of N-Ac-PCBC, a result that distinguishes between the metabolic pathways that lead from N-Ac-PCBC to a thioketene or to N-Ac-PCBC-sulfoxide. N-Ac-PCBC-sulfoxide has not been tested for carcinogenicity or genotoxicity.

Figure 1. Proposed metabolic pathway for the formation of a DNA reactive metabolite of HCBD. Adapted from ATSDR (1994).



Additional genotoxic products could result from CYP-dependent oxidative metabolism of HCBD. Reichert *et al*. (1984) proposed that the spontaneous rearrangement of a short-lived monoepoxide intermediate metabolite of HCBD could result in the formation of PCBAC, which upon subsequent hydrolysis would form the free acid, PCBA. PCBAC and PCBA were each mutagenic towards *Salmonella typhimurium* in the presence of a metabolic activation system (Section 3.3.2) (Reichert *et al.*, 1984). Other data suggesting a role for oxidative metabolism in the bioactivation of HCBD to DNA reactive species include observations of HCBD genotoxicity in CHO cells under conditions where CYP-dependent oxidative metabolism was present (Galloway *et al.*, 1987), and increases in HCBD covalent binding to liver and kidney protein under conditions of CYP enzyme induction by phenobarbital pretreatment, and decreases in covalent binding to proteins under conditions of CYP enzyme inhibition by piperonyl butoxide (Reichert *et al.*, 1985). Interpretation of the protein binding studies is complicated, however, by the ability of phenobarbital to induce GSTase, and the ability of piperonyl butoxide to induce rather than inhibit some CYP isoenzymes (reviewed by Hodgson, 1994).

In summary, HCBD is activated through a pathway that includes GSH, enzymes of the mercapturate pathway, deacylase activity and β-lyase activity. Deacylase and β-lyase activities are present in the kidney, which is the target site of HCBD tumorigenesis in the rat. Inhibitors of GGT, the first enzyme in mercapturate acid synthesis, and of β-lyase activity result in decreased genotoxic activity of HCBD and its GSH conjugate. Although the GSH conjugate of HCBD may be metabolized in bile, liver and intestinal tissue to the mercapturic acid and then transported to the kidney, the GSH conjugate itself may be transported intact to the kidney where it is metabolized to the ultimate β-lyase-derived reactive metabolite. Oxidative metabolism of HCBD to DNA reactive metabolites may also occur to a lesser extent.

3.3.4 Structure-Activity Comparisons

HCBD is a chlorinated alkene. Two other chlorinated alkenes that have been identified as causing cancer under Proposition 65 are TCE and PERC. TCE and PERC were each classified by IARC (1995a; 1995b) as probably carcinogenic to humans (group 2A) and the classifications were based on limited evidence in humans and sufficient evidence in experimental animals. Renal tubular adenomas and carcinomas were induced in male rats exposed to PERC by inhalation (IARC, 1995a). Renal tubular adenomas were also observed among two strains of rats that received TCE by gavage and in one strain, a renal tubular adenocarcinoma was observed (IARC, 1995b).

Similarities in the site and type of tumor induced in rodents, the genotoxic activity in short-term test systems, and the metabolism of HCBD, TCE and PERC suggest that the three chlorinated alkenes may share a common mechanism of action in the induction of kidney tumors. The GSH conjugates of TCE and PERC, similar to the GSH conjugate of HCBD, were each genotoxic towards porcine kidney cells (Vamvakas *et al.*, 1989). The cysteine conjugates of TCE and PERC, similar to the cysteine conjugate of HCBD, were each mutagenic toward *Salmonella typhimurium* (TA100) in the presence of rat kidney extracts without NADPH (Green and Odum, 1985). In addition, the cysteine conjugates

of HCBD, TCE and PERC are each N-acetylated in rat tissues, to the corresponding mercapturates (Birner *et al.*, 1997). Thus, each of these chlorinated ethylenes can be metabolized through the same GSH/mercapturate/β-lyase pathway, forming genotoxic metabolites capable of reaching the kidney. Each of these compounds also undergoes oxidative metabolism via CYP-dependent monooxygenases. This latter pathway appears to play an important role in the bioactivation of TCE and PERC (IARC, 1995a; 1995b), while the GSH/mercapturate/β-lyase pathway appears to be more important in the carcinogenicity of HCBD.

The structural similarity between HCBD and these two chlorinated alkenes may be extended to chlorinated alkanes in terms of the role of GSH in bioactivation to a reactive metabolite. 1,2-Dichloroethane (DCE) has been listed as causing cancer under Proposition 65 and classified as possibly carcinogenic to humans (Group 2B) by IARC (1999a). Similar to HCBD, DCE is also mutagenic towards *Salmonella typhimurium* in the presence of liver cytosol and GSH but not in the presence of liver microsomal fractions and NADPH (Anders *et al.*, 1988). Although DCE can be metabolized through either an oxidative or GSH-dependent pathway, the GSH pathway is considered more important for DCE-induced DNA damage (IARC, 1999a).

3.3.5 Pathology

The kidney tumors described by Kociba *et al*. (1977a; 1977b) as being related to treatment with HCBD were of renal tubular epithelial origin. These neoplastic lesions were observed unilaterally and bilaterally. All but two of the neoplasms were classified as either adenomas or adenocarcinomas. Two cases of undifferentiated renal tubular carcinoma were reported, one each in the high-dose males and females. In two cases, adenocarcinomas were associated with metastasis to the lung. In the rat, the progression of renal tubular adenomas to carcinomas, and the potential for renal tubular carcinomas to metastasis to the lung has been noted (IARC, 1992).

3.4 Mechanism

Although the mechanism of HCBD carcinogenesis has not been determined, there is evidence for a role for genotoxicity. The major metabolic pathway for HCBD, and the one postulated to result in formation of the ultimate DNA reactive metabolite, is the GSH/mercapturate/β-lyase pathway. This pathway consists of a series of enzyme catalyzed reactions that are initiated with the conjugation of HCBD with GSH in the liver and completed with the formation of a DNA reactive thioketene in the kidney, the site of tumor formation in the rat. A minor role for the bioactivation of HCBD through CYP-dependent oxidative metabolism may exist; however, the contribution of this pathway to the formation of DNA reactive metabolites remains to be determined.

Evidence in support of a genotoxic mechanism of action includes positive mutagenicity studies of HCBD in the *Salmonella typhimurium* reverse mutation assay under conditions where the GSH/mercapturate/β-lyase pathway was operative. Metabolites of HCBD formed through the action of enzymes in this pathway were also mutagenic in this assay. The mutagenicity of HCBD and PCBG was decreased in the presence of an inhibitor to

β-lyase. HCBD was also mutagenic towards *Salmonella typhimurium* in a forward mutation assay. HCBD-induced genotoxicity has been observed in mammalian cells *in vitro,* with SCEs, but not chromosome aberrations, induced in CHO cells and UDS and morphologic transformation induced in SHE cells. In addition, the GSH conjugate of HCBD induced UDS in porcine kidney cells under conditions where cytotoxicity was not evident, and HCBD induced cross-linking, but not DNA single-strand breaks, in isolated rabbit renal tubule preparations. *In vivo* evidence of HCBD genotoxicity includes the induction of DNA adducts in kidney, the target organ, in rats and mice, and increased renal DNA repair in rats.

HCBD acted as a tumor promoter, increasing the incidence of renal cell adenomatous hyperplasia, adenoma and carcinoma in rats initiated with EHEN (Nakagawa *et al*., 1990). Increased hyperplasia of the renal tubular epithelium was also reported by Kociba *et al.* (1977a; 1977b) in rats treated with HCBD. Thus in addition to genotoxicity, other mechanisms may contribute to HCBD carcinogenicity.

4 OTHER REVIEWS

The International Agency for Research on Cancer reviewed the evidence of HCBD carcinogenicity in 1979 (IARC, 1979) and 1987 (IARC, 1987) and classified HCBD as a group 3 carcinogen (not classifiable as to carcinogenicity). The classification was based on inadequate data in humans and limited data in experimental animals. The classification remained unchanged as a result of IARC's most recent evaluation (IARC, 1999b). The U. S. EPA (Integrated Risk Information Service) (1991) classified HCBD as a possible human carcinogen (group C) based on renal neoplasms in male and female rats. More recently, HCBD was classified by U.S. EPA (1998) in a criteria document as "likely to be carcinogenic to humans", based on renal tumor finding in both sexes of rats. The National Institute for Occupational Safety and Health (NIOSH, 2000) identified HCBD as a potential occupational carcinogen based on kidney tumors in animals.

5 SUMMARY AND CONCLUSIONS

5.1 Summary of Evidence

No studies on the long-term health effects of human exposure to HCBD were reported. Carcinogenic activity of HCBD has been observed in studies in experimental animals. HCBD induced renal tubular neoplasms (undifferentiated carcinoma, adenocarcinoma, and adenoma) in female and male Sprague-Dawley rats that received the compound in the diet (Kociba *et al.*, 1977a; 1977b). No treatment-related tumors were observed in male Wistar rats, exposed by gavage to HCBD in sunflower oil for nearly two years (Chudin *et al*., 1985). In female mice receiving dermal applications of HCBD in acetone three times/week for 440 to 594 days, benign lung tumors were increased ($p<0.05$) in treated groups, as compared with untreated, but not vehicle controls (Van Duuren *et al*.,

1979). No treatment-related tumors were observed in the short-term bioassay of HCBD in A/St male mice (Theiss *et al*., 1977); however, this finding is not unexpected, given the reported insensitivity of this experimental animal model to the tumorigenic effects of aliphatic chlorides. When tested in a mouse initiation/promotion model, dermal application of HCBD did not result in a treatment-related increase in tumors; however, a decrease in the time to first tumor was observed in the HCBD-initiated animals, as compared with those receiving only the promoter (Van Duuren *et al*., 1979). HCBD was shown to act as a tumor promoter, increasing the combined incidence of renal cell adenoma and carcinoma in rats initiated with EHEN (Nakagawa *et al.*, 1998).

HCBD has been shown to be genotoxic in multiple test systems, inducing reverse and forward mutations in *Salmonella typhimurium*, SCEs in CHO cells, UDS and morphological transformation in SHE cells, and increased repair of renal DNA in rats. Known or predicted metabolites of HCBD have also been shown to induce reverse mutations in *Salmonella typhimurium*, DNA cross-links in isolated rabbit renal tubule preparations, UDS in porcine kidney cells, and UDS and morphological transformation in SHE cells. Studies conducted in *Salmonella typhimurium* and mammalian cells *in vitro* indicate that the genotoxicity of HCBD requires metabolism to one or more DNA reactive metabolites. Mutagenicity findings in *Salmonella typhimurium* were mostly negative under conditions favoring oxidative metabolism and mostly positive under conditions favoring GSH- and β-lyase-mediated metabolism. The available data therefore suggest that the GSH/mercapturate/β-lyase pathway is likely to be the predominant means of bioactivation of HCBD to a DNA reactive species, while oxidative metabolism to one or more DNA reactive metabolites may occur, but to a lesser extent. Additional support for this conclusion is provided by positive genotoxicity studies of GSH metabolites of HCBD in *Salmonella typhimurium* and mammalian cells *in vitro*, and by observations that two postulated oxidative metabolites of HCBD were mutagenic in *Salmonella typhimurium* and genotoxic to mammalian cells *in vitro*. *In vivo* binding of HCBD to renal and hepatic mitochondrial and nuclear DNA was detected in mice administered HCBD by gavage (Schrenk and Dekant, 1989). *In vivo* alkylation of kidney DNA has also been observed in rats (Stott *et al.,* 1981).

The studies on pharmacokinetics and metabolism suggest that the carcinogenicity and genotoxicity of HCBD is primarily due to metabolism through a pathway that includes GSH, enzymes of the mercapturate pathway, deacylase activity and β-lyase activity. Oxidative metabolism of HCBD to one or more DNA reactive metabolites may also occur, but the extent to which this pathway contributes to the genotoxicity and tumorigenicity of HCBD is not known. Metabolic intermediates formed as a result of metabolism of HCBD by the GSH/mercapturate/β-lyase pathway, and detected in experimental animal studies include PCBG (Nash *et al.*, 1984), PCBcysteinylglycine (Nash *et al.*, 1984), PCBC (Gietl and Anders, 1991) and N-Ac-PCBC (Reichert and Schutz, 1986; Birner *et al.*, 1995). PCBC may occur as the second degradation product of PCBG (Nash *et al.*, 1984; Gietl and Anders, 1991) or the deacylation product of N-Ac-PCBC (Pratt and Lock, 1988; Uttamsingh and Anders, 1999). The ultimate DNA reactive species is thought to be a thioketene. The GSH conjugate of HCBD may be metabolized in bile, liver and intestinal tissue to the mercapturate and then transported to

the kidney, which possesses deacylase and β-lyase activities, and is the site of HCBD tumorigenesis in the rat. In addition, the GSH conjugate itself may be transported intact to the kidney where it is metabolized to a β-lyase-derived thioketene.

Two other chlorinated alkenes, TCE and PERC, have been classified by IARC (1995a; 1995b) as probable human carcinogens (group 2A) and have been identified as causing cancer under Proposition 65. Both TCE and PERC have been shown to induce renal tubular neoplasms in long-term studies in rats. Similarities in the site and type of tumor induced in rodents, the genotoxic activity in short-term test systems, and the metabolism of HCBD, TCE and PERC suggest that the three chlorinated alkenes may share a common mechanism of action in the induction of kidney tumors. However, oxidative metabolism via CYP-dependent monooxygenases appears to play a greater role in the bioactivation of TCE and PERC than HCBD.

Although the mechanism of HCBD carcinogenesis has not been determined, there is evidence for a role for genotoxicity. The major metabolic pathway for HCBD, and the one postulated to result in formation of the ultimate DNA reactive metabolite, is the GSH/mercapturate/β-lyase pathway. However, more than one mechanism may be operative, as HCBD acted as a renal tumor promoter and increased the incidence of hyperplasia of the renal tubular epithelium in rats.

5.2 Conclusion

There is evidence for the carcinogenicity of HCBD, based on the development of renal tubular neoplasms in female and male rats that received HCBD in the diet for approximately two years. Contributing to the weight of evidence are observations of mutagenicity in bacteria under conditions that favor the GSH/mercapturate/β-lyase pathway, genotoxicity in mammalian cells, and *in vivo* DNA binding in rats and mice. Chemical structural, functional, and metabolic analogies with recognized carcinogens, and evidence of tumor promoter activity further contribute to the weight of evidence.

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APPENDIX: CHEMICAL NAME ABBREVIATIONS

Chemical name abbreviations used in this document:

DCE	dichloroethane
HCBD	1,3-hexachlorobutadiene
N-Ac-PCBC	N-acetyl-S-(1,2,3,4,4-pentachlorobutadienyl)cysteine, the mercapturate of hexachlorobutadiene
N-Ac-PCBC-sulfoxide	N-acetyl-S-(1,2,3,4,4-pentachlorobutadienyl)cysteine-sulfoxide
PCBA	pentachlorobutanoic acid
PCBAC	pentachlorobutanoic acylchloride
PCBC	S-(1,2,3,4,4-pentachlorobutadienyl)cysteine, the cysteine conjugate of hexachlorobutadiene
PCBG	S-(1,2,3,4,4-pentachlorobutadienyl)glutathione, the glutathione conjugate of hexachlorobutadiene
PCCMTB	pentachloro-carboxymethylthio-1,3-butadiene
PCMTB	pentachloro-1-methylthio-1,3-butadiene
PERC	tetrachloroethylene
TCE	trichloroethylene