mammary adenomas were also increased in both sexes receiving the high dose. The NOAEL for hepatocellular tumours was estimated to be 200 ppm (16-21 mg/kg BW/day) and the LOAEL for all tumours was 1,000 ppm (81-103 mg/kg BW/day).

In a long-term rat inhalation study, no carcinogenic effects were observed (Torkelson et al., 1974). Male A/J mice administered 1,4-dioxane by intraperitoneal injection 3 times per week for 8 weeks for total doses of 400, 1,000 and 2,000 mg/kg BW exhibited increase in the multiplicity of lung tumours to 0.97 per mouse at the high dose compared with 0.28 per mouse in controls given vehicle alone (Maronpot et al., 1986). In a mouse-lung adenoma assay, 1,4-dioxane produced a significant increase in the incidence of lung tumours in males given an intermediate intraperitoneal dose whereas no such increase was noted in males given a lower or higher intraperitoneal dose or in females given the 3 intraperitoneal doses or in either males or females given 1,4-dioxane orally (Stoner et al., 1986).

Male SD rats were administered 1,4-dioxane by gavage once a day, 5 days per week for 7 weeks at doses of 100 or 1,000 mg/kg BW beginning 5 days after partial hepatectomy and injection of a single dose of 30 mg/kg BW N-nitrosodiethylamine (NDEA) to initiate hepatocarcinogenesis (Lundberg et al., 1987). The high dose increased the multiplicity of hepatic foci to 4.7 per cm^2 compared with 1.3 per cm^2 with NDEA initiation alone. Without partial hepatectomy or the NDEA initiation, 100 or 1,000 mg/kg BW 1,4-dioxane alone did not induce foci. Application of 0.2 mL to the skin of Swiss-Webster mice 3 times a week after initiation with dimethylbenzanthracene (DMBA) resulted in increase in the numbers of tumors in skin, lungs and kidneys (King e t al, 1973).

6. Effects on humans

Two cases of lethality due to in occupational exposure to 1,4-dioxane have been described (DeRosa et al., 1996). Hemorrhagic nephritis, centrilobular liver necrosis, severe epigastric pain, convulsion, and coma were found as the major effects. The levels or length of exposure could not be estimated in one case. In the other, the workers were exposed to 1,4-dioxane at levels ranging between 208 to 650 ppm for 1 week.

In volunteer short time exposure studies (200 or 300 ppm for 15 min; 1,600 ppm for 10 min; 5,500 ppm for 1 min), mucous irritation in eyes, nose and throat was noted as a clinical sign (DeRosa et al., 1996). After exposure to 50 ppm of 1,4-dioxane for 6 hr, only mild eye irritation was noted with no other clinical signs, demonstrated by chest X-ray, electrocardiograms, respiratory function tests, blood determinations, and urinalysis (Young et al., 1977).

In a cohort study of 74 workers exposed to 1,4-dioxane for an average duration of 25 years, with an estimated exposure to 0.02 to 48 mg/m^3 (0.006 to 13.3 ppm), no clinical signs or mortality were related to the chemical exposure (Thiess et al., 1976). No increase of chromosomal

aberrations in peripheral lymphocytes of 6 workers was noted as compared with controls. High serum transaminase levels were found in 6 of 24 current workers, but the authors concluded that these changes could have been related to habitual alcohol consumption. In another occupational cohort study of 165 workers exposed for at least 1 month over about 20 years to 1,4-dioxane at between 0.1 and 17 ppm, the observed number of cancer deaths was not different from that expected (Buffler et al., 1978).

A comparative mortality study in Denmark was conducted with 19,000 cases in the cancer register (Hansen, 1993). In male workers at companies dealing with 1,4-dioxane, the standard proportionate incidence ratio (SPIRS = 1.64) for liver cancer was significantly increased. Although alcohol consumption could not account for this increase, data for co-exposure to chemicals other than 1,4-dioxane or the exposure period and dose were not controlled.

7. Guideline value

1,4-Dioxane caused hepatic and nasal cavity tumors in rodents in most long-term oral studies conducted. Tumors in peritoneum, skin and mammary gland were also observed in rats given a high dose. Lung tumors were specifically detected after intraperitoneal injection. Although cohort studies of workers did not reveal any elevation in the incidence of death by cancer, significant increase of liver cancer was found in a comparative mortality study. However, the evidence is inadequate for human carcinogenicity assessment because of small samples or lack of exposure data. IARC has classified 1,4-dioxane as Group 2B; possibly carcinogenic to human (IARC 1999).

Although possibly weak genotoxic potential of 1,4-dioxane has been suggested, use of the linear multistage model for estimating cancer risk was chosen because the compound clearly induces multiple tumors in various organs. As results of calculations for the most sensitive sites, concentrations of 54 and 88 μg/L in drinking water associated with a lifetime excess cancer risk of 10^{-5} without body surface correction were derived from data for nasal carcinomas (NCI, 1978) and hepatic tumors (Yamazaki et al. 1994) from drinking water studies of rats.

On the other hand, assuming 1,4-dioxane is not genotoxic in humans at low doses, the TDI approach could be used for derivation of the guideline value. For a non-cancer endpoint, a TDI of 96 μg/kg BW/day has been calculated by applying an uncertainty factor of 100 (inter- and intra-species variation) to a NOAEL of 9.6 mg/kg BW/day from a long-term drinking water study in rats (Kociba et al., 1974). With a cancer endpoint, a TDI of 16 μg/kg BW/day has been calculated by applying an uncertainty factor of 1,000 (100 for inter- and intra-species variation, 10 for non-genotoxic carcinogenicity) to the NOAEL of 16 mg/kg BW/day from a long-term drinking water study of rats (Yamazaki et al. 1994). The equivalent concentration in the drinking water was

calculated to be 48 μg/L based on 10% allocation of the lower TDI from the cancer endpoint.

As similar values of 54 and 48 μg/L were derived with two different approaches, a rounded figure of 50 μg/L is rational as guideline value for 1,4-dioxane.

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Epichlorohydrin (ECH)

4. Kinetics and metabolism in laboratory animals and humans

The pharmacokinetics of ECH have been reviewed by the WHO (1984) and the U.S. EPA (1987). ECH is rapidly and extensively absorbed following oral administration. There is also evidence that it is similarly absorbed with both inhalation and dermal exposure.

Following oral administration of [^{14}C]-ECH to rats, peak tissue levels occur after 2 hr in males and from 2-8 hr in females, depending on the tissues (Weigel et al., 1978). These containing the highest levels of radioactivity are the kidneys, liver, pancreas, spleen and adrenals. Absorption and elimination of ECH are rapid in mice (Rossi et al., 1983a). After 200 mg/kg oral administration, the peak concentration (C_{max}), its time (T_{max}) and half-life ($t_{½}$) in the blood were found to be about 0.5 μg/mL, 5 min and 5 min for ECH, and 84 μg/mL, 10 min and 63 min for α-chlorohydrin (3-chloro-1,2-propanediol), respectively. ECH is rapidly removed from blood and is not likely to accumulate during chronic exposure. Metabolites of ECH, however, are much more persistent and have some potential for accumulation with in tissues.

The major metabolites in the urine have been identified as N-acetyl-S-(3-chloro-2-hydroxypropyl)-L-cysteine, formed by conjugation with glutathione, and α-chlorohydrin, accounting for about 31-36 and 1-4 % of the administered dose, respectively (Gingell et al., 1985; Rooij et al., 1996). ECH is a substrate for an epoxide hydratase, resulting in the formation of α-chlorohydrin (Gingell et al., 1987).

Following either oral administration or inhalation, ECH metabolites are rapidly excreted in the urine and expired air. After an oral dose of 6 mg/kg BW to male rats, approximately 38 % of the dose was exhaled as CO_2, and 50 % was excreted as metabolites in urine, while 3 % was present in the feces within 3 days (Gingell et al., 1985). The half-life of initial elimination of radioactivity in the urine and expired air was about 2 hr. After an oral dose of 10 mg/kg BW, 18-21% of the dose was exhaled as CO_2, 38-40 % was excreted as metabolites in the urine, and minor amounts (4 %) were excreted in the feces over 72 hr (Weigel et al., 1978). Unmetabolized ECH was not been detected in any excreta.

5. Effects on laboratory animals and *in vitro* test systems

5.1 Acute exposure

EHC is a strong irritant and acutely toxic following oral, percutaneous, subcutaneous or respiratory exposure (Lawrence et al., 1972). Death is due to effects on the central nervous system and the respiratory center (Freuder & Leake, 1941). Oral LD_{50} values for ECH have been reported

to range from 90 to 260 mg/kg BW in rats (US EPA, 1987). With intraperitoneal application in male ICR mice and male SD rats, they were 170 and 113 mg/kg BW, respectively (Lawrence et al., 1972).

The dermal LD_{50} in male New Zealand White rabbits was 754 mg/kg BW. Sensitization potential was evident using the guinea pig maximization test, the lowest concentration of ECH producing irritating activity, being 0.625 % (v/v) in cotton seed oil (Lawrence et al., 1972).

5.2 Short-term exposure

In a 90-day oral study in both sexes of rats (FDRL strain), the maximum tolerated dose of ECH was established to be 45 mg/kg BW/day, based on depressed growth and mortality (Oser et al., 1975). Oral administration of ECH by gavage in distilled water to SD rats of both sexes at doses of 3, 7, 19 and 46 mg/kg BW for 10 days, and 1, 5 and 25 mg/kg BW for 90 days resulted in weight loss, increased weights of kidney and liver, and decreased erythrocyte counts with the high doses in both studies (Daniel et al., 1996). The most pronounced effect on histopathological examination was a dose-related increase in mucosal hyperplasia and hyperkeratosis in forestomach. The lowest observable adverse effect level was 3 mg/kg BW/day in the 10 days study and 1 mg/kg BW/day in the 90 days study.

Intraperitoneal administration of ECH to male SD rats for 12 weeks (3 days/week) casued a dose-related decrease in haemoglobin values and an increase in segmented neutrophils at 56 mg/kg BW/day (Lawrence et al., 1972).

5.3 Long-term exposure

Oral administration of ECH in water by gavage 5 times a week to weanling Wistar rats of both sexes for 104 weeks was associated with gradual increase in mortality with clinical symptoms including dyspnea and weight loss, decrease in leukocytes and hyperplasia in the forestomach at doses of 2 and 10 mg/kg BW (equivalent to 1.43 and 7.14 mg/kg BW/day) (Wester et al., 1985).

Inhalation of ECH by noninbred male SD rats for 6 hr/day, 5 days/week in lifetime caused weight loss, high mortality, severe inflammatory changes in the nasal cavity, lung congestion and pneumonia, tubular dilatation and dose dependent tubular degeneration in the kidney at 38 and 114 mg/m^3 (Laskin et al., 1980).

5.4 Reproductive toxicity, embryotoxicity, and teratogenicity

Although no microscopic changes or changes in weight of the testes were observed in

male Wistar rats given a single oral dose of ECH at 25 or 50 mg/kg BW, an increased percentage of abnormal sperm heads at 50 mg/kg BW and a reduced number of sperm heads at 25 mg/kg BW were found (Cassidy et al., 1983). Male and female Long-Evans rats were given EHC by gavage at 12.5, 25, or 50 mg/kg BW/day for 21 days. Mating trials with untreated rats were then performed for the highest dose (Toth et al., 1989). None of the females that mated with the males given ECH at 50 mg/kg BW/day became pregnant. Adverse effects on the vigor and swimming pattern of sperms were found at 12.5 mg/kg BW/day and higher. The authors suggested that these effects due to ECH were mediated principally through its metabolite, α-chlorohydrin.

Male New Zealand White rabbits and male SD rats were exposed to ECH via inhalation for 6 hr/day, 5 days/week, at 5, 25, or 50 ppm for 10 weeks (John et al., 1983). No adverse effects on male fertility or sperm parameters in rabbits were observed. A decrease in number of implantation sites in unexposed females mated with males exposed at 25 ppm and an increase in number of infertile males at 50 ppm were found in the rats. A single inhalation of ECH for 4 hr at 100 ppm caused transient decrease in sperm velocity in F-344 rats (Slott et al., 1990), but a single subcutaneous injection of ECH at 31.3 mg/kg BW did not cause any changes in body weight, testicular weight, epididymal weight, sperm count, or incidence of sperm abnormalities (Omura et al., 1995). When ECH at 0.025, 0.05, 0.1 and 0.2 mg/kg BW/day was intraperitoneally injected into (CBA X BALB/c) F_1 male mice for 5 days, no increase in abnormal sperm heads was detected after 5 weeks (Topham, 1980).

ECH was given by gavage to CD rats at 40, 80 or 160 mg/kg BW/day and to CD-1 mice at 80, 120 or 160 mg/kg BW/day on days 4-15 of pregnancy (sperm in virginal plugs= day 0) (Marks et al., 1982). In rats, ECH was maternally lethal at 160 mg/kg BW/day, but no adverse effects on fetuses were found. In mice, ECH was maternally toxic at 160 mg/kg BW/day and a decrease in fetal weight was detected at 120 mg/kg BW/day. Female Long-Evans rats were given EHC by gavage at 25, 50, or 100 mg/kg BW/day for 14 days prior to mating trials with untreated rats (Toth et al., 1989). Treated females were further dosed until delivery, but no adverse effects on reproductive parameters were detected. Similarly, reproductive function was unchanged after exposure of female SD rats to ECH via inhalation for 6 hr/day, 5 days/week, at 5, 25, or 50 ppm for 10 weeks (John et al., 1983).

5.5 Mutagenicity and related end-points

ECH induced base-change type mutations in *Salmonella typhimurium* and *Escherichia coli* in the absence of metabolic activation (Bridges, 1978). It also induced gene mutations in *Klebsiella pneumonia* without exogenous metabolic activation (Voogd et al., 1981; Knaap et al., 1982). It has been shown to cause chromosomal aberrations (Terada et al., 1992), gene mutations (Amacher &

Dunn, 1985; Perocco et al., 1983) and sister chromatid exchange (von der Hude et al., 1991) in mammalian cells *in vitro* but proved negative in the mouse micronucleus assay (Kirkhart, 1981; Asita et al., 1992) and in the mouse dominant lethal assay (Epstein et al., 1972). It was found to induce DNA damage, gene conversion (Zimmermann & Scheel, 1981), recombination (Vashishat et al., 1980), aneuploidy (Parry & Sharp, 1981) and mutation (Mehta & von Borstel, 1981) in *Saccharomyces cerevisiae* and gene mutations in *Schizosaccharomyces pombe* (Rossi et al., 1983b) and *Neurospora crassa* (Kolmark & Giles, 1955). It was also mutagenic in the *Drosophila melanogaster* sex-linked recessive lethal mutation assay (Vogel et al., 1981; Knaap et al., 1982) and induced DNA single-strand breaks (Garberg et al., 1988) but not unscheduled DNA synthesis (Probst et al., 1981) in mammalian cell cultures. In a single study, ECH bound to DNA of mice and rats treated *in vivo* (Prodi et al., 1986). Positive results were also reported in the mouse host-mediated assay in one of three studies (Srám et al., 1976).

5.6 Carcinogenicity

Male Wistar rats that had received ECH at 18, 39 or 89 mg/kg BW/day in drinking water for 81 weeks showed increased incidences of forestomach proliferative lesions: hyperplasia, 0/10, 7/9, 9/10 and 12/12; papillomas, 0/10, 0/9, 1/10 and 7/12; carcinomas, 0/10, 0/9, 1/10 and 2/12 in the control, low-dose, mid-dose and high-dose groups, respectively (Konishi et al., 1980). Thus, the NOAEL for forestomach tumours was estimated to be 18 mg/kg BW/day.

Wistar rats of both sexes given ECH by gavage for 2 years, 5 times a week at doses of 2 or 10 mg/kg BW developed squamous cell carcinomas in the forestomach in both 2 mg/kg BW (2/44 for females, 6/49 for males) and10 mg/kg BW (24/39 for females, 35/49 for males) groups, this tumour not being found in control animals (Wester et al., 1985). Thus, a NOAEL for forestomach tumours could not be derived.

Male SD rats were exposed through whole-body inhalation to 10 or 30 ppm (38 or 113 mg/m^3) ECH for 6 hr per day on 5 days per week for lifetime or to 100 ppm (380 mg/m^3) for 6 hr per day on 30 days followed by observation for the lifetime (Laskin et al., 1980). In rats exposed to 30 ppm ECH and rats exposed 30 times to 100 ppm and observed for lifespan, papillomas and squamous cell carcinomas of the nasal cavity were noted whereas no neoplastic changes were reported at 10 ppm ECH and in control groups. Among 100 rats, lifetime exposure to 30 ppm yielded only one squamous cell carcinoma of the nasal cavity plus one nasal papilloma.

Subcutaneous injection of ECH in ICR/Ha Swiss mice induced local sarcomas and adenocarcinomas (Van Duuren et al., 1974).

In a strain A lung adenoma assay, intraperitoneal injection of total doses of 20, 50 or 100 mg/kg BW given 3 times per week for 8 weeks significantly increased the number of lung tumours

per mouse in males treated with the highest dose but not in other groups (Stoner et al., 1986).

ECH was active as an initiator in a two-stage skin carcinogenesis study in mice although it gave negative results after continuous skin painting (Van Duuren et al., 1974).

6. Effects on humans

Acute toxic responses following dermal exposure are characterized by an initial redness and an itching or burning sensation (Schultz, 1964). With time, the redness intensifies and the tissue becomes swollen and blistered. The initial symptoms following inhalation are local irritation, burning of eyes and throat, swelling of the face, nausea, vomiting and severe headache.

In a case-study, long-term effects due primarily to damage of the liver and kidney were still present 2 years after exposure (Shultz, 1964). In ECH workers, increased incidences of chromatid and chromosomal breaks in peripheral lymphocytes and decrease of blood cell counts were observed (Sram et al., 1980). DNA adducts (7-(3-chloro-2-hydroxypropyl)guanine) in white blood cells were detected by the ^{32}P-post-labelling assay in 7 of 16 workers exposed to ECH (Plna et al., 2000). This adduct was not detected in any of 13 controls.

Another cohort study was undertaken for 863 workers with probable exposure to ECH at two chemical plants (Enterline et al., 1990). All cancers, leukemia, and most other causes of death were related to the estimated levels of exposure to ECH. The most consistent relationship was between exposure level and heart disease. However, a ten-year extension of the follow up in this study did not support an association between exposure to ECH and heart disease or lung cancer (Tsai et al., 1996). In addition, there were no additional deaths from leukaemia with this update and the overall mortality and cancer mortality of employees potentially exposed to ECH continued to be lower than that of the local population.

A retrospective cohort mortality study of 1,064 workers, who were employed in the epoxy resin, glycerin and allyl chloride/epichlorohydrin production areas of a large chemical facility between 1957 and 1986, was conducted (Olsen et al., 1994). Follow-up was carried out until 1989 and the mortality was compared with national rates and company rates for other facilities. Exposure to ECH was estimated to be up to 5 ppm [18.9 mg/m^3]. No associations between site-specific cancer risks and exposure to ECH were observed.

Nested case–control studies for lung and central nervous system neoplasms were conducted by Barbone et al. (1992, 1994) using the full cohort of dye and resin manufacturing workers reported by Delzell et al. (1989). For 51 lung cancer cases, a weak association with ECH exposure (odds ratio: 1.7) was observed, but risk was not related to the level of exposure. For 11 central nervous system tumour cases, an association was observed with potential exposure to ECH (odds ratio: 4.2) and the magnitude of the association increased with both duration of exposure and

cumulative exposure level.

However, an opposite association for lung cancer was obtained with another nested case–control study (Bond et al., 1986), conducted using a cohort of 19,608 male chemical workers, who were employed for 1 year or more between 1940 and 1980 at a large facility which produced chlorinated solvents, plastics, chlorine, caustic soda, ethylene, styrene, epoxy latex, magnesium metal, chloro-nitrogen agricultural chemicals and glycols. Exposure to ECH was associated with a decreased risk of lung cancer (odds ratio: 0.3).

The fertility status of 64 glycerine workers exposed to ECH, ally chloride, and 1,3-dichloropropane was compared with that of a control group of 63 workers who had not handled chlorinated hydrocarbons for more than 5 years (Venable et al., 1980). No association was found between levels, duration, or intensity of exposure and sperm characteristics or hormone levels. A similar negative result for sperm count and hormone levels was obtained for a group of 128 workers from two plants compared with external chemical plant workers who had not been exposed to any chemical known to be toxic to the testes (Milby et al., 1981). In one of these plants, most of the employees were exposed to ECH concentrations below 3.8 mg/m^3. The rate of non-participating employees was high in both plants, 172 workers in total.

7. Provisional guideline value

Major toxic effects of ECH are local irritation and damage to the central nervous system. It induces forestomach tumors with oral administration and squamous cell carcinomas in the nasal cavity on inhalation. It has been shown to be genotoxic *in vitro* and *in vivo*. For human carcinogenicity, results in epidemiological studies are contradictory and all are based on relatively small numbers. IARC has placed ECH in Group 2A (probably carcinogenic to human) (IARC 1999).

Adverse effects of ECH were observed on sperm with oral or inhaled administration to rats with a LOAEL for reproductive toxicity considered to be 12.5 mg/kg BW/day. However, effects were not observed in rats with subcutaneous injection or in other species (mice and rabbits). Therefore, data for induction of sperm abnormalities by ECH exposure are inconclusive.

Although ECH is a genotoxic carcinogen, the use of the linear multistage model for estimating cancer risk has been considered inappropriate because tumors are seen only at the site of administration where ECH is highly irritating. A TDI of 0.143 μg/kg BW/day has been calculated by applying an uncertainty factor of 10,000 (100 for inter- and intra-species variation, 10 for use of LOAEL instead of NOAEL, 10 for carcinogenicity (forestomach tumors)) to the LOAEL of 1.43 mg/kg BW/day (2 mg/kg BW, 5 days/week) obtained in a long-term gavage study of rats (Wester et al., 1985). This gives a provisional guideline value of 0.4 μg/L (round figure) based on an

allocation of 10 % of the TDI to drinking water. A practical quantitation level for ECH is in the order of 30 μg/L, but concentrations in drinking water can be controlled by product specification.

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HEXACHLOROBUTADIENE (HCBD)

4. Kinetics and metabolism in laboratory animals and humans

HCBD has been found to be a good skin penetrant in rabbit (Duprat & Gradiski, 1978) and about 95 % of the ingested dose was absorbed by 16 hr after a single oral dose of [^{14}C]-HCBD 200 mg/kg BW in corn oil in rat, although 85 % of the radioactivity was still present in the small intestine as unchanged HCBD at 4 hr (Nash et al., 1984). After a single oral dose of 1 or 50 mg/kg BW of [^{14}C]-HCBD in female rats, 11 - 31 % of the radioactivity were excreted in the urine by 72 hr, while the major portion (42 - 69 %) were excreted in the feces (Reichert et al., 1985). At the high dose, the absorption of HCBD appeared to be saturated with the result that unchanged HCBD constituted the major portion of the 69 % of radioactivity eliminated in the feces.

The distribution of [^{14}C]-HCBD in rats is determined by the lipophilicity, with largest amounts of radioactivity detected in the kidney, liver and brain (Reichert et al., 1985). Covalent binding to protein was found in the kidney and liver. Autoradiograms showed a specific distribution of radioactivity, especially in the outer medulla of the kidney at 4 hr after an oral dose of [^{14}C]-HCBD in rats (Nash et al., 1984). When a mixture of chlorinated hydrocarbons with HCBD was orally administered to rats at 4 mg/kg BW/day for up to 12 weeks, bioaccumulation of HCBD in adipose tissue was observed up to 8 mg/kg fat (Jacobs et al., 1974).

HCBD is metabolized via conjugation with glutathione (GSH) by GSH-S-transferase, to 1-(glutathione-S-yl)-1,2,3,4,4-pentachlorobuta-1,3-diene (PCBD-GSH) and 1,4-bis(glutathione-S-yl)-1,2,3,4-tetrachlorobuta-1,3-diene (TCBD-diGSH) in rats and mice (Wolf, et al., 1984; Dekant et al., 1988a). The GSH conjugate of HCBD is extensively excreted in the bile of rats and mice, and further metabolized in the intestinal tract and kidney to a number of water soluble metabolites which are predominantly excreted in the urine (Davis et al., 1980, Nash et al., 1984, Reichert & Schutz, 1986). At least 80 % of biliary metabolites of HCBD underwent biliary recycling in one study (Payan et al., 1991). The experimental evidence suggests that the metabolism of PCBD-GSH involves, in part, degradation to S-(1,2,3,4,4-pentachlor-1,3-butadienyl)-cysteine (PCBD-Cys) which is nephrotoxic (Jaffe et al., 1983). PCBD-Cys is N-acetylated to give the mercapturic acid, N-acetyl-S-(1,2,3,4,4-pentachlor-1,3-butadienyl)-Cys (PCBD-NAc) (Reichert & Schutz, 1986, Schrenk et al., 1988). N-Acetylation is the presumable detoxification reaction for PCBD-Cys, but the mercapturate may undergo cycling within the kidney to regenerate the toxic Cys conjugate. The Cys conjugate requires further bioactivation via Cys conjugate β-lyase, for which PCBD-Cys is a substrate (Jones et al., 1986, Pratt & Lock, 1988, Dekant et al., 1988b), resulting in formation of the electrophile trichlorovinyl-chlorothioketene which binds covalently to cellular macromolecules

(Dekant et al., 1986; Ishmael & Lock, 1986).

Other experiments showed that only liver microsomes from male rats catalyze the formation of a toxic electrophile, PCBD-NAc sulfoxide (Birner et al., 1995). Specific inhibition by troleandomycin and induction of rat liver microsomal sulfoxidation of PCBD-NAc by dexamethasone suggest that P450 3A1/2 sulfoxidizes PCBD-NAc and P450s of the 3A family might be responsible for the PCBD-NAc nephrotoxicity (Werner et al., 1995).

The major metabolite excreted by female rats was found to be PCBD-NAc with small amounts of PCBD-Cys detected (Birner et al., 1995). Unmetabolised HCBD has been reported to be present in urine of male but not female rats, this being due to be binding potential to α_{2U}-globulin, a male rat specific protein (Pähler et al., 1997).

After a single oral administration of 200 mg/kg BW [^{14}C]-HCBD to rats, the principal route of excretion was in the bile with 17 to 20 % of the initial dose excreted on each of the first 2 days (Nash et al., 1984). Extensive enterohepatic circulation must have occurred because fecal elimination amounted to only 5 % of the total dose of radioactivity/day. Similarly, between 11 and 16 % and between 3 and 5 % of the radioactivity was excreted in the feces and urine, respectively, with 1-2 % eliminated as unchanged HCBD by 48 hr after a single oral dose of 200 mg/kg BW [^{14}C]-HCBD in rats (Birner et al., 1995).

Another study revealed that between 42 and 69 % and between 11 and 31 % of the radioactivity was excreted in the feces and urine by 72 hr, respectively, after a single oral dose of 1 or 50 mg/kg BW [^{14}C]-HCBD in the rat (Reichert et al., 1985). Elimination in expired air was consistent over 72 hr at about 5 % as unchanged HCBD. Similar results were obtained in mice after a single oral dose of 30 mg/kg BW [^{14}C]-HCBD (67-77 % in feces, 6.6-7.6 % in urine, 4-5 % in exhaled air and 6.7-14 % in the carcass after 72 hr) (Dekant et al., 1988c). The total excretion within 72 hr was at least 65 % of the dose after a single oral administration of [^{14}C]-HCBD up to 50 mg/kg BW to rats and mice.

The biotransformation of HCBD in rats appears to be a saturable process considering the reduced excretion of CO_2 and renal metabolites with increasing doses (Davis et al., 1980; Reichert et al., 1985; Payan et al., 1991).

5. Effects on laboratory animals and *in vitro* test systems

5.1 Acute exposure

Oral LD_{50} values has been reported to be 200 to 400 mg/kg BW and 504 to 667 mg/kg BW, respectively, in adult female and male rats (Schwetz et al., 1977). Oral or intraperitoneal administration of HCBD to rats of both sexes resulted in nephrotoxicity, evidenced by increase in relative kidney weights and blood urea nitrogen, and renal tubular cell necrosis (Hook et al., 1983a

& 1983b; Kuo & Hook, 1983; Harleman & Seinen, 1979; Kociba et al., 1971). At 48 hr after a single oral dose of 200 mg/kg BW in male and female Wistar rats, necrosis in the proximal tubules was increased in male rats rather than female rats. Male rats also demonstrated slight centrilobular toxicity in liver, but again no changes were found in females (Birner et al., 1995).

The dermal LD_{50} in rabbits was 0.72 mL/kg BW after 8 hr exposure, cutaneous necrosis occurring at the site of application (Duprat & Gradiski, 1978). A single intraperitoneal dose of 100 mg/kg BW to male SD rats caused a reduction in urine osmolality and body weights (Berndt & Mehendale, 1979). A single intraperitoneal dose of 170 mg/kg BW to male Wistar rats resulted in severe proximal tubular damage 24 hr after the treatment (Kirby & Bach, 1995). Advanced regeneration and repair were evident at 21 days.

5.2 Short-term exposure

When weanling Wistar rats were administered HCBD by gavage at 0, 0.4, 1, 2.5, 6.3 or 15.6 mg/kg BW/day for 13 weeks (Harleman & Seinen, 1979), relative kidney weights were increased at 6.3 and 15.6 mg/kg BW/day in both sexes, and increased incidences of degeneration in the proximal renal tubules were observed at 2.5 mg/kg BW/day or more in females, and at 6.3 mg/kg BW/day or more in males. Increased incidences of cytoplasmic basophilia of hepatocytes associated with the increased liver weight occurred with the two highest doses in males.

5.3 Long-term exposure

SD rats were administered HCBD with 0, 0.2, 2 or 20 mg/kg BW/day in diet for 2 years. Relative and absolute kidney weights increased at 20mg/kg BW/day in males. At 2 and 20 mg/kg BW/day, increased incidences of renal tubular hyperplasia and focal adenomatous proliferation of renal tubular cells were observed. The NOAEL was established as 0.2 mg/kg BW/day (Kociba et al., 1977).

5.4 Reproductive toxicity, embryotoxicity, and teratogenicity

Male and female SD rats were given dietary HCBD at 0.2, 2.0, or 20 mg/kg BW/day for 90 days prior to mating, for 15 days during mating, and subsequently throughout gestation and lactation (Schwetz et al., 1977). No effects on pregnancy or neonatal survival and development were found. Decreases in body weight and food consumption and histopathological changes in the kidney in adult rats at 2.0 and 20 mg/kg BW/day and low body weight in 21-day-old offspring at 20 mg/kg BW/day were observed. These findings indicated a NOAEL of 0.2 mg/kg BW/day, based

on maternal toxicity, evidenced by decreases in body weight and food consumption, and histopathological changes in the kidney.

Female Wistar-derived rats were given dietary HCBD at 150 or 1,500 ppm for 4 weeks prior to mating with untreated males, and treatment continued until the offspring were 18 weeks of age (Harleman & Seinen, 1979). At 1,500 ppm, female rats lost weight and displayed weakness of the hind legs and unsteady gait, and no conception occurred. At 150 ppm, the offspring had reduced birth weights and subsequent growth.

Pregnant SD rats were intraperitoneally injected with HCBD at 10 mg/kg BW/day on days 1-15 of pregnancy (Harris et al., 1979). The number of fetuses with soft tissue anomalies was 3 times as high as controls, but no specific anomaly prevailed. Pregnant Wistar rats were exposed to HCBD via inhalation at 2, 5, 10, or 15 ppm for 6 h/day during days 6-20 of pregnancy (Saillenfait et al., 1989). Although no teratogenicity was detected, maternal body weight gain and fetal body weights were decreased at 15 ppm.

5.5 Mutagenicity and related end-points

In the Ames test, although negative results have been obtained for mutagenicity of HCBD in certain *Salmonella* strains (DeMeester et al., 1981, Reichert et al., 1983), the compound has been reported to be mutagenic to *Salmonella typhimurium* (Reichert et al, 1984). Metabolites and derivatives of HCBD were mutagenic to *Salmonella* test strains with metabolic activation (Reichert & Schutz, 1986, Green et al., 1983), and possible metabolites of HCBD were found to be mutagenic in *S. typhimurium* TA98, TA100, and TA2638 without S9 mix activation (Dekant et al., 1986). In addition, HCBD produced a dose-dependent increase in the Ara^r forward mutation frequency in *S. typhimurium* that was more marked in the absence than in the presence of an exogenous metabolic system (Roldán-Arjona et al., 1991). HCBD did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (Woodruff et al., 1985), but increased the frequency of sister chromatid exchange in Chinese hamster ovary cells in both the absence and presence of an exogenous metabolic system from liver, while not inducing chromosomal aberrations under the same experimental conditions (Galloway et al., 1987).

5.6 Carcinogenicity

SD rats of both sexes administered HCBD in the diet at a dose of 20 mg/kg BW/day for 2 years caused renal tubular adenomas and adenocarcinomas, but no renal tubular neoplasmas were observed in rats ingesting 2.0 or 0.2 mg/kg BW/day (Kociba et al., 1977). The authors concluded that HCBD-induced renal neoplasma occurred only at doses higher than those causing discernible

renal injury. Thus, the NOAEL for renal tumours was estimated to be 2.0 mg/kg BW/day.

Induction of lung adenomas was not observed in male strain A mice following intraperitoneal administration of HCBD (4 or 8 mg/kg BW) 3 times per week until total exposures of 52 or 96 mg (Theiss et al., 1977). Female Ha: ICR Swiss mice that had received applications of 2 or 6 mg HCBD in 0.2 mL acetone on the dorsal skin 3 times per week for 440–594 days showed no tumours of the skin or distant organs (Van Duuren et al., 1979).

Male Wistar rats were given 0.1 % N-nitroso ethylhydroxyethylamine (NEHEA) in drinking water for 2 weeks and then 0.1 % HCBD in the diet for 30 weeks. Three other groups received either HCBD or NEHEA alone according to the same regimen or basal diet for 32 weeks. The incidence of renal tubular tumours in the group given NEHEA plus HCBD (15/21) was greater than that in rats given NEHEA alone (5/10), and the incidence of preneoplastic renal tubular hyperplasia was also increased (21/21 versus 4/10) (Nakagawa et al., 1998).

Female Ha: ICR Swiss mice that had received a single application of 15 mg HCBD in 0.2 mL acetone on the dorsal skin, followed 14 days later by 5 μg phorbol myristate acetate in 0.2 mL acetone 3 times a week for 428–576 days did not develop any tumours of the skin (Van Duuren et al., 1979).

6. Effects on humans

There have been limited studies in humans exposed to HCDB. Farm workers exposed intermittently for 4 years exhibited higher incidences of hypotension, myocardial dystrophy, nervous disorders, smell and liver function disorders, and respiratory tract lesions, but they were also exposed to other substances (Krasniuk et al., 1969). In a preliminary study, exposure to HCBD was associated with dose-dependent increases in a number of individual and summed bile acid parameters (Driscoll et al., 1992). In a cytogenetic study of workers occupationally exposed in the production of HCDB, an increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes was observed, but this was not linked with the period of employment (German, 1986).

7. Guideline value

In a long-term oral study, an increased incidence of kidney tumors was caused by HCBD administration, but no carcinogenic effects were detected with intraperitoneal or dermal administration. Positive and negative results for HCBD have been obtained in *in vitro* genotoxicity test systems, but several metabolites have given positive results. IARC has placed HCDB in Group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1999). No adverse effects were detected at low doses in reproductive and developmental studies, other than maternal toxicity.