IPCS

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

Environmental Health Criteria 156

Hexachlorobutadiene

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Environmental Health Criteria 156

HEXACHLOROBUTADIENE

First draft prepared by Dr T. Vermeire,
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The Netherlands

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The **International Programme on Chemical Safety (IPCS)** is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are kindly requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (Telephone No. 9799111).

* * *

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ENVIRONMENTAL HEALTH CRITERIA FOR HEXACHLOROBUTADIENE

A Task Group on Environmental Health Criteria for Hexachlorobutadiene met at the Institute of Hygiene and Epidemiology, Brussels, Belgium, from 10 to 15 December 1992. Dr C. Vleminckx welcomed the participants on behalf of the host institution and Professor F. Valić opened the meeting on behalf of the three cooperating organizations of the IPCS (UNEP/ILO/WHO). The Task Group reviewed and revised the draft monograph and made an evaluation of the risks for human health and the environment from exposure to hexachlorobutadiene.

The first draft of this monograph was prepared by Dr T. Vermeire, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Professor F. Valić was responsible for the overall scientific content of the monograph and for the organization of the meeting, and Dr P.G. Jenkins, IPCS, for the technical editing of the monograph.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

ABBREVIATIONS

ACPB	1-(*N*-acetylcystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene
BCTB	1,4-(bis-cystein-*S*-yl)-1,2,3,4-tetrachloro-1,3-butadiene
BGTB	1,4-(bis-glutathion-*S*-yl)-1,2,3,4-tetrachloro-1,3-butadiene
CMTPB	1-carboxymethylthio-1,2,3,4,4-pentachloro-1,3-butadiene
CPB	1-(cystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene
GPB	1-(glutathion-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene
GSH	reduced glutathione
HCBD	hexachlorobutadiene
ip	intraperitoneal
iv	intravenous
MTPB	1-methylthio-1,2,3,4,4-pentachloro-1,3-butadiene
NIOSH	National Institute of Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PATPB	1-(pyruvic acid thiol)-1,2,3,4,4-pentachloro-1,3-butadiene
PBSA	1,2,3,4,4-pentachloro-1,3-butadienyl sulfenic acid
TBA	2,3,4,4-tetrachloro-1,3-butenoic acid
TPA	12-*o*-tetradecanoylphorbol-13-acetate
TPB	1-thiol-1,2,3,4,4-pentachloro-1,3-butadiene
UDS	unscheduled DNA synthesis

1. SUMMARY

1.1 Identity, physical and chemical properties, analytical methods

Hexachlorobutadiene is a non-flammable, incombustible, clear, oily and colourless liquid at ordinary temperature and pressure. It is poorly soluble in water but miscible with ether and ethanol.

The substance can be detected and determined quantitatively by gas chromatographic methods. The detection limits are 0.03 $\mu g/m^3$ in air, 0.001 μg/litre in water, 0.7 μg/kg wet weight in soil or sediment, and 0.02 μg/litre in blood. A level of 0.47 μg/kg wet weight has been determined in tissue.

1.2 Sources of human and environmental exposure

Hexachlorobutadiene is not reported to occur as a natural product. It is chiefly produced as a by-product of the manufacture of chlorinated hydrocarbons where it occurs in the heavy fractions (Hex-waste). The world annual production of the compound in heavy fractions was estimated in 1982 to be 10 000 tonnes.

Hexachlorobutadiene can be used for recovery of chlorine-containing gas in chlorine plants and as a wash liquor for removing certain volatile organic compounds from gas streams. It has further been used as a fluid in gyroscopes, as heat transfer, transformer, insulating and hydraulic fluids, as a solvent for elastomers, and as an intermediate and fumigant.

1.3 Environmental transport, distribution and transformation

The main pathways of entry into the environment are emissions from waste and dispersive use. Intercompartmental transport will chiefly occur by volatilization, adsorption to particulate matter, and subsequent deposition or sedimentation. Hexachlorobutadiene does not migrate rapidly in soil and accumulates in sediment. In water, it is considered persistent unless there is high turbulence. Hydrolysis does not occur. The substance seems to be readily biodegradable aerobically, though biodegradability has not been investigated thoroughly. Hexachlorobutadiene photolyses on surfaces. In addition to deposition, reaction with hydroxyl radicals is assumed to be an important sink of hexachlorobutadiene in the troposphere, and the estimated atmospheric half-life is up

to 2.3 years. The substance has a high bioaccumulating potential as has been confirmed by both laboratory and field observations. Average steady-state bioconcentration factors of 5800 and 17 000, based on wet weight, have been determined experimentally in rainbow trout. Biomagnification has not been observed either in the laboratory or in the field.

1.4 Environmental levels and human exposure

Hexachlorobutadiene has been measured in urban air: in all cases levels were below 0.5 $\mu g/m^3$. Concentrations in remote areas are less than 1 pg/m^3. In lake and river water in Europe concentrations of up to 2 μg/litre have been recorded, but mean levels are usually below 100 ng/litre. In the Great Lakes area of Canada, much lower levels (around 1 ng/litre) were measured. Bottom sediment levels here can be as high as 120 μg/kg dry weight. Older sediment layers from around 1960 contained higher concentrations (up to 550 μg/kg wet weight). The sediment concentration was demonstrated to increase with particle size in the sediment.

Concentrations of hexachlorobutadiene in aquatic organisms, birds and mammals indicate bioaccumulation but not biomagnification. In polluted waters, levels of over 1000 μg/kg wet weight have been measured in several species and 120 mg/kg (lipid base) in one species. Present levels generally remain below 100 μg/kg wet weight away from industrial outflows.

The compound has been detected in human urine, blood and tissues. Certain food items containing a high lipid fraction have been found to contain up to about 40 μg/kg and, in one case, over 1000 μg/kg.

One study reported occupational exposures of 1.6-12.2 mg/m^3 and urine levels of up to 20 mg/litre.

1.5 Kinetics and metabolism

Hexachlorobutadiene is rapidly absorbed following oral administration to experimental animals, but the rate of absorption following inhalation or dermal exposure has not been investigated. In rats and mice, the compound distributes mainly to the liver, kidneys and adipose tissue. It is rapidly excreted. Binding to liver and kidney protein and nucleic acids has been demonstrated.

The biotransformation of the compound in experimental animals appears to be a saturable process. This process proceeds

mainly through a glutathione-mediated pathway in which hexachlorobutadiene is initially converted to *S*-glutathione conjugates. These conjugates can be metabolized further, especially in the brush-border membrane of renal tubular cells, to a reactive sulfur metabolite, which probably accounts for the observed nephrotoxicity, genotoxicity and carcinogenicity.

1.6 Effects on organisms in the environment

Hexachlorobutadiene is moderately to very toxic to aquatic organisms. Fish species and crustaceans were found to be the most sensitive, 96-h LC_{50} values ranging from 0.032 to 1.2 and 0.09 to approximately 1.7 mg/litre for crustaceans and fish, respectively. The kidney was demonstrated to be an important target organ in fish.

Based on several long-term tests with algae and fish species, a no-observed-effect level (NOEL) of 0.003 mg/litre was established; this classifies the compound as very toxic to aquatic species. End-points investigated include general toxicity, neurotoxicity, biochemistry, haematology, pathology, and reproductive parameters. In one 28-day early-lifestage test with fathead minnows, reproduction was unaffected at concentrations of up to 0.017 mg/litre, whereas increased mortality and a decreased body weight were observed at 0.013 and 0.017 mg/litre. The NOEL was 0.0065 mg/litre.

Only one reliable test with terrestrial organisms has been described. In a 90-day test with Japanese quail, receiving a diet containing the compound at concentrations from 0.3 to 30 mg/kg diet, the survival of chicks was decreased at 10 mg/kg diet only.

1.7 Effects on experimental animals and *in vitro* test systems

1.7.1 General toxicity

Hexachlorobutadiene is slightly to moderately toxic to adult rats, moderately toxic to male weanling rats, and highly toxic to female weanling rats following a single oral dose. The major target organs are the kidney and, to a much lesser extent, the liver.

Based on animal data, the vapour of hexachlorobutadiene is irritating to mucous membranes and the liquid is corrosive. The substance should be regarded as a sensitizing agent.

In the kidneys of rats, mice and rabbits, hexachlorobutadiene causes a dose-dependent necrosis of the renal proximal tubules. Adult male rats are less sensitive to renal toxicity than adult females or young males. Young mice are more susceptible than adults, no sex difference being apparent. In adult female rats the lowest single intraperitoneal dose at which renal necrosis was observed was 25 mg/kg body weight, and in adult male and female mice it was 6.3 mg/kg body weight. Biochemical changes and distinct functional alterations in the kidneys occurred at doses similar to or higher than those at which necrosis occurred.

In six short-term oral tests, two reproductive studies and one long-term diet study with rats, the kidney was also the major target organ. Dose-related effects included a decreased relative kidney weight and tubular epithelial degeneration. The no-observed-adverse-effect level (NOAEL) for renal toxicity in rats in a 2-year study was 0.2 mg/kg body weight per day. In mice the NOAEL in a 13-week study was 0.2 mg/kg body weight per day. In both species, adult females were more susceptible than adult males.

In one short-term inhalation test (6 h/day for 12 days), similar effects on the kidneys were observed with a nominal vapour concentration of 267 mg/m^3, at which concentration respiratory difficulties and cortical degeneration in the adrenal glands were also observed.

1.7.2 *Reproduction, embryotoxicity and teratogenicity*

Two reproduction diet studies in rats at doses up to 20 and 75 mg/kg body weight per day, respectively, revealed reduced birth weight and neonatal weight gain at maternally toxic doses of 20 and 7.5 mg/kg body weight, respectively. The highly toxic dose of 75 mg/kg body weight per day was sufficient to prevent conception and uterine implantation. Skeletal abnormalities were not observed.

In two teratogenicity tests, where rats were exposed either to hexachlorobutadiene vapour at concentrations between 21 and 160 mg/m^3 for 6 h/day (from days 6 to 20 of pregnancy) or intraperitoneally to 10 mg/kg body weight per day (from days 1 to 15 of pregnancy), fetuses demonstrated developmental toxicity, including reduced birth weight, delay in heart development and dilated ureters, but no gross malformations. The retarded development was observed at levels which were also toxic to the dams.

1.7.3 Genotoxicity and carcinogenicity

Hexachlorobutadiene induces gene mutations in the Ames Salmonella test under special conditions favouring the formation of glutathione conjugation products. It induced chromosomal aberrations in one *in vivo* study but not in two *in vitro* studies. In one *in vitro* test the frequency of sister chromatid exchanges was increased in Chinese hamster ovary cells. High mutagenic potency by sulfur metabolites of hexachlorobutadiene was reported. In *in vitro* studies, the compound induced unscheduled DNA synthesis in Syrian hamster embryo fibroblast cultures but not in rat hepatocyte cultures. It induced unscheduled DNA synthesis in rats *in vivo*, but did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.

In the only long-term (2 years) study, in which rats received a diet containing hexachlorobutadiene at doses of 0.2, 2 or 20 mg/kg body weight per day, an increased incidence of renal tubular neoplasms was observed only at the highest dose level.

1.7.4 Mechanisms of toxicity

The nephrotoxicity, mutagenicity and carcinogenicity of hexachlorobutadiene is dependent on the biosynthesis of the toxic sulfur conjugate 1-(glutathion-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (GPB). This conjugate is mainly synthesised in the liver and is further metabolized in the bile, gut and kidneys to 1-(cystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene(CPB).The activation of CPB, dependent on cysteine conjugate β-lyase, to a reactive thioketene in the proximal tubular cells finally results in covalent binding to cellular macromolecules.

1.8 Effects on humans

No pathogenic effects in the general population have been described.

There have been two reports of disorders among agricultural workers using hexachlorobutadiene as a fumigant, but they were also exposed to other substances. An increased frequency of chromosomal aberrations was found in the lymphocytes of peripheral blood of workers engaged in the production of hexachlorobutadiene and reported to be exposed to concentrations of 1.6-12.2 mg/m^3.

1.9 Evaluation of human health risks and effects on the environment

1.9.1 Evaluation of human health risks

As there have been very few human studies, the evaluation is mainly based on studies in experimental animals. However, limited human *in vitro* data suggest that the metabolism of hexachlorobutadiene in humans is similar to that observed in animals.

Hexachlorobutadiene vapour is considered to be irritating to the mucous membranes of humans, and the liquid is corrosive. The compound should also be regarded a sensitizing agent.

The main target organs for toxicity are the kidney and, to a much lesser extent, the liver. On the basis of short- and long-term oral studies in rats and mice, the NOAEL is 0.2 mg/kg body weight per day. In one short-term inhalation study in rats (12 days, 6 h/day), the NOAEL was 53 mg/m^3.

Reduced birth weight and neonatal weight gain was observed only at maternally toxic doses, as was developmental toxicity.

Hexachlorobutadiene has been found to induce gene mutations, chromosomal aberrations, increased sister chromatid exchanges and unscheduled DNA synthesis, although some studies have reported negative results. There is limited evidence for the genotoxicity of hexachlorobutadiene in animals, and insufficient evidence in humans.

Long-term oral administration of hexachlorobutadiene to rats was found to induce an increased frequency of renal tubular neoplasms, but only at a high dose level causing marked nephrotoxicity. There is limited evidence for carcinogenicity in animals and insufficient evidence in humans.

On the basis of the NOAEL for mice or rats of 0.2 mg/kg body weight per day, a NOAEL of 0.03-0.05 mg/kg body weight per day has been estimated for humans. There is a margin of safety of 150 between the estimated NOAEL and the estimated maximum total daily intake assuming absorption of the compound via contaminated drinking-water and food of high lipid content.

1.9.2 *Evaluation of effects on the environment*

Hexachlorobutadiene is moderately to highly toxic to aquatic organisms; crustaceans and fish are the most sensitive species. An environmental concern level of 0.1 μg/litre has been established. It is estimated that the maximum predicted environmental concentration away from point sources is twice the extrapolated environmental concern level and, consequently, aquatic organisms may be at risk in polluted surface waters. Adverse effects on benthic organisms cannot be excluded.

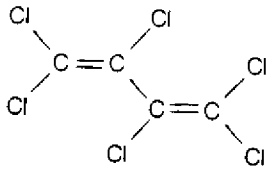
Considering the toxicity of hexachlorobutadiene to mammals, consumption of benthic or aquatic organisms by other species may cause concern.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 Identity

Chemical formula: C_4Cl_6

Chemical structure:



Common name:	hexachlorobutadiene
Common synonyms:	1,3-hexachlorobutadiene, 1,1,2,3,4,4-hexachloro-1,3-butadiene, perchlorobutadiene
Common trade names:	C-46, Dolen-pur, GP40-66: 120, UN2279
Common abbreviation:	HCBD
CAS registry number:	87-68-3
RTECS registry number:	EJ 0700000
Relative molecular mass:	260.8

2.2 Physical and chemical properties

Hexachlorobutadiene is a non-flammable, incombustible, clear, colourless and oily liquid at ordinary temperature and pressure. Its odour is described as turpentine-like. The odour threshold for the compound in air is reported to be 12 mg/m^3 (Ruth, 1986). In water an odour threshold of 0.006 mg/litre has been reported (US EPA, 1980). The compound is poorly soluble in water but is miscible with ether and ethanol.

Hexachlorobutadiene is very stable to acid and alkali in the absence of an appropriate solvent and has no tendency to polymerize even under high pressure. It reacts with chlorine under severe reaction conditions, often with cleavage of the carbon skeleton (Ullmann, 1986).

Some physical and chemical data on hexachlorobutadiene are presented in Table 1.

Table 1. Some physical and chemical properties of hexachlorobutadiene[a]

Physical state	liquid
Colour	clear, colourless
Melting point	-18 °C
Boiling point	212 °C at 101.3 kPa
Water solubility	3.2 mg/litre at 25 °C[b]
Log *n*-octanol-water partition coefficient (K_{ow})	4.78[b], 4.90[c]
Density	1.68 g/cm^3 at 20 °C
Relative vapour density	9.0
Vapour pressure	20 Pa (0.15 mmHg) at 20 °C[d]
Autoignition temperature	610 °C

[a] Unless otherwise stated, the data are selected from secondary sources.
[b] Experimentally derived by Banerjee et al. (1980)
[c] Experimentally derived by Chiou (1985)
[d] McConnell et al. (1975)

2.3 Conversion factors

1 ppm = 10.67 mg/m^3 air at 25 °C and 101.3 kPa (760 mmHg)
1 mg/m^3 air = 0.094 ppm.

2.4 Analytical methods

A summary of relevant methods of sampling and gas chromatographic analysis is presented in Table 2.

The analytical method for air, reported by Dillon (1979) and Boyd et al. (1981) has been approved by NIOSH and was published in the NIOSH Manual of Analytical Methods (NIOSH, 1979, 1990).

Table 2. Sampling, preparation and analysis of hexachlorobutadiene

Medium	Sampling method	Analytical method	Detection limit	Sample size	Comments	Reference
Air	adsorption on Chromosorb 101; extraction by hexane	gas chromatography with electron capture detection		360 litre	developed for personal sampling in industry	Mann et al. (1974)
Air	adsorption on Amberlite XAD-2; extraction by hexane	gas chromatography with electron capture detection	10 $\mu g/m^3$	3 litre	suitable for personal and area monitoring; validation range 10-2000 $\mu g/m^3$	Boyd et al. (1981); Dillon (1979)
Air	adsorption on Tenax-GC; purging of water vapour, oxygen, etc., by nitrogen; desorption by heating	gas chromatography with flame ionization detection	11 $\mu g/m^3$	2 litre	suitable for continuous area monitoring	Melcher & Caldecourt (1980)
Air	adsorption on Tenax-GC; desorption by heating under a helium flow; cryofocussing	gas chromatography (capillary column) with mass spectrometric detection	0.03 $\mu g/m^3$ [a]		developed for the analysis of ambient air	Krost et al. (1982); Pellizari (1982); Barkley et al. (1980)
Water	extraction by hexane; concentration; drying with Na_2SO_4; clean-up by silica gel chromatography	gas chromatography (capillary column) with electron capture detection	0.05 μg/litre	16 litre	developed for the analysis of surface water	Oliver & Nicol (1982)

Table 2 (contd).

Water	extraction by dichloromethane-acetone; drying; concentration by N_2 stream	gas chromatography with electron capture detection	0.0014 µg/litre	0.8-1 litre	US EPA Method 8120	Lopez-Avila et al. (1989)
Water	extraction by dichloromethane; drying; concentration	gas chromatography with electron capture detection	0.001 µg/litre	12 litre	developed for monitoring of domestic and process waters	Zogorski (1984)
Water	extraction by dichloromethane; drying; concentration and exchange to hexane; clean-up by fluorisil chromatography	gas chromatography with electron capture detection	0.34 µg/litre	1 litre	US EPA Method 612; developed for the analysis of municipal and industrial discharges	US EPA (1984a)
Water	extraction by dichloromethane at pH >11, then at pH <2; drying; concentration	gas chromatography	0.9 µg/litre	1 litre	US EPA Method 625; developed for the analysis of municipal and industrial discharges	US EPA (1984b)
Water	purging by helium; trapping; desorption by heating	gas chromatography (capillary column) with mass spectrometric detection	0.4 µg/litre	0.1 litre	developed for the analysis of volatile organics in waters	Otson & Chan (1987); Eichelberger et al. (1990)
Soil, sediment	extraction by acetone-benzene	gas chromatography with electron capture detection	0.7 µg/kg wet weight			Laseter et al. (1976)

Table 2 (contd).

Medium	Sampling method	Analytical method	Detection limit	Sample size	Comments	Reference
Soil	add water; adjust to pH >12; extraction by dichloromethane; centrifugation; drying; concentration	gas chromatography (capillary column) with flame ionization and mass spectrometric detection			developed for screening of soil for priority pollutants	Kiang & Grob (1986)
Sediment	add water; adjust to pH ≥ 11; extraction by dichloromethane; centrifugation; drying; concentration; clean-up by silica gel chromatography	gas chromatography (capillary column) with flame/electron capture/mass spectrometric detection			developed for screening of sediment for priority pollutants	Lopez-Avila et al. (1983)
Sediment	extraction by hexane-acetone; removal of acetone by water extraction; drying; concentration; clean-up by silica gel chromatography and agitation with mercury	gas chromatography (capillary column) with electron capture detection	13 µg/kg[a]	10-15 g dry weight		Oliver & Nicol (1982)
Biota	homogenization; filtration; separation; extraction by hexane; clean-up by fluorisil chromatography	gas chromatography with electron capture detection	0.7 µg/kg		method applied to analysis of fish	Laseter et al. (1976)

Table 2 (contd).

Biota	grind and mix edible tissue; extraction; clean-up by fluorisil chromatography	gas chromatography with electron capture detection	0.005 mg/kg wet weight or 0.04 mg/kg fat	25 g (eggs) wet weight 50 g (fish) wet weight 3 g (milk fat) 100 g (vegetables) wet weight		Yurawecz et al. (1976)
Biota	grinding with Na_2SO_4; extraction by hexane-acetone; back-extraction of acetone by water; concentration; clean-up by silica gel chromatography	gas chomatography (capillary column) with electron capture detection	0.47 µg/kg[a]	15 g	method applied to analysis of fish	Oliver & Nicol (1982)
Biota	extraction by benzene-acetone; filtration; concentration; redissolution in hexane; clean-up including fluorisil-silicic acid chromatography	gas chromatography with electron capture detection	1 µg/kg wet weight[a]	2 g	method applied to analysis of chlorinated hydrocarbon residues in human adipose tissue and human milk	Mes et al. (1982; 1985; 1986)
Biota	extraction by hexane containing an internal standard; centrifugation; direct injection	gas chromatography with electron capture detection	0.0182 µg/litre	100 mg	method applied to whole (rat) blood analysis	Kastl & Hermann (1983)

[a] lowest reported level measured

The method was validated for the concentration range of 10-2000$\mu g/m^3$ in 3 litre air samples. The lowest detectable quantity for this method was reported to be 20 ng, the desorption efficiency 98%, and the relative standard deviation 9%. Melcher & Caldecourt (1980) described a gas chromatographic method for the direct determination of organic compounds in air using a collection precolumn from which the compounds are directly injected into the analytical column by rapid heating of the precolumn. The method was reported to be suitable for the analysis of aqueous samples by purging the precolumn following injection of the sample (0.01-0.2 cm^3). The analytical method developed for volatile halogenated compounds by Krost et al. (1982) was applied by Pellizari (1982) and Barkley et al. (1980). Barkley et al. (1980) also described the analysis of volatile halogenated compounds in water, blood and urine using a modification of this method: the substances are recovered from water by heating and from biological matrices by heating and purging and are subsequently trapped on a Tenax column.

A spectrophotometric method for the determination of hexachlorobutadiene in blood and urine has been reported. The method involves extraction by heptane and determination by either UV spectroscopy or colorimetry after derivatization with pyridine. Reported detection limits were 0.05 mg/litre for the UV method and 5 mg/litre for the colorimetric method (Gauntley et al., 1975). Interference by other chlorinated hydrocarbons can be expected.

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

3.1 Natural occurrence

Hexachlorobutadiene has not been reported to occur as a natural product.

3.2 Anthropogenic sources

3.2.1 Production levels and processes

The available data are in general of poor quality and not up-to-date. Commercial production of hexachlorobutadiene was reported to occur in Germany and Austria (SRI, 1984). In the USA, commercial production was apparently terminated around 1970 (Mumma & Lawless, 1975). The compound was and is chiefly produced as by-product of the manufacture of chlorinated hydrocarbons, often in association with hexachlorobenzene. In the USA, the manufacture of tetrachloroethene, trichloroethene and carbon tetrachloride accounted in 1972 for over 99% of this production of hexachlorobutadiene in heavy fractions, the so-called Hex-waste, and amounted to 3310-6580 tonnes (Brown et al., 1975; Mumma & Lawless, 1975; Yurawecz et al., 1976; see also section 3.2.3). It was also reported to be a by-product of the manufacture of vinyl chloride, allyl chloride and epichlorohydrin by chlorinolysis processes (Kusz et al., 1984). Hexachlorobutadiene has been identified in the effluents of sewage treatment plants (section 5.2) and as a by-product of the pyrolysis of trichloroethene (Yasuhara & Morita, 1990) and plastics (Singh et al., 1982). The annual world production of hexachlorobutadiene in heavy fractions was estimated in 1982 to be 10 000 tonnes (Hutzinger, 1982). No data have been found regarding the amount of hexachlorobutadiene, if any, which is now recovered from this waste.

Apart from the possible commercial production of hexachlorobutadiene by recovery from Hex-waste, three pathways for chemical synthesis are known: the chlorination and dehydrochlorination of hexachlorobutene; the chlorination of polychlorobutanes; and the catalytic chlorination of butadiene (Mumma & Lawless, 1975; CESARS, 1981). There is no evidence, however, that the latter reactions have ever been used commercially.

The fraction of hexachlorobutadiene released to the environment during its industrial life cycle (not defined) has been estimated to be between 1 and 3% (SRI, 1984). The fraction of hexachlorobutadiene lost to the environment during its production at a tetrachloroethene manufacturing plant in the USA was estimated to be 1.5% (Brown et al., 1975). Using a simple model describing the troposphere, the global annual emission rate was calculated to be 3000 tonnes of hexachlorobutadiene based on air sampling data of 1985 (Class & Ballschmiter, 1987; see also section 4.2.2).

3.2.2 Uses

Hexachlorobutadiene can be used for the recovery of "snift", which is chlorine-containing gas in chlorine plants, and as a wash liquor for removing volatile organic compounds from gas streams. It can be used as a fluid in gyroscopes, as heat transfer, transformer, insulating and hydraulic fluids, and as solvent for elastomers. It can be an intermediate in the manufacture of lubricants and rubber compounds. In the ex-USSR, the substance was reported to find widespread application as a fumigant for treating *Phylloxera* on grapes, and 600-800 tonnes was used for this purpose in 1975 (Brown et al., 1975; Mumma & Lawless, 1975).

3.2.3 Waste disposal

Hex-waste containing hexachlorobutadiene may be destroyed by incineration, placed in landfill, or simply stored. Another procedure involves recycling the compound by catalytic chlorination and subsequent high temperature chlorinolysis to carbon tetrachloride and tetrachloroethene (Markovec & Magee, 1984).

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION AND TRANSFORMATION

4.1 Transport and distribution between media

The main pathways for entry of hexachlorobutadiene into the environment are its emission via industrial waste (section 3.2.3) and following dispersive use (section 3.2.2). The compound may enter surface and ground water, soil and air. In view of its physical properties, intercompartmental transport of hexachlorobutadiene is expected to occur by volatilization and adsorption to suspended particulate matter.

Considering the vapour pressure of the compound, i.e. 20 Pa at 20 °C (McConnell et al., 1975), transfer across soil-air boundaries may be significant. Depending on the soil type, adsorption will hinder this transport (see below). In a field study in the ex-USSR, concentrations of hexachlorobutadiene in air above a vineyard were found to be 0.08 and 0.003 mg/m^3 at 1 day and 3 months, respectively, following a spring application of 250 kg/ha. The method of analysis was not reported. Volatilization of the compound from light soils was more rapid than from heavy soils (Litvinov & Gorenshtein, 1982).

The Henry coefficient of hexachlorobutadiene is 0.43 (1040 Pa.m^3.mol^{-1}) at 25 °C (Shen, 1982) and 0.3 at 22 °C (Hellmann, 1987a). These values are comparable to those of other chlorinated aliphatic alkenes. They indicate possible transfer of the compound across water-air boundaries leading to a wide distribution, with aerial transport playing a major role (McConnell et al., 1975). In a model experiment, hexachlorobutadiene was allowed to evaporate from a 20-mg/litre aqueous-methanolic solution, containing 10% methanol, in a porcelain basin with slow magnetic stirring at 22 °C. UV spectrophotometry recorded a 25% loss within 28 min. It was shown that methanol decreased the disappearance time. For the transfer of this and other model results to flowing waters, a reduction factor of 30 was proposed for the rate of evaporation on the basis of limited data for two compounds (Hellmann, 1987a).

In a model experiment, UV spectrophotometric analysis of solutions of hexachlorobutadiene in deionized water to which 1 g/litre of clay mineral (Fuller's earth) was added revealed a clay-water partition coefficient of 500 litre/kg, showing limited

adsorption to pure clay minerals comparable to that of other chlorinated alkenes (Hellmann, 1987b). Based on the log octanol-water partition coefficient (log K_{ow}) of 4.78-4.90 (Table 1), hexachlorobutadiene is expected to adsorb strongly to organic matter. The organic carbon-water partition coefficient (K_{oc}) can be estimated to be 25 120 litre/kg on the basis of a log K_{ow} of 4.8 using the semi-empirical equation of Karickhoff (1981). Oliver & Charlton (1984) determined a K_{oc} value of 158 500 litre/kg on the basis of sediment and water concentrations in the Niagara River, USA. Partition coefficients of approximately 200-260 litre/kg were found for two unspecified types of soil in model experiments employing gas chromatographic analysis of solutions of hexachlorobutadiene in water (Leeuwangh et al., 1975; Laseter et al., 1976). In field experiments conducted along the Mississippi river in the USA in 1974-1975, some water samples were found to contain 1.0-1.5 μg/litre, whereas levee soil samples at the same sites contained 62-1001 μg/kg dry weight. At a more polluted site near a Hex-waste landfill, water samples contained 0.04-4.6 μg/litre and mud samples 270-2370 μg/kg dry weight. These studies show that soil-water partition coefficients can range over 2 to 4 orders of magnitude assuming equilibrium (Laseter et al., 1976). It can be concluded that the compound does not migrate rapidly in soils and will accumulate in sediment. It should be noted that the micro-particles onto which hexachlorobutadiene is absorbed may themselves migrate in the sub-surface resulting in facilitated transport. The degree of adsorption to soil is highly dependent on the content of organic matter and is less pronounced in sandy soils.

On the basis of data for Dutch surface waters, the half-lives of hexachlorobutadiene were estimated to be 3-30 days in rivers and 30-300 days in lakes and ground water. This suggests that turbulence, and therefore increased aerobic biodegradation, volatilization and adsorption, account for the shorter half-lives in river water, that the compound is difficult to degrade both biologically and chemically (see below), and that, overall, the compound is persistent in water (Zoeteman et al., 1980).

4.2 Abiotic degradation

4.2.1 Photolysis

Hexachlorobutadiene absorbs light within the solar spectrum. Irradiation of a solution of hexachlorobutadiene in benzene at 254 nm for 15 min resulted in the formation of numerous products

having a relative molecular mass greater than that of hexachlorobutadiene itself (Laseter et al., 1976). The extent of mineralization of the compound adsorbed to silica gel and exposed to oxygen was examined following irradiation with ultraviolet light filtered by quartz (wavelength < 290 nm) or by pyrex (simulating tropospheric UV with a wavelength > 290 nm). After 6 days, 50-90% mineralization to hydrogen chloride and/or chlorine, and carbon dioxide was observed (Gäb et al., 1977). These experiments indicate that hexachlorobutadiene present as a virtual monolayer on silica gel undergoes quite rapid photolysis.

4.2.2 Photooxidation

Using a steady-state mathematical model for the troposphere (describing it as 2 boxes one north one south of the equator) and on the basis of gas chromatographic analysis of air samples from sites far away from anthropogenic sources, the tropospheric lifetime of hexachlorobutadiene was estimated to be 2.3 years for the northern hemisphere and 0.8 years for the southern hemisphere. It was assumed that the reaction with hydroxyl radicals in the troposphere is the main sink for hexachlorobutadiene, by analogy with other halocarbons. The calculated lifetimes at -8 °C correspond to a pseudo-first order rate constant of $(2 \pm 1) \times 10^{-14}$ $cm^3.molecules^{-1}.sec^{-1}$ at estimated hydroxyl radical concentrations of 7×10^5 $molecules.cm^{-3}$ for the northern hemisphere and 17×10^5 for the southern hemisphere (Class & Ballschmiter, 1987). Experimentally, a half-life of 1 week was determined when hexachlorobutadiene was exposed to air in flasks outdoors. This relatively short disappearance time was possibly due to heterogeneous reactions on the vessel walls, as suggested by the authors of the report. Hydrogen chloride was found to be the main degradation product after exposure of samples to xenon arc radiations (wavelength > 290 nm) (Pearson & McConnell, 1975).

4.2.3 Hydrolysis

Hexachlorobutadiene is highly resistant to chemical degradation by strong acids and alkalis in the absence of appropriate solvents, although it is readily degraded by ethanolic alkali (Roedig & Bernemann, 1956). Based on the measured hydrolysis rate of the compound in a 1:1 acetone-water mixture, a half-life of over 1800 h was calculated (Hermens et al., 1985).

4.3 Biodegradation

Hexachlorobutadiene, at concentrations of 5 or 10 mg/litre, was completely degraded by adapted aerobic microorganisms within 7 days in a static-culture flask screening procedure at 25 °C, as shown by gas chromatography and by determination of total and dissolved organic carbon. The inoculum was taken from settled domestic waste water (Tabak et al., 1981). Approximately 70% adsorption to sludge and 10% degradation was found to occur within 8 days in a pilot low-loaded biological sewage treatment plant (Schröder, 1987).

Anaerobic degradation of hexachlorobutadiene at 100 mg/litre was not observed in 48-h batch assays at 37 °C using an inoculum from a laboratory digester (Johnson & Young, 1983).

4.4 Bioaccumulation

Considering the low water solubility of 3.2 mg/litre and the high log K_{ow} of 4.78-4.90 (Table 1), a strong bioaccumulating potential would be expected. Both laboratory and field data support this prediction. In flow-through laboratory tests with algae, crustaceans, molluscs and fish in fresh or marine waters, bioconcentration factors (on a wet weight basis) were between 71 and 17 000. The results appear to be highly dependent on the exposure period and there is great variability between organisms (Leeuwangh et al., 1975; Pearson & McConnell, 1975; Laseter et al., 1976; Oliver & Niimi, 1983). Steady state was clearly demonstrated to be reached in only one of these tests. Oliver & Niimi (1983) exposed rainbow trout (*Salmo gairdnerii*) to aqueous solutions of hexachlorobutadiene at 0.10 and 3.4 ng/litre and found average bioconcentration factors of 5800 and 17 000, steady states having been reached after 69 and 7 days, respectively. When Oligochaete worms were exposed via spiked Lake Ontario sediments to a pore water concentration of 32 ng/litre in a flow-through system, steady state was reached within 4 to 11 days and the average bioconcentration factor was 29 000, based on dry weight of which about 8% is lipid (Oliver, 1987). Biomagnification, the concentrating of a substance through a food chain, was not observed for hexachlorobutadiene in two limited laboratory experiments with fish fed contaminated food (Pearson & McConnell, 1975; Laseter et al., 1976).

The bioaccumulation factors found in plankton, crustaceans, molluscs, insects and fish in surface waters are comparable to

those observed in the laboratory: available bioaccumulation factors based on wet weight range between 33 and 11 700 (Goldbach et al., 1976; Laseter et al., 1976). No biomagnification was observed when levels in fish were compared with those of detritus and several invertebrates (Goldbach et al., 1976). The latter was confirmed by a trophodynamic analysis in the Lake Ontario ecosystem (Oliver & Niimi, 1988).

Limited bioaccumulation of hexachlorobutadiene was observed in the fat of rats following exposure for 4 to 12 weeks to a mixture of this compound and 1,2,3,4-tetrachlorobenzene, hexachlorobenzene, 1,3,5-trichlorobenzene, *o*-dichlorobenzene and γ-hexachlorocyclohexane in food (each compound at 2 or 4 mg/kg body weight per day). Fat concentrations of up to 8 mg/kg were observed at the higher dose rates (Jacobs et al., 1974).

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Environmental levels

5.1.1 Air

Concentrations of hexachlorobutadiene measured in air at different locations are summarized in Table 3.

5.1.2 Water

Concentrations of hexachlorobutadiene measured in water at different locations are summarized in Table 4.

5.1.3 Soil and sediment

Concentrations of hexachlorobutadiene measured in soil and sediment at different locations are summarized in Table 5.

5.1.4 Biota

Concentrations of hexachlorobutadiene measured in aquatic organisms, birds and mammals are summarized in Table 6.

5.2 General population exposure

Levels of hexachlorobutadiene encountered in the food and drinking-water of the general population are summarized in Table 7.

Hexachlorobutadiene was not detected in the urine or blood of nine individuals living near Old Love Canal, USA, whereas trace levels were found in the breath of one of them (Barkley et al., 1980). In another investigation the compound could not be detected in the blood of 36 Love Canal area residents (Bristol et al., 1982). Hexachlorobutadiene was found at levels of 0.8-4 μg/kg wet weight (fat) and 1.2-13.7 μg/kg wet weight (liver) in postmortem tissues from 6 out of 8 United Kingdom residents in 1970 (McConnell et al., 1975). In the adipose tissue of accident victims in Canada (1976), levels of 1 to 8 μg/kg wet weight were measured in 128 out of 135 samples (Mes et al., 1982, 1985). In Canada (1982), hexachlorobutadiene could not be detected in any of 210 samples of breast milk (Mes et al., 1986).

Table 3. Levels of hexachlorobutadiene in environmental air

Type of air	Year	Location	Detection limit (ng/m^3)	Levels determined[a] (ng/m^3)	Reference
Ambient	1985	Atlantic Ocean, lower troposphere in a south-north cross section, 8 sites		0.0001-0.0004 (r) 0.0003 (m, north) 0.0001 (m, south)	Class & Ballschmiter (1987)
Urban	1978	USA, Niagara Falls, inside homes near dump site		nd (n = 9)	Barkley et al. (1980)
		USA, Niagara Falls, outside homes near dump site		nd (n = 6) trace (n = 3)	
		USA, Niagara Falls area		nd (n = 3) trace (n = 1) 50-390 (r, n = 2)	
Urban	1980-1981	USA, 7 cities		nd-117 (r,m) nd-251 (r)	Singh et al. (1982)

Table 3 (contd).

Type of air	Year	Location	Detection limit (ng/m^3)	Levels determined[a] (ng/m^3)	Reference
Polluted	1975	USA, 9 sites with chemical industries, on plant property		nd-460 000 (r)	Li et al. (1976)[b]
		USA, 9 sites with chemical industries, off plant property		nd-22 000 (r)	
Polluted	1978	USA, Niagara Falls, household basement near dump site		< 45 (n = 1)	Barkley et al. (1980)
Polluted	1978	idem		30-410 (r, n = 4)	Pellizari (1982)
Polluted	1982	USA, liquid waste lagoon	2	nd (n = 2) 3-160 (n = 4)	Guzewich et al. (1983)

[a] nd = not detectable; r = range of individual values; r,m = range of mean values; m = mean; n = number of samples

[b] The highest levels were associated with the production of tetrachloroethene and trichloroethene. At other plants, levels of hexachlorobutadiene remained below 3 ng/m^3. Waste holding areas (especially when involving open storage) were often the most significant sources of hexachlorobutadiene, contaminated soil being a secondary source. The total number of samples examined was 405.

Table 4. Levels of hexachlorobutadiene in environmental water

Type of water	Year	Location	Detection limit (ng/litre)	Levels determined[a] (ng/litre)	Reference
Surface		Canada, Niagara River	50	1.5	Oliver & Nicol (1982)
Surface	1982	Canada, Niagara River		0.82 (m, n = 5)	Oliver & Charlton (1984)
Surface	1981-1983	Canada, Niagara River	0.01	0.78 (m, n = 104) 0.67 (median) 0.27-3.2 (r)	Oliver & Nicol (1984)
Surface	1981	Canada, Niagara River		nd-0.6 (n = 1)	Fox et al. (1983)
Surface	1972-1973	Netherlands, River IJssel, Keteimeer, IJsselmeer		50-130 (r, n = 5)	Goldbach et al. (1976)
Surface	1976-1978	Netherlands, River Rhine		1000-2000	Zoeteman et al. (1980)
Surface	1975	USA, 9 sites with chemical industries, on plant property		nd-240 000 (r)	Li et al. (1976)
		idem, off plant property		nd-23 000 (r)	

Table 4 (contd).

Type of water	Year	Location	Detection limit (ng/litre)	Levels determined[a] (ng/litre)	Reference
Surface	1976	Germany, River Rhine, 865 km	10	10 (50-percentile) 180 (90-percentile)	Alberti (1983)
	1978	Germany, idem	10	20 (50-percentile) 60 (90-percentile)	
	1981	Germany, idem	10	< 10 (50-percentile) 40 (90-percentile)	
	1980-1981	Germany, 4 River Rhine tributaries	10	nd	
		Germany, River Lippe	10	40-200	
Surface	1979-1981	Germany, River Rhine,		≤ 50	Haberer et al. (1988)
	1979-1981	Germany, River Main		≤ 1000	
Surface	1983	Netherlands, River Rhine, River Lek		< 100 (m, n=52)	Meijers (1988)
		idem, before dune infiltration		70 (m, n=13)	

Table 4 (contd).

Surface	1984-1985	Germany, River Rhine Germany, River Elbe		10-20 10-150	Petersen (1986)
Estuarine		USA, Calcasieu River estuary, vicinity of industrial outfall		1298	Pereira et al. (1988)
Sea	1972-1973	United Kingdom, Liverpool Bay	1	4 (m, n = 150) nd-30 (r)	Pearson & McConnell (1975)
Sea	1977	USA, Gulf of Mexico,			Sauer (1981)
		open ocean	1	nd (n = 4)	
		coast	1	nd-15 (n = 4)	
Ground water		Switzerland, aquifer contaminated by leachate from a chemical waste disposal site		200-300 (r)	Giger & Schaffner (1981)

[a] nd = not detectable; r = range of individual values; r,m = range of mean values; m = mean; n = number of samples; x percentile = x percent of samples with values up to that given

Table 5. Levels of hexachlorobutadiene in soil and sediment

Type of soil or sediment	Year	Location	Levels determined[a] (μg/kg)	Reference
Soil, agricultural		vineyards infected with *Phylloxera* and treated at 250 kg/ha	≤ 7300 (8 mo) ≤ 2990 (32 mo)	Vorobyeva (1980)
Soil	1975	USA, 9 sites with chemical industries, on plant property	nd-980 000 (r)[b]	Li et al. (1976)
		idem, off plant property	nd-110 (r)[b]	
Sediment	1975	idem, on plant property	nd-33 000 (r)[b]	Li et al. (1976)
		idem, off plant property	nd-40 (r)[b]	
Sediment, marine		United Kingdom, Liverpool Bay	< 1 (n = 110) > 1 (n = 30)	Pearson & McConnell (1975)
Sediment, river/lake		Canada, Niagara Falls	18	Oliver & Nicol (1982)
Sediment, lake	1980	Canada, Lake Ontario	nd (n = 9) trace (n = 3) 8.7 (n = 1)	Kaminsky et al. (1983)

Table 5 (contd).

Sediment, river	1981	Canada, Niagara River, downstream idem, upstream	9.6-37 (n = 5, dwt)[c] nd (n = 1, dwt)	Fox et al. (1983)
Sediment, river	1982	Germany, River Rhine, 707 km idem, 815 km	0.002 (dwt) 0.005 (dwt)	Alberti (1983)
Sediment, lake	1981	Canada, Lake Ontario	12-120 (n = 5, dwt)	
Sediment, lake	1968-1978 1959-1962 1980-1981 1868-1981	Canada, Niagara Falls sediment core near Niagara River	nd 550 18 nd-550	Durham & Oliver (1983)
Sediment, lake	1980-1982 1980 1982 1982	Canada, lakes Canada, Lake Huron Canada, Lake St. Clair Canada, Lake Erie	0.04-9.3 (r, n = 57) 0.08 (m, n = 9, dwt) 7.3 (m, n = 2, dwt) 0.2-1.6 (r,m, n = 46, dwt)	Oliver & Bourbonniere (1985)
Sediment, lake	1982	Canada, Niagara Falls, settling particulates at 20 m depth idem, settling particulates at 68 m depth bottom sediment	nd (n = 1) 2.9-11 (r, n = 5), 5.9 (m) 7.4 (m) 32 (m, n = 12)	Oliver & Charlton (1984)

Table 5 (contd).

Type of soil or sediment	Year	Location	Levels determined[a] (µg/kg)	Reference
Sediment, lake		Canada, Lake Ontario	0.1-75 (r, n = 3)	Oliver (1984)
Sediment, sea harbour		USA, Eagle Harbour, creosote contaminated sediment, 3 sites	< 0.79 (m, n = 15, dwt)	Malins et al. (1985)
Sediment, sea harbour		USA, President Point, 1 reference site	< 2.0 (n = 1, dwt)	
Sediment estuarine		USA, Calcasieu River estuary, vicinity of industrial outfall	85 (bottom) 1.7 (suspended)	Pereira et al. (1988)

[a] dwt = dry weight; nd = not detectable; r = range of individual values; r,m = range of mean values; m = mean; mo = months after treatment; n = number of samples

[b] The highest levels were associated with the production of tetrachloroethene and trichloroethene. Waste holding areas (especially when involving open storage) were often the most significant sources of hexachlorobutadiene, contaminated soil being a secondary source.

[c] surficial sediment; the sediment concentration increased with fraction size

[d] surficial sediment

Table 6. Concentrations of hexachlorobutadiene in aquatic organisms, birds and mammals

Type of biota	Year	Location	Levels determined[a] (μg/kg wwt)	Reference
Detritus (bottom)	1972-1976	Netherlands, surface water	200	Goldbach et al. (1976)
Detritus (floating)			220	
Invertebrates				
Plankton	1972-1973	United Kingdom, sea water	nd-2.0	Pearson & McConnell (1975)
Ragworm,				
Nereis diversicolor			0.06	
Mussel,				
Mytilus edulis			nd-3.8	
Crab,				
Cancer pagarus			nd-1.1	
Others			nd	
Cerastoderma edule				
Ostrea edulis				
Buccinum undatum				
Crepidula fornicata				
Carcinus maenus				
Eupagurus bernhardus				
Crangon crangon				
Asterias rubens				
Solaster sp.				
Echinus esculentus				

Table 6 (contd).

Type of biota	Year	Location	Levels determined[a] (µg/kg wwt)	Reference
Snail	1972-1976	Netherlands, surface water		Goldbach et al. (1976)
Lymnaea peregra			30, 1670	
Clam,				
Sphaerium sp.			2410	
Oligochaetes			0.3 (m, n=3)	
Oligochaetes	1981	Canada, Lake Ontario	nd-37 (dwt)	Fox et al. (1983)
Amphipods			7.5-62 (dwt)	
Mysids			6 (dwt)	
Benthic organisms in stomachs of fish	1983-1984	USA, sea water	< 5 (dwt)	Malins et al. (1985)
Clam,	1982-1983	Canada, Great Lakes area		Kauss & Hamdy (1985)
E. complanatus			nd-83 (r, n=34)	
Marine algae	1972-1973	United Kingdom, sea water		Pearson & McConnell (1975)
Enteromorpha compressa			nd	
Ulva lactuca			nd	
Fucus vesiculosis			8.9	
Fucus serratus			0.6	
Fucus spiralis			0.6	

Table 6 (contd)

Fish				
Ray,	1972-1973	United Kingdom, sea water		Pearson & McConnell (1975)
Raja clavata (flesh)			0.1-0.4	
Raja clavata (liver)			0.2-1.5	
Plaice,				
Pleuronectes platessa (flesh)			nd-0.4	
Pleuronectes platessa (liver)			0.2-1.2	
Dab,				
Limanda limanda (flesh)			< 0.1	
Limanda limanda (liver)			nd	
Mackerel,				
Scomber scombrus (flesh)			nd-2.6	
Cod,				
Gadus morrhua (flesh)			< 0.1	
Gadus morrhua (air bladder)			0.35	
Others (liver and/or flesh),			nd	
Platycthus flesus				
Solea solea				
Aspitrigla cuculus				
Trachurus trachurus				
Trisopterus luscus				

Table 6 (contd).

Type of biota	Year	Location	Levels determined[a] (µg/kg wwt)	Reference
Trout, *Salmo gairdneri*		USA, Niagara River, Lake Ontario	0.47	Oliver & Nicol (1982)
Trout	1981	Canada, Lake Ontario	1.3 (dwt)	Fox et al. (1983)
Catfish (flesh)	1973	USA, surface water near chemical plants manufacturing tetrachloroethene	trace-4600	Yurawecz et al. (1976)
Gaspergoo (flesh)			200	
Buffalo fish (flesh)			100	
Mullet (flesh)			trace	
Sea trout (flesh)			trace	
Sheepshead minnow (flesh)			trace	
Catfish	1973	USA, < 40 km from tetrachloroethene or trichloroethene manufacturing plants	10-1200	Yip (1976)
Carp			62	
Gaspergoo			12-30	
Buffalo fish			120	
Whiting			20	
Drum			10	

Table 6 (contd).

Pike perch, *Stizostedion lucioperca*	1972-1976	Netherlands, Ketelmeer (lake)	440 (m, n = 8)	Goldbach et al. (1976)
		Netherlands, IJsselmeer (lake)	23 (m, n = 4)	
Perch, *Perca fluviatilis*		Netherlands, Ketelmeer	130, 400 (n = 2)	
Pike, *Esox lucius*		Netherlands, Ketelmeer	260	
Tench, *Tinca tinca*		Netherlands, Ketelmeer	950	
Common bream, *Abramis brama*		Netherlands, Ketelmeer	1520 (m, n = 5)	
		Netherlands, IJsselmeer	33 (m, n = 5)	
White bream *Blicca bjoerkna*		Netherlands, Ketelmeer	360 (m, n = 3)	
Roach, *Rutilis rutilis*		Netherlands, Ketelmeer	910 (m, n = 10)	
		Netherlands, IJsselmeer	61 (m, n = 4)	
Eel, *Anguilla anguilla*		Netherlands, IJsselmeer	33 (m, n = 4)	
Smelt *Osmerus eperlanus*		Netherlands, IJsselmeer	43 (m, n = 3)	

Table 6 (contd)

Type of biota	Year	Location	Levels determined[a] (µg/kg wwt)	Reference
English sole (liver)	1983-1984	USA, sea water	< 9 (dwt)	Malins et al. (1985)
English sole (muscle)			< 0.2 (dwt)	
Catfish		USA, vicinity of industrial outfall in Calcasieu River estuary	46 000-120 000 (lipid base)	Pereira et al. (1988)
Atlantic croaker		idem, in Calcasieu River	41 000 (lipid base)	
Blue crab			12 000 (lipid base)	
Spotted sea trout			15 000 (lipid base)	
Blue catfish			46 000 (lipid base)	
Coho salmon	1980	USA, Great Lakes	nd (n = 31)	Clark et al. (1984)
			trace-10 (r, n = 5)	
Several species	1983	USA, 14 Lake Michigan tributaries and embayments	nd	Camanzo et al. (1987)
Birds				
Guillemot, *Uria aalge* (eggs)	1972-1973	United Kingdom	1.6-9.9	Pearson & McConnell (1975)

Table 6 (contd).

Swan,				Pearson & McConnell (1975)
Cygnus olor (liver)			5.2	
Cygnus olor (kidney)			nd	
Moorhen,	1972-1973	United Kingdom		Pearson & McConnell (1975)
Gallinula chloropus (liver)			0.8	
Gallinula chloropus (muscle)			2.6	
Gallinula chloropus (eggs)			nd	
Others			nd	
Sula bassana (liver, eggs)				
Phalacrocerax aristotelis (eggs)				
Alca torda (eggs)				
Rissa tridactyla (eggs)				
Anas platyrhyncos (eggs)				
Mammals	1972-1973	United Kingdom		Pearson & McConnell (1975)
Grey seal,				
Halichoerus grypus (blubber)			0.4-3.6	
Halichoerus grypus (liver)			nd-0.8	
Common shrew,				
Sorex araneus			nd	

* dwt = dry weight; r = range of individual values; m = mean of individueal values; n = number of samples; nd = not detectable; wwt = wet weight

Table 7. Levels of hexachlorobutadiene in food and drinking-water

Type of food or drinking-water	Year	Location	Levels determined[a] (µg/kg wwt or µg/litre)	Reference
Tap water	1978	USA, houses bordering Old Love Canal, Niagara Falls	nd-trace (r, n = 3) 0.06–0.17 (r, n = 6)	Barkley et al. (1980)
Well water	1978	USA, Tennessee, contaminated by leachate from waste dump	nd–2.53 (r, n = 28) 0.15 (m, n = 22)	Clark et al. (1982)
Fresh milk		United Kingdom	0.08	McConnell et al. (1975)
Butter			2	
Cheese, eggs			nd	
Meat (3 types)			nd	
Oils/fats (4 out of 5 types)			nd	
Vegetable cooking oil			0.2	
Beverages (5 out of 6 types)			nd	
Light ale			0.2	
Fruits/vegetables (5 out of 7 types)			nd	
Tomatoes		United Kingdom, reclaimed lagoon	0.8	
Black grapes		United Kingdom, import	3.7	

Table 7 (contd).

Type of food or drinking-water	Year	Location	Levels determined[a] (μg/kg wwt or μg/litre)	Reference
Fresh bread		United Kingdom	nd	
Eggs	1973	USA, < 40 km from tetrachloro-	nd (n = 15)	Yip (1976)
Milk		ethylene or trichloroethylene	nd (n = 19)	
		manufacturing plants, 6-7 sites	1320 (n = 1, fat basis)	
Vegetables (7 types)			nd (n = 20)	
Condensed milk	1975	Germany, Bonn	4	Kotzias et al. (1975)
Milk (products) (2 types)			nd	
Eggs (white)			nd	
Eggs (yolk)			42	
Meats (4 types)			nd	
Tinned fish (2 types)			nd	
Onion bread			nd	
Chicken feed			39	
Chicken meal			2	

[a] nd = not detected; m = mean of individual values; n = number of samples; r = range; wwt = wet weight

When 15 samples of hazardous waste from incineration facilities in the USA were analysed, 4 sites were found to contain hexachlorobutadiene, but the levels were reported to be below 10 mg/kg (Demarini et al., 1987). In sewage sludge, Alberti & Plöger (1986) measured levels of below 1 μg/kg dry weight (3 samples of municipal or municipal/industrial sludge), up to 0.6 μg/kg dry weight (1 sample of municipal/industrial sludge), and 15 μg/kg dry weight (1 sample of industrial sludge).

5.3 Occupational exposure

Hexachlorobutadiene levels of 1.6-12.2 mg/m^3 air have been measured in the workplace, resulting in reported urine levels of up to 20 mg/litre in workers at the end of the day (German & Viter, 1985).

6. KINETICS AND METABOLISM

6.1 Absorption and distribution

Whole body autoradiography of longitudinal sagittal sections of male rats after administration of a single oral dose of 200 mg uniformly labelled hexachlorobutadiene/kg body weight in corn oil demonstrated that intestinal absorption of the parent compound was virtually complete by 16 h. The radioactivity in the gastrointestinal tract at this point in time was mainly due to water-soluble metabolites, whereas 85% of the radioactivity in the small intestine was still present as unchanged hexachlorobutadiene 4 h after the administration. At all points in time radioactivity levels in the stomach were low compared to those in the intestines. The autoradiogram showed a specific distribution of radioactivity, especially in the outer medulla of the kidney (Nash et al., 1984).

Reichert et al. (1985) orally administered 1 or 50 mg of labelled hexachlorobutadiene/kg body weight in tricaprylin to female rats and recovered, at 72 h, approximately 7% of the label in carcass and tissues, mainly liver, brain and kidneys. Most of the label was excreted via urine or faeces within this time period (section 6.4). In mice given 30 mg of labelled hexachlorobutadiene per kg body weight in corn oil, over 85% of the label was excreted within 72 h (section 6.4); 6.7-13.6% was found in the carcass, especially in adipose tissue (Dekant et al., 1988a). This report on mice supports the study by Reichert et al. (1985) on rats with respect to the amount of labelled hexachlorobutadiene absorbed.

6.2 Metabolism

The extent of metabolic transformation and the identity of excretion products found in studies with rodents are summarized in Table 8. The available evidence suggests that hexachlorobutadiene is metabolized in a glutathione-dependent reaction to toxic sulfur metabolites. The glutathione-S-conjugate 1-(glutathion-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (GPB) is formed in the liver and excreted with bile. GPB is reabsorbed from the gut both intact and after degradation to 1-(cystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (CPB). Finally, these sulfur conjugates and the corresponding mercapturic acid 1-(*N*-acetylcystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene(ACPB) are delivered to the kidney. In the kidney, high concentrations of CPB are present due to renal accumulation, enzymes with acylase

Table 8. Tracer studies with [^{14}C] hexachlorobutadiene

Species	Route	Dose (mg/kg body weight)	Medium	Metabolite[a]	Fraction of dose (%)	Time after dosing (h)	Reference
Rat	ip	0.1	urine	total	29	48	Davis et al. (1980)
				water-soluble	25	48	
			faeces	total	40	48	
		300.1	urine	total	7	48	
				water-sofuble	6	48	
			faeces	total	7	48	
Rat	oral	200	urine	total	11	120	Nash et al. (1984)
				PBSA	1	120	
				non-ether soluble	7	120	
			faeces	total	39	120	
Rat	oral	1	expired air	total	8.9	72	Reichert et al. (1985)
				HCBD	5.3	72	
				CO_2	3.6	72	
			urine	total	30.6	72	
			faeces	total	42.1	72	
		50	expired air	total	6.6	72	
				HCBD	5.4	72	

Table 8 (contd).

				CO_2	1.2	72	
			urine	total	11.0	72	
			faeces	total	69	72	
Rat	oral	100	urine	total	5.4	24	Reichert et al. (1985);
				S-containing	ca 4.3	24	Reichert & Schütz (1986)
				ACPB, MTPB, CMTPB	0.5	24	
			faeces	total	60	72	
	oral	1	expired air	total	7.45	72	
				CO_2	2.2		
			urine	total	17.5		
			faeces & git[b]	total	61.8		
			carcass	total	10.5		
		100	expired air	total	7.57	72	
				CO_2	0.7		
			urine	total	9.0		
			faeces & git[b]	total	72.1		
			carcass	total	5.8		

Table 8 (contd).

Species	Route	Dose (mg/kg body weight)	Medium	Metabolite[a]	Fraction of dose (%)	Time after dosing (h)	Reference
Rat	iv	1	expired air	total	8.54	72	Payan et al. (1991)
				CO_2	2.6		
			urine	total	21.1		
			faeces & git[b]	total	59.3		
			carcass	total	12.9		
		100	expired air	total	8.11	72	
				CO_2	0.9		
			urine	total	9.2		
			faeces & git[b]	total	71.5		
			carcass	total	11.1		
Mouse	oral	30	expired air	total = HCBD	4.5	72	Dekant et al. (1988a)
			urine	total	7.2	72	
			faeces	total	72.0	72	
				HCBD	> 57	72	
				GPB	7.2	72	

[a] For abbreviations see Fig. 1; "total" indicates that no individual chemicals were specified
[b] git = gastrointestinal tract

activity and gamma-glutamyltranspeptidase. CPB is finally cleaved by renal cysteine conjugate β-lyase to the electrophile trichlorovinyl-chlorothioketene. The renal accumulation of sulfur conjugates and the location of β-lyase along the nephron (MacFarlane et al., 1989) explain the organ- and site-specific toxicity of hexachlorobutadiene (Lock, 1987a,b; Anders et al., 1987; Dekant et al., 1990a,b; Koob & Dekant, 1991).

6.2.1 In vitro *studies*

Incubation of hexachlorobutadiene with rat or mouse liver or kidney subcellular fractions caused a depletion of non-protein sulfhydryl groups, which was not due to oxidation (Kluwe et al., 1981).

The formation of GPB and of 1,4-(bis-glutathion-*S*-yl)-1,2,3,4-tetrachloro-1,3-butadiene (BGTB) is catalysed by glutathione-*S*-transferase in rat and mouse liver microsomes and cytosol (Wolf et al., 1984; Wallin et al., 1988; Dekant et al., 1988a,b). GPB formation has also been observed in human liver microsomes and those from several other species (Oesch & Wolf, 1989; McLellen et al., 1989). Conjugation in mouse liver microsomes, but not in those from rat liver, is significantly faster in females than in males (Wolf et al., 1984; Dekant et al., 1988a).

GPB formation has also been demonstrated in the isolated perfused rat liver; in this system, GPB formed in the liver was almost exclusively excreted with bile by a carrier-mediated active transport mechanism; only after infusing very high concentrations of hexachlorobutadiene was sinusoidal excretion of GPB into the perfusate observed (Gietl & Anders, 1991).

A large number of studies have used GPB, CPB and ACPB to further delineate the fate of hexachlorobutadiene in the organism. These studies have demonstrated that CPB is the penultimate intermediate in hexachlorobutadiene metabolism. CPB is a substrate for renal cysteine conjugate β-lyase and is metabolized by this enzyme to 2,3,4,4-tetrachlorobutenoic acid and 2,3,4,4-tetrachlorothionobutenoic acid (Dekant et al., 1988a). Trichlorovinyl-chlorothioketene has been identified as the ultimate reactive intermediate in hexachlorobutadiene metabolism catalysed by β-lyase (Dekant et al., 1991). ACPB accumulated by the renal organic anion transporter is cleaved to CPB by renal acylases (Vamvakas et al., 1987; Pratt & Lock, 1988).

6.2.2 In vivo studies

In *in vivo* studies, hexachlorobutadiene caused a marked, dose-related depletion of renal nonprotein sulfhydryl (NP-SH) in mice at single intraperitoneal doses of 33-50 mg/kg body weight but little or no decrease in hepatic NP-SH (Kluwe et al., 1981; Lock et al., 1984). This pattern was also observed in female rats at single intraperitoneal doses from 300 mg/kg body weight (Hook et al., 1983). Conversely, the compound caused a marked, dose-related depletion of hepatic NP-SH in male rats from 300 mg/kg body weight intraperitoneally, but no decrease (or even an increase) in renal NP-SH (Kluwe et al., 1981, 1982; Lock & Ishmael, 1981; Baggett & Berndt, 1984).

When cannulated male rats were given intravenously either a tracer dose of 0.971 mg radiolabelled hexachlorobutadiene/kg body weight or the same dose at 24 h after an intraperitoneal nephrotoxic dose of 300 mg/kg body weight in corn oil, 13 and 10% of the label was recovered in the bile, respectively, within the 3 h following the tracer dose. The labelled material was completely water soluble (Davis et al., 1980).

In a study by Payan et al. (1991), rats with cannulated bile ducts received once, either orally or intravenously, 1 or 100 mg of radiolabelled hexachlorobutadiene/kg body weight. At 72 h after exposure, fractional urinary excretion (7.5% of the dose) was independent of the dose and route of administration, in contrast to the situation in intact rats (see section 6.4). Fractional biliary excretion decreased with increasing dose following oral administration (66.8% versus 58%) and intravenous injection (88.7% versus 72%). Fractional faecal excretion was minimal following intravenous injection (3.1% following the low oral dose and 16.2% following the high oral dose). In a group of bile duct-duodenum cannula-linked rats given one dose of 100 mg/kg body weight, all tissue concentrations (kidney, liver, plasma, carcass) and the urinary excretions at 30 h after dosing were higher in bile donor rats than in recipient rats. The biliary contribution to both urinary and tissue concentrations was calculated to be 40%. Of the biliary metabolites entering the recipients, 80% was found to be reabsorbed.

Nash et al. (1984) administered 200 mg labelled hexachlorobutadiene in corn oil/kg body weight to male rats with exteriorized bile flow. They recovered 35% of the label in the bile during the 48 h following treatment, 40% of which was identified

as GPB (Fig. 1) and 12% as CPB. In another investigation into the identity of biliary excretion products, male rats were given intravenously an aqueous suspension of 0.026 mg of labelled hexachlorobutadiene. During the next two hours over 30% of the label was recovered in bile; 35% of this radioactivity was identified as GPB and 6% as BGTB (Fig. 1), but the remaining labelled material was not identified. Since some of the unidentified peaks disappeared after treatment of bile with inhibitors of gamma-glutamyltranspeptidase, they probably represent degradation products of GPB and BGTP (Jones et al., 1985).

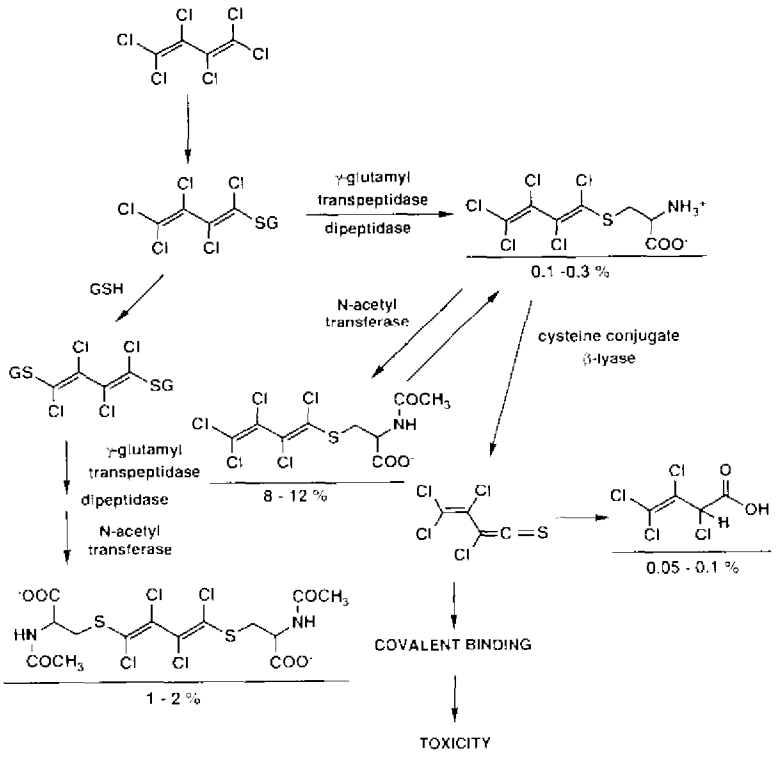


Fig. 1. Metabolism of hexachlorobutadiene in rats (From: Vamvakas et al., 1993)

The intestinal absorption of GPB and CPB was studied in rats by infusing the compounds into the intestine via a biliary cannula. When GPB was infused, both GPB and CPB were found in the blood in approximately equal concentrations. Higher blood CPB concentrations were found after CPB infusion than after GPB infusion (Gietl et al., 1991).

In studies with radiolabelled hexachlorobutadiene, several urinary metabolites were identified. The structure of these metabolites further supported the hypothesis that hexachlorobutadiene is bioactivated by glutathione conjugation.

ACPB was found to be the main metabolite (representing approximately 80% of the radioactivity present in urine) excreted after the administration of [^{14}C] hexachlorobutadiene (200 mg/kg) in female rats (Reichert & Schütz, 1986). The same authors also identified 1-carboxymethylthion-1,2,3,4,4-pentachlorobuta-1,3-diene and 1-methylthio-1,2,3,4,4-pentachloro-1,3-butadiene (MTPB) as urinary metabolites (Reichert et al., 1985). It is the opinion of the Task Group that the identification of MTPB by diazomethane treatment of the urinary extract is questionable.

In male rats, 1,2,3,4,4-pentachloro-1,3-butadienyl sulfenic acid (PBSA) is the only metabolite excreted in urine that has so far been identified. The data presented suggest that ACPB is not a major urinary metabolite of hexachlorobutadiene in male rats (Nash et al., 1984). In urine of mice exposed to radiolabelled hexachlorobutadiene (30 mg/kg), CPB, ACPB and 2,3,4,4-tetrachlorobutenoic acid were identified as urinary metabolites (Dekant et al., 1988a).

It is probable that 2,3,4,4-tetrachlorobutenoic acid is formed by reaction of the intermediate thioketene with water and further hydrolysis of the thionol acid thus formed (Dekant et al., 1988a).

The weight of evidence suggests that oxidative reactions involving cytochrome P-450 have little role in the metabolism of hexachlorobutadiene (Wolf et al., 1984; Dekant et al., 1988a).

6.3 Reaction with body components

The covalent binding of [^{14}C]-hexachlorobutadiene-related radioactivity to tissue proteins has been shown to be time dependent, with the highest level occurring during the first 6 h after treatment. The half-life of hexachlorobutadiene binding was

22 h in both liver and kidney (Reichert, 1983; Reichert et al., 1985).

In a DNA binding study, rats received a single oral dose of 20 mg [^{14}C]-hexachlorobutadiene, and DNA was isolated from the kidneys of these rats at 6, 18.5 and 30 h after dose administration. Although the results have been reported only in summary form, various levels of radioactivity were recovered with the DNA, but there was a marked variation in the level of radioactivity between samples. Furthermore complete analysis of the DNA was not performed and protein may have been associated with the DNA (Stott et al., 1981).

Covalent binding to mouse liver and kidney DNA was demonstrated after the oral administration of radiolabelled hexachlorobutadiene (30 mg/kg body weight) in corn oil (Schrenk & Dekant, 1989). In the liver and kidneys, the binding capacity of mitochondrial DNA was significantly higher than that of nuclear DNA. The level of binding to nuclear DNA in the liver was indistinguishable from that of controls. HPLC separation of the hydrolysed DNA indicated the presence of three distinct peaks of radioactivity.

6.4 Excretion

Following oral administration in rats and mice of single doses of hexachlorobutadiene up to 100 mg/kg body weight, the total excretion within 72 h was at least 65% of the dose. In mice, less than half of a dose of 30 mg/kg body weight was metabolized (Dekant et al., 1988a). In rats, assuming that the faeces mostly contain unchanged compound and no non-resorbed conjugates, 44% of an orally administered low dose of hexachlorobutadiene (1 mg/kg body weight) was metabolized (Reichert et al., 1985). At higher doses the percentage of hexachlorobutadiene metabolized decreased dramatically. The biotransformation of hexachlorobutadiene in rats appears to be a saturable process considering the reduced excretion of carbon dioxide and renal metabolites at increasing doses (Davis et al., 1980; Reichert et al., 1985; Reichert & Schütz, 1986; Payan et al., 1991). This could be explained by saturation of the gastrointestinal absorption, which was observed by Reichert et al. (1985). It should be noted, however, that the observed increase in the amount of unchanged hexachlorobutadiene in faeces with increasing dose applies only up to 100 mg/kg body weight. At higher dose levels, the amount of unchanged hexachlorobutadiene in faeces decreases, probably due to a decrease in faecal output (Davis et al., 1980; Nash et al., 1984).

The results of studies of Payan et al. (1991) on bile-duct cannulated (see section 6.2.2) and intact (see Table 8) rats show that saturation of gastrointestinal absorption indeed occurs following oral administration.

Pharmacokinetic data concerning the fate of hexachlorobutadiene in organisms were not available to the Task Group.

7. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

7.1 Aquatic organisms

7.1.1 Short-term toxicity

A summary of short-term aquatic toxicity data is presented in Table 9. In most of these studies the concentration of hexachlorobutadiene was not reported. Therefore, the actual effect concentrations may be lower than the nominal ones. In several cases these nominal values far exceed the solubility limits. Based on these data the substance is moderately to highly toxic to aquatic organisms (Canton et al., 1990).

Adverse effects reported in some of these acute tests included loss of equilibrium, erratic swimming (Leeuwangh et al., 1975; Laseter et al., 1976), decreased activity, increased rate of opercular movement, jumping (Leeuwangh et al., 1975), inverted positions, fin fibrillation and muscle tetany (Laseter et al., 1976). In a special investigation into kidney pathology, groups of five goldfish (*Carassius auratus*) received one intraperitoneal injection of hexachlorobutadiene at a dose level of 500 mg/kg body weight in corn oil. They were subsequently fasted for up to 6 days and sacrificed at different points in time up to day 7. Controls received corn oil only. The temperature was kept between 18 and 21 °C. By 24 h the fish showed decreased activity, swam with dorsal fins down, and had difficulty in following food. By day 4 exophthalmos, distended abdomen and ascites were observed. These signs of toxicity were all reversible. Relative kidney weights were elevated on day 4 only. From 12 h after exposure, P_2 and P_3 renal epithelial cells exhibited marked vacuolation and necrosis, which persisted up to day 7. Increased gamma-glutamyltransferase (EC 2.3.2.2) staining was seen in P_2 and P_3 segments (Reimschüssel et al., 1989).

In a report of this experiment, the fish were sacrificed at different points in time up to day 70 after exposure. In one of the two experiments the fish also received 5-bromo-2'-deoxyuridine 4 h prior to sacrifice. Morphometric analysis of developing nephrons showed an increase in the percentage of volume occupied by basophilia clusters and developing nephrons from day 14 onwards. The apparent number of basophilic clusters and developing nephrons per unit surface area was also increased from day 14 (Reimschüssel et al., 1990).

Table 9. Short-term aquatic toxicity of hexachlorobutadiene

Organisms	Species	Temperature (°C)	pH	Dissolved oxygen (mg/litre)	Hardness (mg $CaCO_3$ per litre)	Stat/flow[a] open/closed	Exposure period (h)	Parameter	Concentration (mg/litre)	Reference
Fresh water										
Algae	*Haematococcus pluvialis*	20				stat, closed	24	EC_{10}[b]	> 2	Knie et al. (1983)
Bacteria	*Pseudomonas putida*	25	7.0			stat, closed	16	TT[c]	> 25	Bringmann & Kuhn (1977)
Bacteria	*Pseudomonas putida*	20	7.2			stat, open	0.5	EC_{10}[b]	> 0.9	Knie et al. (1983)
Protozoans	*Chilomonas paramecium*	20	6.9			stat, closed	48	TT[c]	> 10	Bringmann et al. (1980)
Molluscs	great pond snail, *Lymnaea stagnalis*	19				stat,[d] closed	24 96	LC_{50} LC_{50}	0.21 0.21	Leeuwangh et al. (1975)[e]
Crustaceans	water flea, *Daphnia magna*	20	7		250	stat, open	24	EC_{50}	0.5	Knie et al. (1983)
	aquatic sowbug, *Asellus aquaticus*	19				stat,[d] closed	96	LC_{50}	0.13	Leeuwangh et al. (1975)[e]

Table 9 (contd).

Fish	goldfish, *Carassius auratus*	17.5				stat,[d] open	96	LC_{50}	0.09	Leeuwangh et al. (1975)[e]
Fish	zebrafish, *Brachydanio rerio*	20	8.0	9.0	180	flow, closed	48	LC_{50}	1	Slooff (1979)
Fish	rainbow trout, *Salmo gairdneril*							LC_{50}	0.320	US EPA (1980)
Fish	bluegill sunfish, *Lepomis macrochirus*							LC_{50}	0.326	US EPA (1980)
Fish	sheepshead minnow, *Cyprinodon variegatus*							LC_{50}	0.557	US EPA (1980)
Fish	golden orfe, *Leuciscus idus*	20	8		270	open	48	LC_{50}	3	Knie et al. (1983)[e]
Fish	fathead minnow, *Pimephales promelas*	25	6.7-7.6	8.0	45	flow, open	96	LC_{50}	0.10	Walbridge et al. (1983)[e]

Table 9 (contd).

Organisms	Species	Temperature (°C)	pH	Dissolved oxygen (mg/litre)	Hardness (mg $CaCO_3$ per litre)	Stat/flow[a] open/closed	Exposure period (h)	Parameter	Concentration (mg/litre)	Reference
Marine										
Crustaceans	harpacticoid copepod	21	7.9	≥ 5		stat, open	96	LC_{50}	1.2	Bengtsson & Tarkpea (1983)
	grass shrimp, *Palaemonetes pugio*							LC_{50}	0.032	US EPA (1980)
	Mysid shrimp, *Mysidopsis bahia*							LC_{50}	0.059	US EPA (1980)
Fish	sailfin molly, *Poecilia latipinna*	22-24	6.6-7.9	8-9		flow, open	26 30 77 115 138	LC_{50} LC_{50} LC_{50} LC_{50} LC_{50}	4.2 4.5 1.4-1.9 1.7 1.2	Laseter et al. (1976)[e,f]
	pinfish, *Lagodon rhomboides*							LC_{50}	0.399	US EPA (1980)

[a] static or flow-through test, open or closed system
[b] effect is 10% reduction in oxygen consumption
[c] TT = toxic threshold for inhibition of cell multiplication
[d] semi-static (daily renewal) test
[e] analysis for hexachlorobutadiene was reported
[f] salinity was 0.25%, 96-h LC_{50} was calculated to be 1.6 mg/litre

7.1.2 Long-term toxicity

The cell multiplication of green algae (*Scenedesmus quadricauda*) was not inhibited after 8 days of static exposure to a nominal concentration of 25 mg/litre (well above pure water solubility) in a closed system at 27 °C and a pH of 7 (Bringmann & Kühn, 1977). A 14-day LC_{50} of 0.4 mg/litre was determined for 2- to 3-month old guppies (*Poecilia reticulata*) in a semi-static test using an open system at 22 °C, a water hardness of 25 mg $CaCO_3$/litre, and a dissolved oxygen concentration of > 5 mg/litre. No analysis for hexachlorobutadiene was reported (Könemann, 1981). In the same test under the same conditions, but with analysis for the compound, the 14-day LC_{50} was 0.16 mg/litre (Hermens et al., 1985).

In a study by Leeuwangh et al. (1975), groups of six goldfish (*Carassius auratus*) were each exposed to hexachlorobutadiene in tap water at measured concentrations of 0, 0.0003, 0.003, 0.0096 or 0.03 mg/litre for 49 and 67 days. The static test in an open system was conducted at 19 °C, a pH of 7.6, and a dissolved oxygen concentration between 3.2 and 6.3 mg/litre. Body weights were decreased after 49 days at 0.03 mg/litre, and body weight gain was still reduced at 67 days. Abnormal behaviour, jumping, incoordination, increased opercular movement and overall immobility were noted at 0.0096 mg/litre. After 49 days at 0.0096 mg/litre (no data at 0.03 mg/litre), relative liver weights were increased, and the activity of liver glucose-6-phosphatase (EC 3.1.3.9) was decreased, whereas the activity of liver glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was increased. After 67 days the activity of liver phenylalanine hydroxylase (EC 1.14.16.1) showed a concentration-related increase. No effects were found on haemoglobin concentration and haematocrit after 49 days or on the activity of serum alanine aminotransferase (EC 2.6.2.1) and serum alkaline phosphatase (EC 3.1.3.1) after 67 days.

Groups of 12 largemouth bass (*Micropterus salmoides*) were each exposed to hexachlorobutadiene for 10 days at measured concentrations of 0.00343 and 0.03195 mg/litre in filtered tap water with a salinity of 0.08–0.1%, at 22–24 °C, a pH of 6.6–7.9 and a dissolved oxygen concentration of 7.6–8.5 mg/litre. A control group comprised 12 water and 12 vehicle (acetone) controls. Plasma cortisol levels were increased at both concentrations, but haematocrit values were not affected. At the higher concentration there was leukocytic infiltration in the kidneys of one of the fish and paleness and accentuated lobulation of parenchyma in the livers (Laseter et al., 1976).

In an early lifestage test, four replicate groups, each of 30 fathead minnow (*Pimephales promelas*) eggs, 2-4 h after spawning, were exposed to measured hexachlorobutadiene concentrations in sand-filtered and sterilized lake water of 0.0017, 0.0032, 0.0065, 0.013 and 0.017 mg/litre in an open system. Following hatching (4-5 days after spawning), four replicate groups of 15 larvae continued to be exposed for 28 days. Control groups of equal size were exposed to slightly contaminated water containing 0.00008 mg/litre. The temperature was 25 °C, pH was 7.4, dissolved oxygen concentration 7 mg/litre, and water hardness 45 mg $CaCO_3$/litre. The hatchability of embryos and the percentage of normal larvae at hatch were not affected. An increased fish mortality and a concentration-related decrease of body weight were observed at the two highest concentrations at the end of the exposure period (Benoit et al., 1982).

7.2 Terrestrial organisms

7.2.1 Short-term toxicity

Except for one test with birds, reliable tests with terrestrial organisms have not been reported.

Groups of 12 female and four male Japanese quails (*Coturnix coturnix japonica*) were exposed to a diet containing hexachlorobutadiene at levels of 0, 0.3, 3, 10 or 30 mg/kg diet for 90 days. Each cage contained three females and one male of the same dose group. Feed analysis indicated levels close to the nominal values. Adults were all histopathologically examined. Eggs were collected on days 37-46, 64-73, and 81-90. Six adults died during the study: 4 at 0.3 mg/kg, 1 at 10 mg/kg, and 1 at 30 mg/kg, but this was not considered to be related to treatment. The survival of chicks from eggs collected on days 81-90 was decreased at 10 mg/kg only. Egg production, the percentage of fertile eggs, the percentage of hatchable eggs, and eggshell thickness were unaffected compared to controls (Schwetz et al., 1974).

8. EFFECTS ON EXPERIMENTAL ANIMALS AND *IN VITRO* TEST SYSTEMS

8.1 Single exposure

The available mortality data are summarized in Table 10.

8.1.1 Inhalation exposure

8.1.1.1 Mortality

In the only reported study of the mortality of hexachlorobutadiene following inhalation, groups of 20 SPF mice of the OF_1 strain were exposed for 6 h (Gage, 1970). The vapour concentrations were measured by gas chromatography, and the concentrations were within 10% of the nominal value. Results are shown in Table 10.

8.1.1.2 Systemic effects

The decrease in respiratory rate (reflex bradypnoea) in groups of six male Swiss OF1 mice was measured following exposure for 15 min to hexachlorobutadiene vapour at concentrations between 886 and 2625 mg/m^3. The vapour concentrations were checked by gas chromatography. The mice were restrained in a body plethysmograph, while the heads were extended into an inhalation chamber. The 50% effect level, calculated from the concentration-effect curve, was 2250 mg/m^3 (De Ceaurriz et al., 1988).

8.1.2 Oral exposure

8.1.2.1 Mortality

Based on acute mortality data, hexachlorobutadiene is slightly to moderately toxic to adult rats, moderately toxic to male weanling rats, and highly toxic to female weanling rats following ingestion. Young rats are far more sensitive than adult rats (Kociba et al., 1977a). Gradiski et al. (1975) observed delayed mortality (after 24 h) in oral LD_{50} studies on rats and mice.

8.1.2.2 Systemic effects

Hexachlorobutadiene mainly affects the kidneys and, to a lesser extent, the liver. The effects on these organs and the related

Table 10. Mortality of hexachlorobutadiene from single exposure

Species/strain	Age	Sex	Route of exposure	Observation period (days)	LD_{50} (mg/kg body weight) or LC_{50} (mg/m^3)[a]	Reference
Mouse			oral		87 (78.1-95.9)	Murzakayev (1963)[b]
OF_1	adult	female	inhalation (6 h)	14	107 (102-113)	Gage (1970)
OF_1	adult	female	oral	14	65 (60-70)	Gradiski et al. (1975)[c]
OF_1	adult	male	oral	14	80 (75-85)	Gradiski et al. (1975)[c]
Alderley Park	adult	male	intraperitoneal	14	67 (53-85)	Lock et al. (1984)[e]
Alderley Park	adult	female	intraperitoneal	14	85 (65-111)	
$C_{57}BL/10J$	adult	male	intraperitoneal	14	57 (41-81)	
C_3H	adult	male	intraperitoneal	14	25-75	
BALB/c	adult	male	intraperitoneal	14	32-40	
DBA/2J	adult	male	intraperitoneal	14	53 (36-76)	
Rat			oral		350 (323-377)	Murzakayev (1963)[b]
OF_2 rat	adult	female	oral	14	270 (250-290)	Gradiski et al. (1975)[c]
OF_2 rat	adult	male	oral	14	250 (220-280)	Gradiski et al. (1975)[c]
Sprague-Dawley	adult	female	oral		200-400	Kociba et al. (1977a)[d]
Sprague-Dawley	adult	male	oral		580 (504-667)	Kociba et al. (1977a)[d]
Sprague-Dawley	weanling	female	oral		46 (26-81)	Kociba et al. (1977a)[d]
Sprague-Dawley	weanling	male	oral		65 (46-91)	Kociba et al. (1977a)[d]

Table 10 (contd).

Species/strain	Age	Sex	Route of exposure	Observation period (days)	LD_{50} (mg/kg body weight) or LC_{50} (mg/m^3)[a]	Reference
Alderley Park	weanling	male	intraperitoneal	7	57 (38-87)	Hook et al. (1983)[e]
Alderley Park	29 days	male	intraperitoneal	7	96 (72-128)	
Alderley Park	adult	male	intraperitoneal	7	360 (325-396)	
Guinea-pig			oral		90 (81.5-98.5)	Murzakayev (1963)[b]
Rabbit						
New Zealand	adult	female	dermal (8 h)	14	1120 (890-1400)	Duprat & Gradiski (1978)[f]

[a] When available, 95% confidence limits are reported between brackets.
[b] Observation period, strain, sex, age (or body weight) and vehicle were not reported.
[c] Vehicle was olive oil.
[d] Confidence limits not calculable; observation until no toxicity was observed any longer; vehicle was corn oil.
[e] Vehicle was corn oil.
[f] Application undiluted using glass vials (3.6 cm^2).

biochemical findings are discussed extensively in sections 8.8.2 (liver) and 8.8.3 (kidney). In oral LD_{50} studies on rats and mice, Gradiski et al. (1975) observed hyper-reactivity just after exposure, followed by decreased activity and staggering.

8.1.3 Dermal exposure

8.1.3.1 Mortality

Hexachlorobutadiene was harmful to rabbits following acute dermal exposure (LD_{50} = 1120 mg/kg body weight; (range, 890-1400 mg/kg; Table 10). After a dose of 780 mg/kg body weight, death occurred within 24 h from respiratory and cardiac failure (Duprat & Gradiski, 1978).

8.1.3.2 Systemic effects

New Zealand rabbits dermally exposed to undiluted hexachlorobutadiene at doses of 1170 and 1550 mg/kg body weight exhibited stupor, dyspnoea and cyanosis (Duprat & Gradiski, 1978).

8.1.4 Other routes of exposure

Hexachlorobutadiene has been shown to be toxic to various strains of mice after intraperitoneal injection (Lock et al., 1984) and harmful to adult rats (Hook et al., 1983). The compound was considerably more toxic to young and weanling rats (Table 10).

Rats intraperitoneally exposed to single lethal doses of hexachlorobutadiene from 500 to 1000 mg/kg in corn oil exhibited piloerection, sedation, hunching, incoordination, loss of muscle tone and hypothermia (Lock & Ishmael, 1979). A macroscopic and haematological investigation of rats intraperitoneally exposed to doses between 121 and 336 mg/kg body weight in olive oil did not reveal any damage to the gastrointestinal tract, spleen, heart or gonads. In the lungs, congestion, haemorrhage, and oedema were observed, but these were attributed by the authors to ether anaesthesia. At dose levels of 213 mg/kg body weight or more, lymphopenia and related neutrophilia were induced (Gradiski et al., 1975).

8.2 Short-term exposure

8.2.1 Inhalation exposure

In the only inhalation study published, groups of four adult Alderley Park SPF rats of each sex were dynamically exposed to nominal concentrations of 53, 107 or 267 mg/m^3 6 h/day for 15 days, 1067 mg/m^3 6 h/day for 12 days, or 2668 mg/m^3 4 h/day for 2 days. Petroleum ether was used as a solvent for concentrations below 1067 mg/m^3. Many other chemicals were tested similarly, and batches of control rats of unknown size were maintained at intervals of 2 months during the whole experimental period. No analysis for hexachlorobutadiene was carried out. Two of the four female rats exposed to 1067 mg/m^3 died, and autopsy revealed pale, enlarged kidneys, adrenal regeneration and renal cortical tubular degeneration with epithelial regeneration. Rats of both sexes lost weight at 1067 mg/m^3 and the weight gain of females was reduced at 107 and 267 mg/m^3. Irritation of eyes and nose was observed at the two highest levels. At 267 and 1067 mg/m^3 rats were in a poor condition, females being more affected than males. Respiratory difficulties were seen at and above 267 mg/m^3. Haematological examination at the end of the exposure period showed slight anaemia in females at 1067 mg/m^3. Urinalysis did not reveal abnormalities at any of the exposure levels. Macroscopically enlarged, pale kidneys were found at 267 and 1067 mg/m^3 and enlarged adrenals at 1067 mg/m^3. Histopathological investigations revealed proximal tubular degeneration in the kidneys and cortical degeneration in adrenals at concentrations of 267 mg/m^3 or more. No toxic signs were observed at the lowest exposure level and autopsy revealed no gross abnormalities (Gage, 1970).

8.2.2 Oral exposure

8.2.2.1 Rats

Groups of five adult male Sprague-Dawley rats were exposed to daily oral doses of hexachlorobutadiene (0, 0.2 or 20 mg/kg body weight) in corn oil for 3 weeks. Only the kidneys were examined histologically. At the higher dose level, body weight gain was decreased and relative kidney weight increased. Histopathological examination of the kidneys revealed damage in the middle and inner cortical region, including loss of cytoplasm, nuclear pyknosis, increased basophilia and mitotic activity, and increased cellular debris. No toxic signs were observed at a dosage of 0.2 mg/kg per day (Stott et al., 1981).

In a study by Kociba et al. (1971), groups of four female Sprague-Dawley rats consumed for 30 days a diet containing hexachlorobutadiene, which resulted in ingested nominal daily doses of 0, 1, 3, 10, 30, 65 and 100 mg/kg body weight. Analysis of the compound in the feed was not reported. Body weights were decreased at the two highest dose levels. At 10 mg/kg or more, a dose-related decrease in body weight gain was observed along with a decrease in food consumption. There was also an increase in haemoglobin concentrations, which, although significant, was not clearly dose related. There was no effect on serum alanine aminotransferase activity (EC 2.6.1.2). A dose-related increase in relative kidney weight was observed at dose levels of 3 mg/kg or more. Histopathological examination, which was restricted to liver and kidneys, showed proximal tubular degeneration, individual cell necrosis, and regenerative changes in the kidneys at doses of 10 mg/kg or more. Hepatocellular swelling was seen at 100 mg/kg. The no-observed-adverse-effect level (NOAEL) was 1 mg/kg body weight per day.

In a 2-week experiment, groups of six weanling Wistar-derived rats of each sex were exposed to measured dietary hexachlorobutadiene levels of 0, 73, 182 or 447 mg/kg (the Task Group considered this equivalent to doses of 0, 7.3, 18.2 and 44.7 mg/kg body weight per day, respectively). At all dose levels, body weight and food conversion efficiency (g of weight gain/g of food) were decreased in a dose-related manner. Food consumption per g of body weight was decreased at 44.7 mg/kg body weight. Relative kidney weights were increased at the two highest dose levels. At all dose levels a dose-related degeneration of kidney tubular cells was observed, especially in the straight limbs of the proximal tubules located in the outer medulla. No toxic signs were observed in the liver. A NOAEL was not found (Harleman & Seinen, 1979).

In a follow-up to the dietary study, groups of 10 weanling Wistar-derived rats of each sex received daily doses by gavage of 0, 0.4, 1, 2.5, 6.3 or 15.6 mg/kg body weight in peanut oil for 13 weeks (Harleman & Seinen, 1979). Body weight gain, food consumption and food utilization efficiency were decreased at 6.3 and 15.6 mg/kg. Polyuria was observed in females at these dose levels after week 10, while a dose-related decrease in urine osmolarity occurred at dose levels of 2.5 mg/kg or more. In males, the latter effect was observed at 15.6 mg/kg. No other changes were observed in urinalysis (after week 10) and haematological investigations (after week 8). A dose-related increase in relative

kidney weight was measured in males of all treatment groups but only at 6.3 mg/kg or more in females. Dose-related increases in the relative weight of liver and spleen were measured at 6.3 mg/kg or more. Histopathological examinations revealed changes in liver and kidneys. In the livers of males dosed with 6.3 mg/kg or more, an increased basophilic, flocky granulation was observed. At dose levels of 6.3 mg/kg or more in males and 2.5 mg/kg or more in females, there was a dose-related degeneration of the renal proximal tubules, as shown by hyperchromatic nuclei, hypercellularity, vacuolation and focal necrosis of epithelial cells and a diminished brush border. No adverse effects were observed at daily doses of 1 mg/kg in females or 2.5 mg/kg in males (Harleman & Seinen, 1979).

8.2.2.2 Mice

In a two-week study, groups of five B6C3F$_1$ mice of each sex were fed a diet containing hexachlorobutadiene at nominal doses of 0, 30, 100, 300, 1000 or 3000 mg/kg feed for 15 days (calculated by the Task Group to be equivalent to 0, 4.3, 14.3, 43, 143 and 430 mg/kg body weight per day, respectively, using standard values for average body weight and food consumption in mice). Analysis of the feed was carried out by gas chromatography, and no more than 9% loss of the chemical was observed in one day (feed was replaced every 2 days). All mice given 143 or 430 mg/kg body weight died or were sacrificed in a moribund condition within 7 days. A dose-related growth retardation was observed. At the two highest doses, the observed toxic effects included renal tubular necrosis, hepatic cytoplasmic vacuolization, and testicular degeneration characterized by the presence of syncytial giant cell formation of spermatocytes. At dose levels of 43 mg/kg body weight or more, minimal to mild depletion of bone marrow (characterized by a decrease in the haematopoietic cells) was seen in two out of five mice of both sexes per dose group. At dose levels of 4.3, 14.3 and 43 mg/kg body weight, at which all animals survived to the end of the study, renal tubular cell regeneration was observed (Yang et al., 1989; Yang, 1991).

In a 13-week study, groups of 10 B6C3F$_1$ mice of each sex were fed a diet containing hexachlorobutadiene at concentrations of 0, 1, 3, 10, 30 or 100 mg/kg feed. Using measurements of food consumption and body weight, the authors determined doses of 0, 0.1, 0.4, 1.5, 4.9 or 16.8 mg/kg body weight per day for males, and 0, 0.2, 0.5, 1.8, 4.5 or 19.2 mg/kg body weight per day for

females. Analysis of the compound was carried out as in the two-week study. No treatment-related clinical signs or deaths were observed. The motility of sperm from treated mice was significantly lower than in controls, although this effect was not dose related (Yang et al., 1989; Yang, 1991). Body weight gain was reduced at dose levels of 4.5 and 19.2 mg/kg body weight in females. Reductions in kidney weight occurred at dose levels of 1.5 mg/kg or more in males and at 19.2 mg/kg body weight in females, and reductions in heart weight occurred at 19.2 mg/kg body weight in males. Necropsy revealed a treatment-related increase in renal tubular regeneration (prominent in the outer stripe of the medulla) at dose levels of 0.2 mg/kg body weight or more in females (Table 11). Although the author concluded that a NOAEL was not observed for females, the Task Group noted that the occurrence of renal tubular regeneration in one out of ten female mice in the 0.2-mg/kg body weight group is insufficient evidence of an adverse effect at this dose level in females.

Table 11. Incidences of renal tubular regeneration in 13-week feed studies on B6C3F$_1$ mice[a]

Concentration (mg/kg feed)	Dose (mg/kg body weight per day)		Number of mice with lesions/number examined	
	Male	Female	Male	Female
0	0	0	0/10	0/10
1	0.1	0.2	0/10	1/10
3	0.4	0.5	0/10	9/10
10	1.5	1.8	0/9	10/10
30	4.9	4.5	10/10	10/10
100	16.8	19.2	10/10	10/10

[a] Modified from: Yang (1991)

8.3 Long-term exposure

No long-term inhalation studies have been reported. A study describing long-term exposure of mice by the dermal route is presented in section 8.7.3.

An oral toxicity/carcinogenicity test in rats has been reported (see section 8.7.2).

8.4 Skin and eye irritation; sensitization

8.4.1 Irritation

The vapour of hexachlorobutadiene has been found to be irritating to the eyes and nose of rats (Gage, 1970; see section 8.2.1).

Groups of six New Zealand rabbits received either 0.78 g (0.5 ml) of undiluted hexachlorobutadiene on the intact or abraded skin for 24 h, or 0.15 g (0.1 ml) in the conjuctival sac of the left eye. Assessment of the degree of irritation was conducted according to Draize et al. (1944) and by calculating the primary irritation index. Hexachlorobutadiene was moderately irritating for the skin (primary irritation index 4) but not irritating for the eyes (primary irritating index 1.5). Moderate conjunctivitis, epithelial abrasion and, at day 7, epithelial keratitis were observed in the eyes (Duprat et al., 1976).

Duprat & Gradiski (1978) applied undiluted hexachlorobutadiene to New Zealand rabbits at doses of 0.39, 0.78, 1.17 and 1.55 mg/kg body weight (0.25, 0.50, 0.75 and 1.00 ml, respectively) under occluded conditions, using glass vials, for 8 h. The observation period was 14 days. The skin was histopathologically examined in all dead animals, in half the survivors at day 15, and in the remaining survivors at day 36. After 12 h of exposure to the two highest doses, epidermis and subcutaneous tissue revealed oedema and polymorphonuclear leukocyte infiltration. In the epidermal cells, degeneration with pyknosis of nuclei and perinuclear oedema, and focal separation from the corium with vesicle formation were seen. After 3 to 5 days of exposure to the three highest doses, dermal necrosis was observed, leading to eschar formation and partial destruction of hair follicles. The effects increased with time, not with dose. Two to five weeks after application, repair was apparent at all dose levels, with scarring and upper dermis fibrosis, and epidermal acanthosis with focal dyskeratosis. Diffuse mononuclear infiltrate was seen in the dermis.

8.4.2 Sensitization

A group of 20 Hartley guinea-pigs were treated according to the Magnusson-Kligman protocol by intradermal injections of 5% hexachlorobutadiene in peanut oil and, after one week and subsequent treatment by sodium lauryl sulfate, by a 48-h dermal

application of a 25% suspension of the chemical in vaseline. The challenge was performed by dermal application of a 20% suspension in vaseline. A group of five controls was induced similarly and challenged by vaseline only. All exposed animals, but none of the controls, showed a positive reaction. The test was repeated in the same fashion without adjuvant in five guinea-pigs: all animals showed positive reactions (Gradiski et al., 1975).

8.5 Reproduction, embryotoxicity and teratogenicity

8.5.1 Reproduction

A group of female albino rats was exposed to one dose of hexachlorobutadiene (20 mg/kg body weight) administered subcutaneously before mating. Within 90 days after exposure, all 86 newborn rats had died, compared with 13 of the 61 controls. The offspring from exposed dams were reported to show excitation, disturbances of motor coordination, a decrease in appetite and a loss of weight, lymphocytosis, neutropenia, myelocytes, Jolly's and Cabeau's bodies, pneumonia, bronchitis, granular dystrophy of renal cells, glomerulonephritis, inflammatory destructive lesions of the gastrointestinal tract and vascular hyperaemia (Poteryayeva, 1966). The Task Group noted major deficiencies and incomplete reporting of the experiment, the unusual route of administration, and the high percentage of mortality in control rats.

Groups of 10-12 male and 20-24 female Sprague-Dawley rats received a diet containing hexachlorobutadiene at dose levels of 0.2, 2.0 or 20 mg/kg body weight per day for 90 days prior to mating, 15 days during mating, and subsequently throughout gestation and lactation. In the mating period, two females were placed with one male of the same dose group. The control group consisted of 17 males and 34 females. The diets were reportedly analysed for the test compound. No mortality was observed. At 20 mg/kg, adults showed decreased food consumption and body weight gain. Blood urea nitrogen, serum alanine aminotransferase (EC 2.6.1.2) and serum creatinine were unchanged compared to controls. The dams had an increased relative brain weight and the male rats had an increased relative liver weight at 20 mg/kg. The relative kidney weights were increased in both sexes at 20 mg/kg. At 2 and 20 mg/kg the kidneys of adult rats revealed dose-related tubular dilatation and hypertrophy with foci of epithelial degeneration and regeneration; however, there was no effect at 0.2 mg/kg. The only adverse reproductive effect in neonates was a

decreased weanling weight at 20 mg/kg. There was no detectable effect on the percentage pregnancy, the period from first cohabitation to delivery, survival indices, sex ratio, histopathology of weanlings, and the incidence of skeletal alterations and abnormalities in neonates (Schwetz et al., 1977).

In a third reproductive study, groups of six female SPF Wistar-derived rats, 10 weeks of age, received a diet containing hexachlorobutadiene at levels of 0, 150 and 1500 mg/kg diet (estimated by the Task Group to be equivalent to 0, 7.5 and 75 mg/kg body weight per day) for 3 weeks prior to mating, 3 weeks during mating, and subsequently throughout gestation and lactation. In the mating period, two untreated males were placed with the females, after which the females were housed individually. The 75-mg/kg female adults were killed in week 10, while those given 0 or 7.5 mg/kg were killed in week 18. Food analysis at the low dose level revealed hexachlorobutadiene levels within 96% of nominal values after 1 week and within 81% of nominal values after 2 weeks. Diets were prepared weekly. There was a reduced body weight gain by female rats in the two groups receiving hexachlorobutadiene. Weakness of hind legs, unsteady gait, incoordination and ataxia were seen at 75 mg/kg. The relative kidney weight was increased at both dose levels. Histopathological investigations revealed hypercellularity of epithelial cells, hydropic degeneration, and necrosis of proximal tubules in the kidneys at 7.5 mg/kg. At 75 mg/kg, slight proliferation of bile duct epithelial cells, fragmentation and demyelination of single fibres of the femoral nerve, and extensive renal degeneration were observed. Again at 75 mg/kg, no conceptions occurred, the ovaries showing little follicular activity, and there was no uterine implantation sites. At 7.5 mg/kg fertility and litter size were reduced, but not significantly. In both the control and 7.5-mg/kg groups, the resorption quotient was low. Compared to controls, pup weights were reduced significantly on days 0, 10 and 20 in the 7.5-mg/kg group. No gross abnormalities were observed (Harleman & Seinen, 1979).

8.5.2 *Embryotoxicity and teratogenicity*

In a teratology study, groups of 24–25 female rats were exposed to hexachlorobutadiene vapour at measured concentrations of 0, 21, 53, 107 or 160 mg/m^3 for 6 h per day from days 6 to 20 of pregnancy. The breathing zone atmosphere was analysed by gas chromatography. Maternal weight gain decreased at 53 and 160 mg/m^3. At the other two exposure levels, the slight decrease in

maternal weight was not significant. The mean number of implantation sites, total fetal losses, resorptions, live fetuses, incidences of pregnancy, and sex ratio were not affected by exposure to hexachlorobutadiene, compared to controls. Fetal body weight was reduced in both sexes at 160 mg/m^3. The incidences of external, visceral, and skeletal alterations were not significantly increased (Saillenfait et al., 1989).

In a study by Hardin et al. (1981), groups of 10-15 mated Sprague-Dawley rats received hexachlorobutadiene in corn oil by intraperitoneal injection at a dose level of 10 mg/kg body weight per day from days 1 to 15 of gestation. It was reported (without further details) that at least two maternal organ weights were changed and that pre- or postimplantation survival was reduced. Maternal tissues did not reveal histopathological effects. Fetuses had a reduced weight or length, a 1-2 day delay in heart development, and dilated ureters. No grossly visible external or internal malformations were observed (Hardin et al., 1981).

It was reported briefly by Badaeva et al. (1985) that daily oral administration of hexachlorobutadiene to pregnant rats at a dose level of 8.1 mg/kg body weight per day resulted in histopathological changes of nerve cells and myelin fibres of the spinal cord in the dams and their offspring.

8.6 Mutagenicity and related end-points

8.6.1 In vitro *effects*

Purified hexachlorobutadiene induces gene mutations in the Ames Salmonella test when specific incubation conditions are employed.

In preincubation assays adapted to include rat liver microsomes and additional reduced glutathione, hexachlorobutadiene induced point mutations in *Salmonella typhimurium* TA100 (Vamvakas et al., 1988a). Assays lacking specialized metabolic activation conditions have generally yielded negative results (Table 12).

Data from bacterial mutagenicity assays are consistent with the proposed scheme for the biotransformation of hexachlorobutadiene in animals (section 6.3; Fig. 1). Activity in *S. typhimurium* TA100, mediated by subcellular fractions of rat kidney, was inhibited by the addition of the β-lyase inhibitor, AOAA (Vamvakas et al., 1988a, 1989a) and the gamma-glutamyltranspeptidase inhibitor, acivicin (Vamvakas et al., 1989a).

Table 12. Studies on mutagenicity of hexachlorobutadiene

Test description	Species/strain/cell type	Conditions[a]	Result[c]	Reference
Reverse mutations	*Salmonella typhimurium* TA98, TA100, TA1530, TA1535, TA1538	+/- rat liver S9, purity 98%, plate incorporation	-	De Meester et al. (1981)
	S. typhimurium TA100	+/- rat liver S9, purity > 99%, plate incorporation	-	Stott et al. (1981)
	S. typhimurium TA98, TA100	+/- rat liver S9, purity not reported, suspension test[b]	-	Reichert et al. (1983)
	S. typhimurium TA98, TA100, TA1535, TA1537	+/- rat liver S9, purity not reported, preincubation test	-	Haworth et al. (1983)
		+ rat liver S9*, purity > 99.5%, preincubation test	+	
	S. typhimurium TA100, TA1535 TA1538	+/- rat liver S9, purity not reported, plate incorporation	-	Chudin et al. (1985)

Table 12 (contd).

Test description	Species/strain/cell type	Conditions[a]	Result[c]	Reference
	S. typhimurium TA100	+/- rat liver S9, - rat liver S9[*]	- +	Reichert et al. (1984)
	S. typhimurium TA100	+ rat liver S9[*], purity >99.5%, preincubation test	+[d]	Wild et al. (1986)
	S. typhimurium TA100	no activation, purity 98%	+	Vamvakas et al. (1988a)
		no activation, purity > 99.5%, preincubation test	-	
	S. typhimurium TA100	+ rat liver microsomes without additional GSH	-	Vamvakas et al. (1988a)
		+ rat liver microsomes and additional GSH, purity > 99.5%, plate incorporation	+[e]	

Table 12 (contd).

Test description	Species/strain/cell type	Conditions[a]	Result[c]	Reference
Sex-linked lethals	*Drosophila melanogaster*	feeding or injection	-	Woodruff et al. (1985)
Chromosome aberrations	CHO cells	+/- rat liver S9	-	Galloway et al. (1987)
Chromosome aberrations	human lymphocytes	- rat liver S9	-	German (1988)
Sister chromatid exchanges	CHO cells	+/- rat liver S9	+	Galloway et al. (1987)
Chromosome aberrations	mouse bone marrow cells	inhalation, 4 h	+	German (1988)
Chromosome aberrations	mouse bone marrow cells	oral gavage	+	German (1988)

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

[b] The extreme toxicity of the compound without S9 was supposed to exclude testing in this system

[c] + = ≥ twice the background rate or, in the case of bacterial studies, a reproducible dose-related increase in the number of revertants per plate; - = negative

[d] 0.23 revertants per nmol

[e] Addition of rat kidney microsomes further increased the number of revertants; positive results were inhibited by the β-lyase inhibitor aminooxyacetic acid

Several of the proposed metabolites of hexachlorobutadiene have been assayed for mutagenic activity in *S. typhimurium* TA100 (Table 13). The mono-glutathione (GPB) and mono-cysteine (CPB) conjugates were mutagenic in the presence or absence of rat kidney S9. Rat liver microsomes and mitochondria that exhibit high gamma-glutamyltranspeptidase activities strongly enhanced the mutagenic potency of GPB in the presence of additional glutathione, in contrast to liver microsomes that exhibit lower gamma-glutamyltranspeptidase activity. Furthermore, AOAA and acivicin both inhibit the activation of GPB mediated by kidney fractions. The di-glutathione (BGTB) and di-cysteine (BCTB) conjugates of hexachlorobutadiene were not mutagenic either in the presence or absence of rat kidney S9 (Green & Odum, 1985; Dekant et al., 1986; Vamvakas et al., 1988a, 1989a).

The mercapturic acid, ACPB, was mutagenic both in the presence and absence of rat liver S9. It has been suggested that the metabolism of ACPB in animals is catalysed by an *N*-deacetylase and by β-lyase (section 6.3) (Reichert et al., 1984). The Task Group considered that *S. typhimurium* possesses both of these enzymes activities. Both MTPB and CMTPB gave negative results in tests with *S. typhimurium* TA100 and are considered to be detoxified metabolites of hexachlorobutadiene (Wild et al., 1986).

Chinese hamster ovary (CHO) cells were exposed to between 5 and 24 mg hexachlorobutadiene/litre for 2 h in the presence of rat liver S9 and throughout the incubation period (8-26 h, depending on cell cycle delay) in the absence of rat liver S9. In comparison with concurrent controls, no significant increase in chromosome aberration frequency was observed (Galloway et al., 1987). In a further study, in which human lymphocyte cultures were exposed to between 0.01 and 0.001 mg hexachlorobutadiene per litre in the absence of S9 for 27 h, there was also no clastogenic effect. At the highest dose level there was a reduction of approximately 60% in the mitotic index of human lymphocyte cultures (German 1988). However, hexachlorobutadiene at a dose level of at least 4 mg/litre did cause a significant increase in the frequency of sister chromatid exchange in CHO cells in both the presence and absence of rat liver S9 (Galloway et al., 1987).

Hexachlorobutadiene was found to induce unscheduled DNA synthesis (UDS) in Syrian hamster embryo fibroblast cultures. Moreover, the magnitude of the response was increased when a preincubation period with rat liver S15 was employed (Schiffman

Table 13. Tests for reverse mutations in *Salmonella typhimurium* TA100 by proposed metabolites of hexachlorobutadiene

Metabolite and abbreviation[a]	Conditions[b]	Result[c]	Reference
1-(glutathion-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (GPB)	no activation	-	Green & Odum (1985)
	+ rat kidney S9	+	
	+/- rat kidney fractions	+[d]	Vamvakas et al. (1988a)
1,4-(bis-glutathion-*S*-yl)-1,2,3,4-tetrachloro-1,3-butadiene (BGTB)	+/- rat kidney fractions	-	Vamvakas et al. (1988a)
1,4-(bis-cystein-*S*-yl)-1,2,3,4-tetrachloro-1,3-butadiene (BCTB)	+/- rat kidney fractions	-	Vamvakas et al. (1988a)
1-(cystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (CPB)	+/- rat kidney S9	+[e]	Green & Odum (1985)
	no activation	+[f]	Dekant et al. (1986)
1-(*N*-acetylcystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (ACPB)	- rat liver S9	-	Wild et al. (1986)
	+ rat liver S9	+[g]	
1-carboxymethylthio-1,2,3,4,4-pentachloro-1,3-butadiene (CMTPB)	+ rat liver S9[h]	-	Wild et al. (1986)

Table 13 (contd).

Metabolite and abbreviation[a]	Conditions[b]	Result[c]	Reference
1-methylthio-1,2,3,4,4-pentachloro-1,3-butadiene (MTPB)	+ rat liver S9	-	Wild et al. (1986)
2,2,3,4,4-pentachloro-3-butenoic acid (PBA)	+/- rat liver S9	+	Reichert et al. (1984)
2,2,3,4,4-pentachloro-3-butenoic acid chloride (PBAC)	+/- rat liver S9	+	Reichert et al. (1984)

[a] See also Figure 1

[b] Plate incorporation assays with, except in the case of the tests by Green & Odum, preincubation; S9* = a fortified S9 mix containing 3 times the normal protein concentration

[c] + = ≥ twice background rate; - = negative

[d] Mutagenic potency enhanced by rat kidney microsomes or mitochondria and less so by cytosol; positive results were inhibited by the β-lyase inhibitor aminooxyacetic acid

[e] Mutagenic potency enhanced by rat kidney S9

[f] Positive results were inhibited by the β-lyase inhibitor aminooxyacetic acid

[g] 18.7 revertants per nmol; mutagenic potency decreased by addition of pyridoxal phospate; activation by cytosol, with and without cofactors, had the same results as S9 mix; microsome mix was inactive

et al., 1984). However, there was no induction of UDS in a study using rat hepatocyte cultures (Stott et al., 1981).

In summary, the Task Group concluded that hexachlorobutadiene was genotoxic *in vitro* and that the negative results reported in some studies may have resulted from the use of inappropriate conditions for metabolic activation.

8.6.2 In vivo *effects*

Hexachlorobutadiene induced a significant increase in the frequency of chromosomal aberrations in mouse bone marrow cells following the administration of acute oral doses of 2 or 10 mg/kg body weight or acute inhalation exposure to 10 mg/m^3 for 4 h. Both experiments used six mice per dose group, and the animals were sacrificed after 24 h (German, 1988).

Six hours after the administration of a single oral dose of 20 mg hexachlorobutadiene/kg body weight to two groups of five male Sprague-Dawley rats, there were statistically significant increases in kidney UDS of 27% and 54% above concurrent control levels. Administration of a positive control substance, dimethylnitrosamine, resulted in an increase of 187% over controls (Stott et al., 1981).

As described in section 6.3, radiolabelled nucleotides were recovered from the kidneys of rats and mice administered ^{14}C-labelled hexachlorobutadiene by gavage (Stott et al., 1981; Schrenk & Dekant, 1989). The Task Group concluded that these studies indicated covalent binding of hexachlorobutadiene or its metabolites to kidney DNA *in vivo*. The study with mice showed that the level of binding to mitochondrial DNA was greater than that to nuclear DNA. In addition, radioactivity was recovered in mitochondrial DNA, but not nuclear DNA, from mouse liver (Schrenk & Dekant, 1989).

Hexachlorobutadiene did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* following treatment of adults either via the diet or by injection (Woodruff et al., 1985).

8.7 Carcinogenicity/long-term toxicity

8.7.1 *Inhalation exposure*

No long-term carcinogenicity studies, where inhalation was the route of exposure, have been reported.

8.7.2 Oral exposure

In a study by Kociba et al. (1977a,b), groups of 39-40 adult Sprague-Dawley rats of each sex received a diet containing hexachlorobutadiene at 0.2, 2 or 20 mg/kg body weight per day for 22 (males) or 24 (females) months. Control groups comprised 90 rats of each sex. Analysis for the compound was not reported. An increased mortality was observed in males at 20 mg/kg. Hexachlorobutadiene caused a depression of the body weight gain in both sexes at the highest dose level without any effect on food consumption. Haematological investigations performed at 12-14 and 22-24 months, revealed a slight, but statistically significant, depression in the red blood cell count of males at 20 mg/kg (22 months). Urinalysis at 12-14 months and 22-24 months did not reveal effects except for a small increase in coproporphyrin excretion. The analysis of the clinical chemistry parameters of blood urea nitrogen, serum alanine aminotransferase (EC 2.6.1.2) and serum alkaline phosphatase (EC 3.1.3.1) at 12 months revealed no treatment-related effects, except for statistically significant decreases in serum alanine aminotransferase in males of the 20-mg/kg dose group and females of the 0.2- or 20-mg/kg dose groups. These changes were considered by the authors to be of questionable toxicological significance. The relative kidney weights were elevated at 20 mg/kg for both sexes, as were the relative weights of the brain in females and of the testes in males. In both sexes, an extensive histopathological examination revealed tubular epithelial hyperplasia at 2 and 20 mg/kg, but not at 0.2 mg/kg, and an increased incidence of renal tubular neoplasms at 20 mg/kg (see Table 14) (Kociba et al., 1977a,b).

8.7.3 Dermal exposure

In a study by Van Duuren et al. (1979), groups of 30 female Ha:ICR Swiss mice received 6.0 mg hexachlorobutadiene in acetone applied 3 times per week to the shaven dorsal skin for between 144 and 594 days. A group of 100 untreated females were included in the study, together with 30 controls which received acetone only. The study duration was described as being between 440 and 594 days. Sections of skin, liver, stomach and kidney were sampled at autopsy, but no increase in the number of distant tumours was observed.

In a two-stage initiation-promotion experiment, each of 20 female Swiss mice received one application of 15.0 mg hexachlorobutadiene in acetone to the dorsal skin. After 14 days, the

mice similarly received 5 μg of the tumour promoter 12-*o*-tetradecanoylphorbol-13-acetate (TPA) three times weekly for between 428 and 576 days. Hexachlorobutadiene administration did not induce a significant increase in the fraction of mice developing skin papillomas in this study (Van Duuren et al., 1979).

Table 14. Renal tubular neoplasms in rats after long-term exposure to hexachlorobutadiene[a]

Dose (mg/kg body weight per day)	Sex	Incidence of renal tubular neoplasms		
		adenoma	adenocarcinoma	total
0	males	1/90	0/90	1/90
0.2		0/40	0/40	0/40
2.0		0/40	0/40	0/40
20		2/39	7/39	9/39 ($P < 0.05$)
0	females	0/90	0/90	0/90
0.2		0/40	0/40	0/40
2.0		0/40	0/40	0/40
20		3/40	3/40[b]	6/40 ($P < 0.05$)

[a] From: Kociba et al. (1977a)
[b] One of these was an undifferentiated carcinoma

8.7.4 *Exposure by other routes*

In a study of repeated exposure to hexachlorobutadiene by ip injection, groups of 20 A/St strain male mice (from 6 to 8 weeks of age) received 12 or 13 ip injections of hexachlorobutadiene (4 or 8 mg/kg body weight) in tricaprylin, respectively. The purity of the hexachlorobutadiene was stated to exceed 99.9%. Urethane was used as a positive control for carcinogenesis, and a negative control group of 50 mice receiving tricaprylin only. Survival was 95% for mice receiving 4 mg/kg and 70% for mice receiving 8 mg/kg, compared to 92% for controls. Mice were sacrificed at 24 weeks after the first injection, and the number of surface adenomas in the lungs was counted. No significant increase in adenomas, compared to the vehicle-treated control, was observed (Theiss et al., 1977). The Task Group noted major deficiencies of this study; including the choice of a sensitive strain of mice, the

short duration of both the exposure period (4 weeks) and the follow-up period (24 weeks), the small group sizes of the experimental animals, the unusual route of administration, and the limited histopathology. The strain A mice used in this study are highly predisposed to spontaneous lung cancer, which is likely to have further compromised the value of the study.

8.8 Other special studies

8.8.1 Effects on the nervous system

Acute high exposure to hexachlorobutadiene has a depressant effect on the central nervous system (see sections 8.1.1.2 and 8.1.2).

Subchronic exposure of rats at high dose levels (1500 mg/kg diet for 13 weeks) also produced some signs of neurotoxicity, which was associated with demyelinization and fragmentation of femoral nerve fibres (Harleman & Seinén, 1979; see also Badaeva et al., 1985, section 8.5.2).

8.8.2 Effects on the liver

8.8.2.1 Acute effects

Hexachlorobutadiene causes hydropic changes in the liver of rats (Gradiski et al., 1975; Lock & Ishmael, 1981; Lock et al., 1982), mice (Lock et al., 1985), and rabbits (Duprat & Gradiski, 1978), sometimes accompanied by fat accumulation (Duprat & Gradiski, 1978; Lock & Ishmael, 1981; Lock et al., 1982).

Male rats, exposed to a single intraperitoneal dose of hexachlorobutadiene (200 or 300 mg/kg body weight) in corn oil showed increased relative liver weights, mitochondrial swelling in liver and bile duct, proliferation of smooth endoplasmic reticulum, lipid accumulation, and increased water content in the liver. Biochemical changes in the liver were a decrease, followed after 1 day by an increase, in non-protein sulfhydryl (NP-SH) concentration, and an increase in potassium content. All effects were reversible within 10 days. Increases in plasma urea and alkaline phosphatase (EC 3.1.3.1.) were also reported. In a separate experiment, the highest dose administered by ip injection which did not cause an increased water content in the liver was 25 mg/kg body weight (Lock et al., 1982).

Male rats exposed to single intraperitoneal doses up to 100 mg/kg body weight showed an increase in serum bile acids and bilirubin (Bai et al., 1992).

Male mice, exposed to single intraperitoneal doses of 50, 100 and 200 mg/kg body weight in corn oil, showed a dose-related increase in relative liver weight at 100 and 200 mg/kg, and, at all dose levels, dose-related, reversible changes in the liver (mitochondrial swelling, proliferation of smooth endoplasmic reticulum, and an increased water content). Reversible biochemical changes included increases in sodium and potassium content, NP-SH concentration in the liver, and serum alanine aminotransferase activity (EC 2.6.1.2) at 50 mg/kg (Lock et al., 1985).

8.8.2.2 Short-term effects

As discussed in section 8.2.2.1, slight hepatotoxic effects have been observed following oral exposure of rats (Kociba et al., 1971; Harleman & Seinen, 1979).

8.8.3 Effects on the kidneys

This section will describe the main features of the renal toxicity induced by hexachlorobutadiene. For more detail the reader is referred to the reviews of Rush et al. (1984), Lock (1988), Yang (1988) and Dekant et al. (1990a).

8.8.3.1 Acute effects

Inhalation exposure of rats produces renal tubular necrosis (Gage, 1970; see section 8.2.1). Enzyme histochemical investigations were performed on groups of 10 male Swiss OF_1 mice 24 h after whole-body inhalation exposure for 4 h to hexachlorobutadiene at measured concentrations of 29.3, 53.4, 106.7 or 266.8 mg/m^3. A concentration-related increase in the percentage of damaged kidney tubules, which had been stained for alkaline phosphatase (EC 3.1.3.1), was observed at all exposure levels. The EC_{50} was calculated to be 76.8 mg/m^3 (De Ceaurriz et al., 1988).

A single oral dose of hexachlorobutadiene (200 mg/kg body weight) in polyethylene glycol caused an increase in plasma urea concentration, a decrease in plasma alanine aminotransferase activity, and, in urine, increases in the levels of glucose, protein, alanine aminotransferase, *N*-acetyl-β-D-glucosaminidase,

γ-glutamyltranspeptidase (EC 2.3.2.2) and alanine aminopeptidase (EC 3.4.11.12) (Nash et al., 1984).

Following *in vivo* administration, hexachlorobutadiene caused dose-dependent necrosis of the renal proximal tubules in rats (Gradiski et al., 1975; Lock & Ishmael, 1979, 1981; Kluwe et al., 1982; Hook et al., 1982, 1983; Ishmael et al., 1982; Ishmael & Lock, 1986), mice (Ishmael et al., 1984) and rabbits (Duprat & Gradiski, 1978). In rats, the lesions were restricted to the pars recta (S_3-segment) and were macroscopically observed as a distinct band of damage in the outer stripe of the medulla (Lock & Ishmael, 1979; Ishmael et al., 1982). In mice and rabbits both the pars recta and the pars convoluta of the proximal convoluted tubules were damaged (Duprat & Gradiski, 1978; Ishmael et al., 1984). The lesion is characterized microscopically by necrotic epithelial cells, most of which are devoid of nuclei. The few remaining nuclei show karyorrhexis, and the cytoplasm is strongly eosinophilic. Many renal tubules contained cellular debris (Duprat & Gradiski, 1978; Lock & Ishmael, 1979; Lock et al., 1984; Ishmael et al., 1984). Vacuolation of the pars convoluta was observed (Duprat & Gradiski, 1978; Ishmael et al., 1982, 1984). Mitochondrial swelling and loss of brush-borders were prominent ultrastructural findings (Ishmael et al., 1982, 1984).

Adult male rats have been found to be less sensitive to the renal toxicity induced by hexachlorobutadiene than adult females and young males (Hook et al., 1983; Kuo & Hook, 1983). When male rats were dosed intraperitoneally with a single dose of 300 mg/kg in corn oil, the earliest pathological change was mitochondrial swelling in proximal tubular cells observed after 1-2 h. Extensive necrosis was evident between days 1 and 4, and active regeneration by day 5 (Ishmael et al., 1982). Similar renal toxicity was seen at a dose level of 25 or 50 mg/kg body weight in female rats and young males, respectively. A similar pattern of pathological changes with comparable intensity was observed in mice at an intraperitoneal dose of 50 mg/kg body weight (Ishmael et al., 1984). In a study by Lock et al. (1984), young mice were found to be more susceptible than adults, but no sex difference was apparent. In both rats and mice, differences in strain susceptibility were observed (Hook et al., 1983; Lock et al., 1984). The lowest intraperitoneal dose at which renal necrosis was observed in adult female rats was 25 mg/kg body weight (Lock & Ishmael, 1985) and in adult male and female mice was 6.3 mg/kg body weight (Lock et al., 1984).

Biochemical changes found following intraperitoneal exposure in both rats and mice were increases in renal water content (Kluwe et al., 1982; Ishmael et al., 1982, 1984; Gartland et al., 1989), plasma urea (Lock & Ishmael, 1979, 1981; Ishmael et al., 1982, 1984; Hook et al., 1983; Lock et al., 1984; Ishmael & Lock, 1986; Stonard et al., 1987; Gartland et al., 1989), plasma alkaline phosphatase (EC 3.1.3.1.) (Lock & Ishmael, 1981), serum alanine aminotransferase (EC 2.6.1.2) (Gradiski et al., 1975; Kuo & Hook, 1983) and serum aspartate aminotransferase (Gradiski et al., 1975; Davis et al., 1980). In the urine of rats, increases in urinary protein, glucose and ketones have been measured (Lock & Ishmael, 1979; Berndt & Mehendale, 1979; Davis et al., 1980; Stonard et al., 1987), as well as increases in the activities of alkaline phosphatase and *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.50) (Lock & Ishmael, 1979; Stonard et al., 1987) and in lactic acid level (Gartland et al., 1989). In the kidneys of rats, increased sodium concentrations were accompanied by equally decreased potassium concentrations (Davis et al., 1980). All these changes occurred at similar or higher intraperitoneal doses than those at which renal necrosis was observed.

Distinct renal functional changes in adult rats have been observed at intraperitoneal doses of 100–400 mg/kg body weight. These include a decrease in urine-concentrating ability (polyuria) (Lock & Ishmael, 1979; Berndt & Mehendale, 1979; Davis et al., 1980; Stonard et al., 1987), a reduced glomerular filtration rate (Davis et al., 1980) and a reduction of *in vivo* renal clearance of inulin, urea, *p*-aminohippuric acid (PAH), tetraethyl-ammonium bromide (TEA) (Lock & Ishmael, 1979) and imipramine (Davis et al., 1980). When organic ion transport was assessed *in vitro* in renal cortical slices of rats and mice that had been exposed to an intraperitoneal dose of 100 mg/kg, 200 mg/kg body weight or more, the transport of anions (PAH) was found to be reduced, but the transport of the cation (TEA), aminoisobutyrate was not (or was only slightly reduced) in rats (Lock & Ishmael, 1979; Berndt & Mehendale, 1979; Kluwe et al., 1982; Hook et al., 1982, 1983). In male adult mice, transport of PAH and TEA was reduced from intraperitoneal doses of 12.5 and 25.0 mg/kg body weight, respectively. The anion transport was reduced in adult females (Lock et al., 1984).

8.8.3.2 *Short- and long-term effects*

The short- and long-term effects of hexachlorobutadiene on the kidneys of experimental animals have already been discussed

in sections 8.2 , 8.5 and 8.7. Based on the studies of Kociba et al. (1971, 1977a,b), Schwetz et al. (1977), Harleman & Seinen (1979), Stott et al. (1981) and Yang et al. (1989), the oral NOAEL for renal toxicity is 0.2 mg/kg body weight per day. Results of the studies are summarized in Table 15. Female rats and mice were found to be distinctly more susceptible than males upon oral exposure for 13 weeks (Yang et al., 1989; Yang, 1991); this was also observed in the single-exposure mortality studies (section 8.1).

8.9 Factors modifying toxicity; toxicity of metabolites

8.9.1 Factors modifying toxicity

8.9.1.1 Surgery

Complete protection from the nephrotoxic effects of hexachlorobutadiene was observed in rats that had been fitted with a biliary cannula before being given a single oral dose of 200 mg/kg body weight. Administration of bile, collected from rats dosed orally with the compound, to naive rats produced marked renal toxicity but no liver toxicity (Nash et al., 1984).

8.9.1.2 Inhibitors and inducers of mixed-function oxidases (MFO)

In the majority of studies, the effects of MFO inhibitors (piperonyl butoxide, SKF 525A) and MFO inducers (Aroclor 1254, isosafrole, β-naphthoflavone, phenobarbitone) on the nephrotoxicity induced by hexachlorobutadiene in rats and mice were absent or negligible (Lock & Ishmael, 1981; Hook et al., 1982; Lock et al., 1984; Davis, 1984). Furthermore, phenobarbitone pretreatment for 7 days at 0.05% in drinking-water enhanced the renal toxicity induced by intraperitoneal doses of hexachlorobutadiene in weanling rats (Hook et al., 1983).

8.9.1.3 Inhibitors of γ-glutamyltranspeptidase (EC 2.3.2.2)

Male rats pretreated with Acivicin (L-(αS, 5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), an inhibitor of γ-glutamyltranspeptidase (down to 3% of control activity in this study), and subsequently exposed intraperitoneally to hexachlorobutadiene, did not show a decrease in nephrotoxicity compared to rats treated with hexachlorobutadiene alone. It was concluded that γ-glutamyltranspeptidase inhibition did not limit the formation of nephrotoxic metabolites (Davis, 1988).

Table 15. No-observed-adverse-effect level (NOAEL) calculated from short-term and long-term studies of exposure to hexachlorobutadiene by oral administration

Species (Strain)	Age	Sex	Number of animals per group	Duration of study	Dose (mg/kg body weight per day)	NOAEL (mg/kg body weight per day)	References
Rat (Wistar-derived)	weanling	male female	6 6	2 weeks	7.3, 18.2, 44.7	< 7.3	Harleman & Seinen (1979)
Rat (Sprague-Dawley)	adult	male	5	3 weeks	0.2, 20	0.2	Stott et al. (1981)
Rat (Sprague-Dawley)	adult	female	4	30 days	1, 3, 10, 30, 65, 100	3	Kociba et al. (1971)
Rat (Sprague-Dawley)	adult	male female	10-12 20-24	3 months	0.2, 2.0, 20	0.2	Schwetz et al. (1977)
Rat (Sprague-Dawley)	adult	male female	39-40 39-40	24 months	0.2, 2.0, 20	0.2	Kociba et al. (1977a,b)
Mouse ($B6C3F_1$)	adult	male female	5 5	2 weeks	4.3, 14.3, 43, 143, 430	< 4.3	Yang et al. (1989); Yang (1991)
Mouse ($B6C3F_1$)	adult	male female	10 10	13 weeks	0.1, 0.4, 1.5, 4.9, 18.8 0.2, 0.5, 1.8, 4.5, 19.2	1.5 < 0.2	Yang et al. (1989); Yang (1991)

Male Swiss OF1 mice, pretreated with Acivicin and subsequently exposed to a single oral dose of hexachlorobutadiene (80 mg/kg body weight), showed a decrease in nephrotoxicity compared to mice treated with hexachlorobutadiene alone, as measured by alkaline phosphatase staining (De Ceaurriz & Ban, 1990). The Task Group noted that only one marker for nephrotoxicity was employed in this study.

8.9.1.4 Inhibitors of cysteine conjugate β-lyase

Male Swiss OF1 mice, pretreated with the two β-lyase inhibitors amino-oxyacetic acid (AOAA) and DL-propargylglycine (PPG) and subsequently exposed to a single oral dose of hexachlorobutadiene (80 mg/kg body weight), showed a decrease in nephrotoxicity compared to mice treated with hexachlorobutadiene alone, as measured by alkaline phosphatase staining (De Ceaurriz & Ban, 1990). The Task Group again noted that only one marker for nephrotoxicity was employed in this study.

8.9.1.5 Inhibitors of organic anion transport

Pre-treatment of male rats with probenecid [(4-(dipropylamino)sulfonyl)] benzoic acid (105 μmol/kg body weight), an inhibitor of organic anion transport, did not alter the increase in plasma urea or decrease in renal clearance of *p*-aminohippuric acid induced by hexachlorobutadiene (Hook et al., 1982). However, in female rats, a higher dose (500 μmol/kg body weight) of probenecid totally protected against the renal toxicity, both functional and morphological, produced by ACPB (Lock & Ishmael, 1985). In addition this dose of probenecid protected female rats against the toxic effects produced by CPB and GPBN as well as the parent chemical (Lock & Ishmael, 1985). Male mice pre-treated with probenecid were also protected against the nephrotoxicity produced by hexachlorobutadiene (Ban & De Ceaurriz, 1988). The Task Group noted that this latter study used only one marker for nephrotoxicity.

8.9.1.6 Non-protein sulfhydryl scavengers

Depletion of hepatic and renal non-protein sulfhydryl content (glutathione) by diethylmaleate in rats appears to potentiate the nephrotoxicity of hexachlorobutadiene as measured by a number of functional markers such as plasma urea (Hook et al., 1982; Baggett & Berndt, 1984, Davis et al., 1986). However, the Task Group noted that no information was available on the metabolism of hexachlorobutadiene to help interpret these studies.

8.9.2 Toxicity of metabolites

This section discusses the renal toxicity of some metabolites of hexachlorobutadiene, the formation of which was discussed in section 6.2. These metabolites are 1-(glutathion-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (GPB), 1-(cystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (CPB), and 1-(*N*-acetylcystein-*S*-yl)-1,2,3,4,4-pentachloro-1,3-butadiene (ACPB). Their mutagenic activity was described along with that of hexachlorobutadiene in section 8.6.

8.9.2.1 In vitro *studies*

GPB decreased the viability of isolated renal epithelial cells of male rats, as measured by leakage of lactate dehydrogenase (EC 1.1.1.27), with a very steep dose-response curve and a lag period of 30 min. No cytotoxicity was observed when the GPB metabolism was blocked by anthglutin, an inhibitor of γ-glutamyl-transpeptidase (EC 2.3.2.2) or amino-oxyacetic acid (AOAA), an inhibitor of renal cysteine conjugate β-lyase (EC 4.4.1.13). The cytotoxicity of GPB was related to an impairment of mitochondrial function, as shown by loss of mitochondrial Ca^{2+} and ATP and inhibition of respiration and thiol depletion (Jones et al., 1986b). Likewise, GPB produced a concentration-dependent nephrotoxicity in the isolated perfused rat kidney, as indicated by the appearance in the urine of alkaline phosphatase, γ-glutamyl-transpeptidase and glucose (Jones et al., 1986a). These changes were prevented by Acivicin and by AOAA (Schrenk et al., 1988a).

In the study of Schrenk et al. (1988a), CPB also caused a marked nephrotoxicity in the isolated perfused kidney, which could be prevented by AOAA. In isolated rabbit renal tubules, CPB was observed to decrease the accumulation of *p*-amino-hippuric acid and tetraethylammonium (Jaffe et al., 1983), to affect mitochondrial function as shown by effects on cell respiration, and to decrease the glutathione content and, after a lag period of 60 min, cell viability (Schnellmann et al., 1987). The effects on respiration resulted initially from the uncoupling of oxidative phosphorylation, followed later by inhibition of state 3 respiration (Schnellmann et al., 1987). Impaired mitochondrial function was observed in CPB-exposed isolated rat renal cortical mitochondria as an inability to retain Ca^{2+}, collapse of the membrane potential, impaired state 3 respiration with succinate as substrate, and nonenzymatic depletion of thiol content. The latter effect was blocked by AOAA. From these results it was concluded

that the reactive intermediate formed from CPB interacts with the inner mitochondrial membrane (Wallin et al., 1987). CPB also inhibited rat kidney mitochondrial DNA, RNA and protein synthesis, and AOAA blocked this effect. Moreover, CPB converted supercoiled DNA to relaxed circular DNA and shorter linear fragments (Banki & Anders, 1989). Chen et al. (1990) observed a decreased viability of isolated human renal proximal tubular cells upon exposure to CPB, which was again blocked by AOAA. Using radiolabelled ACPB and rat renal cortical slices, it was established that ACPB is transported by the same renal mechanisms involved in the movement of many organic anions into tubular fluid. This carrier-mediated transport is reduced by specific inhibitors like probenecid and sulfinpyrazone, a competitive and metabolic inhibitor like 2,4-dinitrophenol, and the transport substrate *p*-aminohippuric acid (Lock et al., 1986). This was confirmed by recent studies on the mechanism of uptake of GPB and CPB in the isolated perfused rat kidney (Schrenk et al., 1988b). Probenecid has also been reported to protect renal proximal tubular cells against ACPB-induced cytotoxicity, as determined by monitoring proline incorporation into renal proteins (Bach et al., 1986).

8.9.2.2 In vivo *studies*

A single oral dose of 138 mg/kg body weight (0.27 mmol) of GPB or a single equimolar oral dose of 100 mg ACPB/kg body weight in polyethylene glycol to male rats caused marked nephrotoxicity similar in both biochemical and histopathological aspects to that observed with an oral dose of 200 mg/kg body weight (0.97 mmol) of hexachlorobutadiene (Nash et al., 1984). When rats received intraperitoneally GPB, CPB or ACPB in polyethylene glycol at single doses between 6.25 and 100 mg/kg body weight, increases in plasma urea level and renal proximal tubular necrosis were observed at dose levels of ≥ 6.25 mg/kg body weight in females and 10 or 12.5 mg/kg body weight in males. The conjugates exhibited a similar pattern of nephrotoxicity at equimolar doses and were more nephrotoxic than the parent compound (Lock & Ishmael, 1985; Ishmael & Lock, 1986). All compounds tested were more toxic to female rats than males (Ishmael & Lock, 1986). Probenecid pretreatment protected the rats against the nephrotoxicity of these metabolites. Probenecid was shown to block the active tubular secretion of ACPB and to reduce the extent of covalent binding to renal protein (Lock & Ishmael, 1985). In mice, GPB and ACPB were also shown to be more toxic than the parent compound: renal necrosis was found

following single intraperitoneal doses of 5.0 mg hexachlorobutadiene/kg body weight, 3.1 mg GPB/kg body weight and 3.0 mg ACPB/kg body weight in corn oil, which were the lowest doses tested (Lock et al., 1984). A single intraperitoneal dose of 10 mg CPB/kg body weight in DMSO and water caused dose-related damage in the pars recta of renal proximal tubules in male mice (Jaffe et al., 1983).

The nephrotoxicity of some structural analogues of the above-mentioned conjugates, e.g. *S*-(1,2-dichlorovinyl)-L-cysteine, has been investigated extensively and has revealed a remarkable similarity (Anders et al., 1987; Lock, 1988).

8.10 Mechanisms of toxicity - mode of action

8.10.1 Mechanisms of toxicity

The following evidence supports the hypothesis that the nephrotoxicity, mutagenicity and carcinogenicity of hexachlorobutadiene is dependent on the biosynthesis of the toxic sulfur conjugate GPB. This conjugate is mainly synthetized in the liver and further metabolized in the bile, gut, and kidneys to the CPB. Cysteine conjugate β-lyase-dependent activation of CPB to a reactive thioketene in the proximal tubular cells finally results in covalent binding to cellular macromolecules.

1. The nephrotoxicity of hexachlorobutadiene in rats was prevented by the implantation of a biliary cannula; administration of bile from hexachlorobutadiene-treated rats to naive rats resulted in nephrotoxicity identical to the nephrotoxicity caused by hexachlorobutadiene (see section 8.9.1.1).

2. Inhibitors of renal organic anion transport protected rats against the nephrotoxicity of hexachlorobutadiene and its sulfur conjugates. Inhibition of the organic anion transport also protected isolated kidney cells against the nephrotoxicity of hexachlorobutadiene-derived sulfur-conjugates (see sections 8.9.1.3 and 8.9.2.1).

3. Anthglutin, Acivicin and aminooxyacetic acid, specific inhibitors of γ-glutamyltranspeptidase and cysteine conjugate β-lyase protected against the cytotoxicity of hexachlorobutadiene-derived sulfur-conjugates in freshly isolated rat renal proximal tubular cells (see section 8.9.2.1).

4. Synthetic sulfur-conjugates of hexachlorobutadiene show a higher nephrotoxicity than the parent compounds in rats and mice and produce renal damage identical to the renal damage induced by hexachlorobutadiene, based on clinical chemistry and histopathological examination (see section 8.9.2.2).

5. Hexachlorobutadiene and its sulfur-conjugates are genotoxic in bacteria; bioactivation by glutathione conjugation is required for hexachlorobutadiene genotoxicity. The ultimate mutagen is formed by cysteine conjugate β-lyase-dependent cleavage of CPB (see section 8.6).

6. Hexachlorobutadiene induces renal tumours in rats only at doses that produce marked nephrotoxicity (see sections 8.7 and 8.8.2.2).

8.10.2 Mode of action

The *in vitro* studies of Jones et al. (1986b), Wallin et al. (1987) and Schnellmann et al. (1987) on the cytotoxicity of sulfur-conjugates to renal tubular cells (section 8.9.1.2) point to renal cortical mitochondria as the major target for sulfur-conjugates of hexachlorobutadiene, analogous to that established for close structural analogues (Dekant et al., 1990b). The hypothesis proposes an interaction of the reactive metabolite with the inner mitochondrial membrane, which ultimately causes respiratory insufficiency.

9. EFFECTS ON HUMANS

9.1 General population exposure

Hexachlorobutadiene has been found in postmortem examinations, but not in living persons. No pathogenic effects have been recorded (see section 5.2).

9.2 Occupational exposure

Two reports on certain disorders among agricultural workers in vineyards where hexachlorobutadiene has been used as a fumigant (Krasniuk et al., 1969; Burkatskaya et al., 1982) cannot be evaluated, since such workers are known to be occupationally exposed to additional substances.

In two cytogenetic studies of occupationally exposed workers from the same plant engaged in the production of hexachlorobutadiene, an increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes was observed (German, 1986). The workers were exposed to hexachlorobutadiene concentrations that ranged from 1.6 to 16.9 mg/m^3. The Task Group noted that exposure concentrations were determined by the factory and that the frequency of chromosome aberrations was not associated with the period of employment.

9.3 *In vitro* metabolism studies

The following studies have been reported:

a) Purified human liver microsomal glutathione-*S*-transferase and human liver cytosol metabolize hexachlorobutadiene to form GPB (McLellan et al., 1989; Oesch & Wolf, 1989).

b) The enzyme cysteine conjugate β-lyase has been isolated and purified from human kidney cytosol (Lash et al., 1990). The activity of the human cytosolic enzymes with a structurally related compound (1,2,2-trichlorovinyl-L-cysteine) is about 10-fold lower than that of rat renal cytosol (Green et al., 1990).

c) Studies in isolated human proximal tubular cells have shown that CPB causes a β-lyase-dependant cytotoxicity (Chen et al., 1990).

These limited studies suggest that humans have the ability to metabolize hexachlorobutadiene to toxic metabolites.

9.4 Extrapolation of NOAEL from animals to humans

Conversion of equivalent doses across species can utilize allometric relationships that relate physiological and anatomical variables across species. Physiological and metabolic rates have been shown to relate closely to body weight to the power 0.75 (Boxenbaum, 1982). The equivalent NOAEL in humans (mg/kg body weight per day) can be determined from the following equation:

$$d_h = d_a \left(\frac{w_a}{w_h}\right)^{0.25}$$

where

d = dose rate (mg/kg body weight per day) in humans (d_h) or animals (d_a)
w_a = weight (kg) of animals (mice 30 g; rats 400 g)
w_h = weight of humans (70 kg)

The NOAEL for humans, based on the NOAEL in mice, is:

$$d_h = (0.2\ mg/kg\ body\ weight\ per\ day) \left(\frac{0.03}{70}\right)^{0.25}$$

= 0.03 mg/kg body weight per day

10. EVALUATION OF HUMAN HEALTH RISKS AND EFFECTS ON THE ENVIRONMENT

10.1 Evaluation of human health risks

10.1.1 Hazard identification

The following evaluation is based on toxicity studies in experimental animals; however, there are some human *in vitro* data which indicate that hexachlorobutadiene metabolism can occur by a similar route to that shown in experimental animals.

Hexachlorobutadiene is slightly to moderately toxic based on acute oral experiments with adult rats, and moderately to highly toxic based on acute oral experiments with weanling rats (based on the WHO pesticide toxicity classification). The specific toxicity of the compound to the kidneys is higher for females than males. Following acute dermal exposure of rabbits, the compound was found to be weakly toxic.

Regardless of species studied and the route of exposure (ip, oral, inhalation, dermal) in both short- and long-term studies, the target organ for toxicity is the kidney. Bioactivation to produce a reactive sulfur metabolite occurs following conjugation with glutathione. The monoglutathione conjugate of hexachlorobutadiene is processed to the cysteine-*S*-conjugate, which is then a substrate for renal cysteine conjugate β-lyase. Hexachlorobutadiene produces a dose-dependent necrosis of the renal proximal tubules, which is followed by regenerative and/or proliferative changes. On the basis of both short- and long-term studies in rats and mice orally exposed to hexachlorobutadiene, the no-observed-adverse-effect level (NOAEL) is 0.2 mg/kg body weight per day. In one short-term inhalation study (12 days, 6 h/day), the NOAEL was 53 mg/m^3.

The vapour of hexachlorobutadiene was found to be irritating to the eyes and nose of rats in one short-term inhalation study. The undiluted compound appeared corrosive in an experiment with rabbits. Based on these limited data, the vapour should be regarded as irritating to human mucous membranes and the liquid should be regarded as corrosive.

In a well-conducted Magnusson-Kligman test hexachlorobutadiene was a sensitizing agent both with and without adjuvant.

Therefore, the compound should be regarded as a sensitizing agent for humans.

In reproductive studies, reduced birth weight and neonatal weight gain in rats were observed, but these effects may be attributed to maternal toxicity. Developmental toxicity to rat fetuses was observed in two teratogenicity tests, but again only at levels that were also toxic to the dams. This developmental toxicity included reduced birth weight, a 1- to 2-day delay in heart development, and dilated ureters, but no gross abnormalities were observed.

In vitro studies have shown that hexachlorobutadiene and, to a much greater extent, its sulfur metabolites induce mutations in *Salmonella typhimurium*. In one study of exposure to hexachlorobutadiene by inhalation or oral administration, an increased frequency of chromosomal aberrations was observed in mouse bone marrow cells. There is limited evidence for the genotoxicity of hexachlorobutadiene in animals, and insufficient evidence in humans.

The long-term oral administration of hexachlorobutadiene to rats induced an increased frequency of renal tubular neoplasms, but only at doses that caused marked nephrotoxicity; at the lowest dose, no adverse effects were observed.

The Task Group concluded that there is limited evidence for the carcinogenicity of hexachlorobutadiene in animals (one study in one rodent strain) and insufficient evidence in humans.

10.1.2 Exposure

Hexachlorobutadiene is mainly a waste product. As such, it can be encountered in different environmental compartments, but predominantly in sediment and biota (see also 10.2.1). Exposure of the general public therefore mainly occurs indirectly via drinking-water and food of high lipid content. Assuming a maximum concentration of 2.5 μg/litre in contaminated drinking-water and 10 μg/kg wet weight in contaminated fatty food items (meat, fish, milk) and daily intakes of 2 litres drinking-water, 0.3 kg meat, 0.2 kg fish and 0.5 kg milk, a maximum total daily intake of 0.2 μg/kg body weight can be calculated for a 70-kg person.

10.1.3 Hazard evaluation

The NOAEL for mice or rats exposed to hexachlorobutadiene is 0.2 mg/kg body weight per day (see Table 15), from which a NOAEL of 0.03-0.05 mg/kg body weight day has been derived for humans (see section 9.4).

The Task Group considered the margin of safety of 150 between the estimated NOAEL in humans and the maximum total daily intake (see section 10.1.2) to be sufficient to protect the general population against the adverse effects of hexachlorobutadiene.

10.2 Evaluation of effects on the environment

10.2.1 Hazard identification

Hexachlorobutadiene is a chemically stable compound. Complete aerobic biodegradation has been observed following adaptation of the inoculum. Partial biodegradation was found to occur in a pilot sewage treatment plant. Based on these observations and the chemical structure, it can be concluded that hexachlorobutadiene is not readily biodegradable, but can be considered to be inherently biodegradable. Experimental photolysis of hexachlorobutadiene in the presence of a surface was rapid, but in the absence of a surface the compound is believed to be persistent. Degradation in the atmosphere is assumed to occur by a rather slow reaction with hydroxyl radicals. A half-life of up to 2.3 years has been calculated.

Once hexachlorobutadiene is released into the environment, intercompartmental transport will occur chiefly by volatilization from water and soil, adsorption to particulate matter in water and air, and subsequent sedimentation or deposition. In view of a strong adsorption potential to organic matter, the compound accumulates in sediment and will not migrate rapidly in soils. Both field and laboratory exposure data support these conclusions.

Field and laboratory data also support the high bioaccumulation potential in aquatic and benthic organisms which can be expected on the basis of the lipophilic nature of the compound. However, no evidence has been obtained for biomagnification.

Hexachlorobutadiene is moderately to highly toxic to aquatic organisms; crustaceans and fish are the most sensitive species. The

lowest E(L)C_{50} for freshwater organisms is 0.09 mg/litre (goldfish). The lowest chronic NOEC is 3 μg/litre (goldfish). Applying the preliminary effect assessment extrapolation procedure, as adopted in the OECD Workshop on Aquatic Effect Assessment (OECD, 1990), an Environmental Concern Level of 0.1 μg/litre can be established.

The toxicity data on terrestrial organisms are insufficient to establish any toxicity threshold.

10.2.2 Exposure

Current environmental levels in surface waters are generally below 0.2 μg/litre, rising to 1.3 μg/litre in highly polluted rivers. Levels in the upper sediment can be as high as 120 μg/kg in heavily polluted rivers or estuaries. In older sediment layers much higher concentrations can be measured. The concentrations in freshwater biota measured since 1980 generally do not exceed 100 μg/kg fresh weight, but in a polluted area can reach 120 mg/kg in the lipid of fish.

10.2.3 Hazard evaluation

It can be concluded that away from point sources the maximum predicted environmental concentration (PEC) is twice the extrapolated Environmental Concern Level of 0.1 μg/litre. Aquatic organisms therefore may be at risk in polluted surface waters.

In view of the rather high concentrations of the compound measured in some sediments, adverse effects on benthic organisms cannot be excluded.

Considering the toxicity of the substance to mammals (the NOAEL for rats or mice is 0.2 mg/kg body weight per day) and its high bioaccumulating potential, the consumption of benthic or aquatic organisms in polluted surface water by other species may give cause for concern. For example, an otter weighing 10 kg and consuming 1 kg fish per day in waters containing 0.2 μg hexachlorobutadiene/litre could ingest 1200 μg/day (assuming a bioconcentration factor for fish of 6000, leading to a concentration of 1200 μg/kg wet weight) or 120 μg/kg body weight per day, which is above the calculated NOAEL value for the otter (calculated as in section 9.4).

11. FURTHER RESEARCH

Hexachlorobutadiene is primarily a waste product and hence an environmental contaminant having only limited use as a fumigant in some parts of the world. The Task Group identified the following areas for which additional information is needed:

a) the degradation of hexachlorobutadiene in the environment focusing on photodegradation and biodegradation;

b) the terrestrial toxicity of hexachlorobutadiene including tests on benthic organisms;

c) the genotoxic activity of hexachlorobutadiene *in vivo*. A further test for micronucleus or chromosome aberration induction in mouse bone marrow cells would strengthen the available data;

d) the metabolism of hexachlorobutadiene and its glutathione-derived conjugates by human liver and renal enzymes and inter-individual variability.

12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

The carcinogenic risk of hexachlorobutadiene was evaluated by the International Agency for Research on Cancer in 1979 (IARC, 1979).

The summary of data reported and the evaluation of the IARC monograph on hexachlorobutadiene is reproduced here.

Experimental data

Hexachlorobutadiene was tested in one experiment in rats by oral administration: it produced benign and malignant tumours in the kidneys of animals of both sexes. It was tested inadequately in one experiment in mice by intraperitoneal injection.

Human data

No case reports of epidemiological studies were available to the Working Group.

The occurrence of hexachlorobutadiene as a by-product in the production of various chlorinated hydrocarbons for over 50 years and its use in some areas as a pesticide indicate that widespread human exposure in both the occupational and general environment occurs. This is confirmed by reports of its occurrence in the environment.

Evaluation

There is limited evidence that hexachlorobutadiene is carcinogenic in rats.

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RESUME

1. Identité, propriétés physiques et chimiques, méthodes d'analyse

L'hexachlorobutadiène est un liquide ininflammable, incombustible, limpide et huileux à la température et la pression ordinaires. Il est peu soluble dans l'eau mais miscible à l'éther et à l'éthanol.

On peut le mettre en évidence et le doser par chromatographie en phase gazeuse. Les limites de détection sont de 0,03 $\mu g/m^3$ dans l'air, 0,001 μg/litre dans l'eau, de 0,7 μg/kg de matière humide dans le sol ou les sédiments et de 0,02 μg/litre dans le sang. Dans les tissus, cette limite est de 0,47 μg/kg de tissus frais.

2. Sources d'exposition humaine et environnementale

L'hexachlorobutadiène n'existe pas à l'état naturel. C'est essentiellement un sous-produit de la fabrication des hydrocarbures chlorés que l'on retrouve dans les fractions lourdes. La production annuelle mondiale (dans les fractions lourdes) a été estimée à 10 000 tonnes en 1982. L'hexachlorobutadiène peut être utilisé pour la récupération des gaz contenant du chlore dans les ateliers de fabrication du chlore et comme liquide de lavage pour éliminer du courant gazeux certains composés organiques volatils. On l'utilise également dans les gyroscopes, comme fluide caloporteur, dans les transformateurs, comme liquide isolant ou liquide hydraulique ainsi que comme solvant des élastomères, comme intermédiaire et comme fumigant.

3. Transport, distribution et transformation dans l'environnement

Les principales voies de pénétration dans l'environnement sont les émissions résultant des déchets et les utilisations qui entraînent la dispersion du produit. Le transport inter-compartimental s'effectue principalement par volatilisation, adsorption sur les matières particulaires puis dépôt ou sédimentation. L'hexachlorobutadiène ne migre pas facilement dans le sol et s'accumule dans les sédiments. Dans l'eau, on le considère comme persistant, sauf turbulences importantes. Il n'y a pas d'hydrolyse. Le produit semble être facilement biodégradable par voie aérobie, encore que le phénomène n'ait pas été étudié à fond. L'hexachlorobutadiène

présent sur les surfaces subit une photolyse. Outre le dépôt, on estime que la réaction de l'hexachlorobutadiène avec les radicaux hydroxyles constitue un mode de piégeage important de ce composé dans la troposphère, la demi-vie atmosphérique estimative de l'hexachlorobutadiène pouvant aller jusqu'à 2,3 ans. Le produit a un potentiel élevé de bioaccumulation, comme l'ont confirmé les observations en laboratoire et sur le terrain. Ainsi, on a trouvé des facteurs de bioconcentration à l'état stationnaire (obtenus expérimentalement par rapport au poids de tissus frais) respectivement égaux en moyenne à 5800 et 17 000 chez la truite arc-en-ciel. On n'a pas observé d'amplification biologique au laboratoire ou sur le terrain.

4. Niveaux dans l'environnement et exposition humaine

Le dosage de l'hexachlorobutadiène dans l'air des villes a donné dans tous les cas des valeurs inférieures à 0,5 $\mu g/m^3$. Dans les régions écartées, les concentrations sont inférieures à 1 pg/m^3. Dans les lacs et les cours d'eau d'Europe, on a enregistré des concentrations pouvant aller jusqu'à 2 μg/litre mais les valeurs moyennes sont généralement inférieures à 100 ng/litre. Dans la région des grands lacs au Canada, on a obtenu des valeurs beaucoup plus faibles (autour de 1 ng/litre). En revanche la teneur des sédiments du fond peut, dans cette zone, atteindre 120 μg/kg de poids sec. Les couches sédimentaires plus anciennes, remontant aux environs de 1960, présentaient des teneurs plus élevées (jusqu'à 550 μg/kg de matière humide). On a montré que la concentration dans les sediments augmentait avec la granulométrie des particules.

A en juger par la concentration de l'hexachlorobutadiène dans les organismes aquatiques, les oiseaux et les mammifères, le composé s'accumule mais ne subit pas d'amplification biologique. Dans les eaux polluées, on a relevé des concentrations dépassant 1000 μg/kg de tissus frais chez plusieurs espèces et même 120 mg/kg (par rapport aux lipides) chez une espèce. Les concentrations actuelles restent généralement inférieures à 1000 μg/kg de poids frais à distance des points de décharge industrielle.

On a décelé la présence du composé dans l'urine, le sang et les tissus humains. Dans certaines denrées alimentaires ayant une fraction lipidique importante, on en a relevé jusqu'à 40 μg/kg et dans un cas, plus de 1000 μg/kg.

D'après une étude, le niveau d'exposition pourrait atteindre 1,6 à 12,2 mg/m^3 et les concentrations urinaires, 20 mg/litre.

5. Cinétique et métabolisme

Après administration par voie orale, l'hexachlorobutadiène est rapidement absorbé chez l'animal de laboratoire mais le taux de résorption après inhalation ou exposition par voie cutanée n'a pas été étudié. Chez le rat et la souris, le composé se répartit principalement dans le foie, les reins et les tissus adipeux. Il est rapidement excrété. On a mis en évidence une fixation aux protéines et aux acides nucléiques dans le foie et les reins.

La biotransformation du composé chez l'animal de laboratoire se révèle être un processus saturable. Elle s'effectue principalement par l'intermédiaire du glutathion, l'hexachlorobutadiène étant d'abord transformé en conjugué du *S*-glutathion. La métabolisation de ce conjugué se poursuit ensuite, en particulier au niveau de la membrane constituant la bordure en brosse des cellules des tubules rénaux, pour donner un métabolite sulfuré réactif qui est probablement responsable des effets néphrotoxiques, génotoxiques et cancérogènes observés.

6. Effets sur les êtres vivants dans leur milieu naturel

L'hexachlorobutadiène est modérément à très toxique pour les organismes aquatiques. Certaines espèces de poissons et de crustacés se sont révélées être les plus sensibles, les valeurs de la CL_{50} à 96 h. allant de 0,032 à 1,2 et de 0,09 à environ 1,7 mg/litre, respectivement pour les crustacés et les poissons. Chez les poissons, le rein est organe-cible important.

On a établi la valeur de la dose sans effets observables à 0,003 mg/litre, à partir des résultats d'un certain nombre d'épreuves à long terme sur certaines espèces d'algues et de poissons; cela permet de considérer ce composé comme très toxique pour les organismes aquatiques. Parmi les points d'aboutissement biologiques étudiés figuraient la toxicité générale, la neurotoxicité, la biochimie, l'hématologie, l'anatomopathologie et la reproduction. Lors d'une étude de 28 jours portant sur les premiers stades de la vie de *Pimephales promelas*, une espèce de vairon, on a observé que la reproduction n'était pas affectée à des concentrations allant jusqu'à 0,017 mg/litre, alors qu'à 0,013 et 0,017 mg/litre il y avait accroissement de la mortalité et réduction du poids du corps. La dose sans effets observables était de 0,0065 mg/litre.

On n'a décrit qu'une seule épreuve fiable portant sur des organismes terrestres. Lors d'une épreuve de 90 jours sur des cailles japonaises qui recevaient une alimentation contenant ce composé à des concentrations allant de 0,3 à 30 mg/kg de nourriture, on a constaté que la survie des oisillons n'était réduite qu'à partir de 10 mg/kg de nourriture.

7. Effets sur les animaux de laboratoire et les systèmes d'épreuves *in vitro*

7.1 Toxicité générale

L'hexachlorobutadiène est légèrement à modérément toxique pour le rat adulte, modérément toxique pour le raton juste sevré et extrêmement toxique pour les rattes juste sevrées après administration d'une seule dose par voie buccale. Les principaux organes-cibles sont le rein et dans une bien moindre mesure, le foie.

D'après les données obtenues sur l'animal d'expérience, les vapeurs d'hexachlorobutadiène sont irritantes pour les muqueuses et le liquide est corrosif. On peut considérer ce composé comme un agent sensibilisateur.

Chez le rat, la souris et le lapin, l'hexachlorobutadiène provoque une nécrose, liée à la dose, des tubules proximaux du rein. Les rats mâles adultes sont moins sensibles à la néphrotoxicité que les femelles ou les jeunes mâles. Les souriceaux sont plus sensibles que les souris adultes sans qu'on puisse observer de différences entre les deux sexes. Chez la ratte adulte, la dose intrapéritonéale unique la plus faible à laquelle on ait observé une nécrose rénale était de 25 mg/kg de poids corporel; elle était de 6,3 mg/kg de poids corporel chez les souris adultes mâles et femelles. A des doses égales ou supérieures à celles qui entraînaient une nécrose, on a observé des modifications biochimiques et une nette amélioration de la fonction rénale.

Lors de six épreuves à court terme où le composé a été administré par la voie orale, deux études de reproduction et une étude d'alimentation à long terme portant sur des rats, c'est également le rein qui s'est révélé être l'organe-cible. Parmi les effets liés à la dose, on notait une diminution du poids relatif des reins et une dégénérescence de l'épithélium des tubules. La dose sans effets nocifs observables au niveau des reins, tirée d'une étude de deux ans sur le rat, était de 0,2 mg/kg de poids corporel

et par jour. Une étude de 13 semaines sur des souris a montré que cette dose était de 0,2 mg/kg de poids corporel et par jour pour cet animal. Chez les deux espèces, les femelles adultes étaient plus sensibles que les mâles adultes.

Lors d'une étude d'inhalation à court terme (six heures par jour pendant 12 jours) on a observé des effets analogues au niveau des reins avec une concentration nominale de vapeur d'hexachlorobutadiène égale à 267 mg/m^3; cette concentration a également entraîné des difficultés respiratoires, ainsi qu'une dégénérescence des corticosurrénales.

7.2 Reproduction, embryotoxicité et teratogétogénicité

Deux études d'alimentation portant sur la reproduction ont été effectuée sur des rats à des doses quotidiennes allant jusqu'à 20 et 75 mg/kg de poids corporel respectivement; elles ont fait ressortir une réduction du poids de naissance et du gain de poids néonatal aux doses toxiques pour la mère. La dose quotidienne de 75 mg/kg de poids corporel, qui était hautement toxique, s'est révélée suffisante pour empêcher la conception et la nidation intra-utérine. On n'a pas observé d'anomalies du squelette.

Lors de deux études de tératogénicité, des rats ont été exposés soit à des vapeurs d'hexachlorobutadiène à des concentrations allant de 21 à 160 mg/m^3, six heures par jour du sixième au vingtième jour de la gestation, soit par voie intrapéritonéale à une dose quotidienne de 10 mg/kg de poids corporel (du premier au quinzième jour de la gestation). Des effets nocifs ont été notés sur le développement des foetus, qui consistaient en une réduction du poids de naissance, un retard dans le développement cardiaque, une dilatation des uretères, mais pas de malformations macroscopiques. Le retard de développement a été observé à des doses qui étaient également toxiques pour les mères.

7.3 Génotoxicité et cancérogénicité

L'hexachlorobutadiène provoque des mutations géniques dans l'épreuve d'Ames sur salmonelle dans des conditions particulières qui favorisent la formation de produits de conjugaison avec le glutathion. Lors d'une étude *in vivo* on a observé des aberrations chromosomiques qui n'ont en revanche pas été constatées lors de deux autres études *in vitro*. Une étude *in vitro* portant sur des cellules ovariennes de hamster chinois a révélé une augmentation de la fréquence des échanges entre chromatides soeurs. On a fait

état de la très forte mutagénicité des métabolites sulfurés de l'hexachlorobutadiène. D'autres études *in vitro* ont montré que ce composé provoquait une synthèse non programmée de l'ADN dans des cultures de fibroblastes embryonnaires de hamsters de Syrie, effets qui n'étaient pas observés dans des cultures d'hépatocytes de rats. Le composé provoque également une synthèse non programmée de l'ADN *in vivo* mais n'induit pas de mutations létales récessives liées au sexe chez *Drosophila melanogaster*.

Lors de la seule étude à long terme (deux ans) qui ait été effectuée, des rats ont reçu une alimentation contenant de l'hexachlorobutadiène à des doses quotidiennes respectives de 0,2, 2 ou 20 mg/kg de poids corporel et seule la dose la plus élevée a provoqué un accroissement de l'incidence des tumeurs malignes au niveau des tubules rénaux.

7.4 Mécanismes de la toxicité

La néphrotoxicité, la mutagénicité et la cancérogénicité de l'hexachlorobutadiène sont liées à la biosynthèse d'un conjugué sulfuré toxique, le 1-glutathion-*S*-yl-1,2,3,4,4-pentachlorobutadiène. Ce conjugué est principalement synthétisé dans le foie et métabolisé ensuite dans la bile, l'intestin et les reins en 1-cystéine-*S*-yl-1,2,3,4,4-pentachlorobutadiène (CPB). L'activation du CPB en thiocétène réactif (qui dépend de la cystéine-conjuguée-béta lyase) au niveau des cellules des tubules proximaux, aboutit en définitive à la formation de liaisons covalentes avec les macromolécules cellulaires.

8. Effets sur l'homme

On n'a pas décrit d'effets pathogènes sur la population dans son ensemble.

On possède deux rapports faisant état de troubles chez des ouvriers agricoles qui utilisaient de l'hexachlorobutadiène comme fumigant mais il est vrai qu'ils avaient également été exposés à d'autres substances. On a également observé un accroissement de la fréquence des aberrations chromosomiques dans les lymphocytes du sang périphériques de travailleurs employés à la production d'hexachlorobutadiène et qui avaient été exposés à des concentrations de 1,6 à 12,2 mg/m^3.

9. Evaluation des risques pour la santé humaine et des effets sur l'environnement

9.1 Evaluation des risques pour la santé humaine

Comme très peu d'études ont été effectuées sur l'homme, l'évaluation repose essentiellement sur les animaux de laboratoire. Toutefois les données *in vitro* limitées dont on dispose au sujet de l'homme incitent à penser que le métabolisme de l'hexachlorobutadiène est analogue chez l'homme et l'animal.

On estime que les vapeurs d'hexachlorobutadiène sont irritantes pour les muqueuses et que le liquide est corrosif. Ce composé doit également être considéré comme un agent sensibilisateur.

Les principaux organes-cibles de son action toxique sont les reins et dans une mesure bien moindre, le foie. Sur la base des études à court et à long terme effectuées sur des rats et des souris, la dose quotidienne sans effets nocifs observables est évaluée à 0,2 mg/kg de poids corporel. On l'a estimée à 53 mg/m^3 lors d'une étude d'inhalation à court terme chez le rat (12 jours, six heures par jour).

L'action toxique sur le développement, de même que la réduction du poids de naissance et du gain de poids néonatal n'ont été observés qu'à des doses toxiques pour la mère.

On a observé que l'hexachlorobutadiène produisait des mutations géniques, des aberrations chromosomiques, un accroissement des échanges entre chromatides soeurs et une synthèse non programmée de l'ADN, encore que certaines études aient donné des résultats négatifs. On ne possède que des indices limités en faveur d'une génotoxicité de l'hexachlorobutadiène chez l'animal, indices qui sont insuffisants en ce qui concerne l'homme.

On a constaté que l'administration d'hexachlorobutadiène par voie orale pendant une longue période à des rats accroissait la fréquence des tumeurs malignes au niveau des tubules rénaux, mais il s'agissait uniquement de doses élevées fortement néphrotoxiques. En ce qui concerne la cancérogénicité de cette substance, les indices sont limités chez l'animal et insuffisants chez l'homme.

En se basant sur la dose quotidienne sans effets nocifs observables estimée à 0,2 mg/kg de poids corporel chez la souris ou le rat, on a fixé à 0,03-0,05 mg/kg de poids corporel la dose quotidienne sans effets nocifs observables chez l'homme. La marge de sécurité entre la dose estimative sans effets nocifs observables et la dose journalière maximale totale ingérée estimée en se basant sur une absorption du composé par l'intermédiaire d'une eau de boisson et de produits alimentaires contaminés à forte teneur en lipides, est égale à 150.

9.2 *Evaluation des effets sur l'environnement*

L'hexachlorobutadiène est modérément à fortement toxique pour les organismes aquatiques: les crustacés et les poissons sont les espèces les plus sensibles. On a fixé à 0,1 µg/litre la concentration écologiquement préoccupante. On estime que la concentration maximale prévisible dans l'environnement à distance des sources ponctuelles de pollution est égale à deux fois la dose écologiquement préoccupante extrapolée et, par voie de conséquence, que les organismes aquatiques peuvent être menacés dans les eaux de surface polluées. On ne peut exclure des effets nocifs sur le benthos.

Compte tenu de la toxicité de l'hexachlorobutadiène pour les mammifères, la consommation de benthos ou d'organismes aquatiques par d'autres espèces pourrait être préoccupante.

RESUMEN

1. Identidad, propiedades físicas y químicas, métodos de análisis

El hexaclorobutadieno es un líquido no inflamable, incombustible, claro, oleoso e incoloro a temperatura y presión ordinarias. Es poco soluble en el agua, pero miscible con éter y etanol.

La sustancia puede detectarse y determinarse cuantitativamente por métodos de cromatografía de gases. Los límites de detección son de 0,03 $\mu g/m^3$ de aire, 0,001 μg/litro de agua, 0,7 μg/kg de peso húmedo en el suelo o en sedimentos y de 0,02 μg/litro de sangre. Se ha determinado un nivel de 0,47 μg/kg de peso húmedo de tejido.

2. Fuentes de exposición humana y ambiental

No hay indicaciones de que el hexaclorobutadieno exista como producto natural. Es principalmente un subproducto de la fabricación de hidrocarburos clorados y se presenta en las fracciones pesadas (como residuo). La producción anual mundial del compuesto en las fracciones pesadas en 1982 se estimó en 10 000 toneladas.

El hexaclorobutadieno puede utilizarse para recuperar gas que contiene cloro en plantas productoras de cloro y como líquido de lavado para eliminar ciertos compuestos orgánicos volátiles de las corrientes de gases. También se ha utilizado como fluido en giróscopos, como transmisor de calor, transformador, fluido aislante y fluido hidráulico, disolvente para elastómeros y como intermediario y sustancia para fumigar.

3. Transporte, distribución y transformación en el medio ambiente

Las principales vías de ingreso en el medio ambiente son las emisiones de residuos y el uso dispersivo. El paso de un entorno a otro ocurre principalmente por volatilización, adsorción a corpúsculos de materia y subsiguiente deposición o sedimentación. El hexaclorabutadieno no migra rápidamente en el suelo y se acumula en el sedimento. Se considera persistente en el agua a menos que haya mucha turbulencia. No produce hidrólisis. La sustancia parece ser fácilmente biodegradable aeróbicamente,

aunque su biodegradabilidad no se ha investigado a fondo. El hexaclorobutadieno se fotoliza en las superficies. Se supone que, además de la deposición, la reacción con radicales hidroxilo es un importante sumidero de hexaclorobutadieno en la troposfera y su semivida atmosférica estimada es de hasta 2,3 años. La sustancia tiene un elevado potencial de bioacumulación, que se ha comprobado mediante observaciones en laboratorio y sobre el terreno. En la trucha arco iris se han determinado experimentalmente factores de bioconcentración en estado estacionario de 5800 y 17 000 como promedio, sobre la base del peso húmedo. No se ha observado biomagnificación en laboratorio ni sobre el terreno.

4. Niveles ambientales y exposición humana

Se ha determinado la presencia de hexaclorubutadieno en el aire urbano; en todos los casos, los niveles eran inferiores a 0,5 $\mu g/m^3$. Las concentraciones en lugares aislados son inferiores a 7 pg/m^3. En las aguas de lagos y ríos de Europa se han registrado concentraciones de hasta 2 μg/litro, pero los niveles medios son generalmente inferiores a 100 ng/litro. En la región de los Grandes Lagos del Canadá se han detectado niveles muy inferiores (de aproximadamente 1 ng/litro). Allí los niveles en el sedimento del fondo pueden ser de 120 μg/kg de peso en seco. En capas más antiguas de sedimento, de 1960 aproximadamente, se encontraron concentraciones más elevadas (de hasta 550 μg/kg de peso húmedo). Se ha demostrado que la concentración en el sedimento aumenta con el tamaño de la partícula de sedimento.

Las concentraciones de hexaclorobutadieno en organismos acuáticos, aves y mamíferos indican bioacumulación pero no biomagnificación. En las aguas contaminadas se han detectado niveles de más de 1000 μg/kg de peso húmedo en varias especies y de 120 mg/kg (base grasa) en una especie. Lejos de los efluentes industriales, los niveles actuales se mantienen en general por debajo de 100 μg/kg de peso húmedo.

Se ha detectado la presencia del compuesto en la orina, en la sangre y en tejidos humanos. En ciertos alimentos que contienen una elevada fracción lipídica se han encontrado hasta unos 40 μg/kg y, en un caso, más de 1000 μg/kg.

Un estudio señala exposiciones ocupacionales de 1,6-12,2 mg/m^3 y en la orina niveles de hasta 20 mg/litro.

5. Cinética y metabolismo

Se ha observado que los animales de laboratorio absorben rápidamente el hexaclorobutadieno después de la administración oral, pero no se ha investigado la velocidad de absorción después de la inhalación o de la exposición dérmica. En ratas y ratones, el compuesto se distribuye principalmente al hígado, a los riñones y al tejido adiposo. Se excreta rápidamente. Se ha demostrado que se fija a las proteínas y ácidos nucleicos del hígado y de los riñones.

La biotransformación del compuesto en animales de experimentación parece ser un proceso saturable. Se produce principalmente a través de una vía mediada por el glutatión, en la cual el hexaclorobutadieno se convierte inicialmente en conjugados de *S*-glutatión. Estos conjugados pueden seguir metabolizándose, especialmente en el ribete en cepillo de las membranas de las células de los tubos renales, produciendo un metabolito sulfuroso reactivo que probablemente explique la nefrotoxicidad, genotoxicidad y carcinogenicidad observadas.

6. Efectos en organismos presentes en el medio ambiente

El hexaclorobutadieno es de moderadamente a muy tóxico para los organismos acuáticos. Los más sensibles que se hayan observado han sido especies de peces y crustáceos; los valores de la CL_{50} en 96 horas oscilan entre 0,032 y 1,2 mg/litro en crustáceos y entre 0,09 y 1,7 mg/litro en peces. Se ha demostrado que el riñón es un órgano muy afectado en los peces.

Sobre la base de varias pruebas a largo plazo con especies de algas y de peces, se ha establecido un nivel sin efectos observados de 0,003 mg/litro; así pues, el compuesto se clasifica como muy tóxico para las especies acuáticas. Los valores extremos investigados comprenden parámetros de toxicidad general, neurotoxicidad, bioquímicos, hematológicos, patológicos y relacionados con la reproducción. En una prueba de 28 días de duración en la que se examinaron las primeras fases de la vida de carpas se observó que la reproducción no se veía afectada con concentraciones de hasta 0,017 mg/litro, mientras que con concentraciones de 0,013 y 0,017 mg/litro se observaron un aumento de la mortalidad y una disminución del peso corporal. El nivel sin efectos observados era de 0,0065 mg por litro.

Se ha descrito una sola prueba fiable con organismos terrestres. En una prueba de 90 días con codornices japonesas alimentadas con una dieta que contenía el compuesto en concentraciones de 0,3 a 30 mg/kg de dieta se observó que la supervivencia de los polluelos disminuía a partir de 10 mg/kg de dieta.

7. Efectos en animales de experimentación y en sistemas de prueba *in vitro*

7.1 Toxicidad general

Después de la ingestión de una dosis oral única, el hexaclorobutadieno es de levemente a moderadamente tóxico para las ratas adultas, moderadamente tóxico para las ratas macho destetadas y muy tóxico para las ratas hembras destetadas. Los principales órganos afectados son el riñón y, en grado mucho menor, el hígado.

Los datos obtenidos con animales indican que el vapor de hexaclorobutadieno es irritante para las membranas mucosas y el líquido es corrosivo. La sustancia debe considerarse como un agente sensibilizador.

En los riñones de ratas, ratones y conejos, el hexaclorobutadieno causa en los tubos proximales del riñón una necrosis que depende de la dosis. Las ratas macho adultas son menos vulnerables a la toxicidad renal que las hembras adultas y que los machos jóvenes. Los ratones jóvenes son más vulnerables que los adultos y no se observaron diferencias entre un sexo y otro. En las ratas hembra adultas la dosis intraperitoneal única más baja con la cual se observó necrosis renal fue de 25 mg/kg de peso corporal y en ratones adultos, machos y hembras, fue de 6,3 mg/kg de peso corporal. Se observaron cambios bioquímicos y alteraciones funcionales marcados en los riñones con dosis iguales o mayores que las asociadas con necrosis.

Asimismo, en seis pruebas orales de corto plazo, dos estudios sobre reproducción y un estudio de largo plazo sobre la dieta realizados con ratas, el riñón fue el principal órgano afectado. Los efectos relacionados con la dosis comprenden una reducción del peso relativo del riñón y una degeneración del epitelio de los tubos. El nivel sin efectos nocivos observados de toxicidad renal en ratas en un estudio de dos años fue de 0,2 mg/kg de peso corporal por día. En un estudio de 13 semanas efectuado en ratones se obtuvo un nivel sin efectos nocivos observados de

0,2 mg/kg de peso corporal por día. Las hembras adultas de ambas especies eran más vulnerables que los machos adultos.

En una prueba de inhalación de corto plazo (6 horas por día durante 12 días) se observaron efectos semejantes en los riñones con una concentración de vapor nominal de 267 mg/m^3, con la cual también se observaron trastornos respiratorios y degeneración de la corteza suprarrenal.

7.2 *Reproducción, embriotoxicidad y teratogenicidad*

Dos estudios sobre dieta y reproducción en ratas con dosis de hasta 20 y 75 mg/kg de peso corporal por dia, respectivamente, mostraron una reducción del peso al nacer y un aumento del peso neonatal cuando se administraban a la madre dosis tóxicas de 20 y 7,5 mg/kg de peso corporal, respectivamente. La dosis altamente tóxica de 75 mg/kg de peso corporal por día fue suficiente para impedir la concepción y la implantación uterina. No se observaron anormalidades del esqueleto.

En dos pruebas de teratogenicidad en las que se expuso a las ratas o bien a vapor de hexaclorobutadieno en concentraciones que oscilaban entre 21 y 160 mg/m^3 durante 6 horas diarias (desde el 6° hasta el 20° día del embarazo) o bien a la administración intraperitoneal de 10 mg/kg de peso corporal por día (desde el 1° al 15° día de embarazo) se observaron en el desarrollo del feto efectos tóxicos tales como una reducción del peso al nacer, un retraso del desarrollo del corazón y uréteres dilatados pero sin grandes malformaciones. El retraso del desarrollo se observó en niveles que también eran tóxicos para las madres.

7.3 *Genotoxicidad y carcinogenicidad*

En la prueba de Ames Salmonella se ha observado que el hexaclorobutadieno induce mutaciones genéticas en condiciones especiales que favorecen la formación de productos de conjugación con el glutatión. En un estudio *in vivo* se observó que había inducido aberraciones cromosómicas, pero no se observaron tales aberraciones en dos estudios *in vitro*. En una prueba *in vitro* se observó que la frecuencia de los intercambios entre cromátidas hermanas había aumentado en las células ováricas de hámsters de China. Se ha señalado el gran potencial mutagénico de los metabolitos sulfurosos del hexaclorobutadieno. En estudios *in vitro*, el compuesto indujo síntesis imprevistas de ADN en cultivos de fibroblastos de embriones de hámsters de Siria, pero no en

cultivos de hepatocitos. Indujo síntesis imprevistas de ADN en ratas *in vivo*, pero no indujo mutaciones letales recesivas ligadas al sexo en *Drosophila melanogaster*.

En el único estudio de largo plazo (dos años), en el cual las ratas recibieron una dieta que contenía hexaclorobutadieno en dosis de 0,2, 2 ó 20 mg/kg de peso corporal por día, se observó una mayor incidencia de neoplasias de los tubos renales únicamente con la dosis más elevada.

7.4 Mecanismos de toxicidad

La nefrotoxicidad, mutagenicidad y carcinogenicidad del hexaclorobutadieno depende de la biosíntesis del conjugado sulfuroso tóxico 1-glutatión-*S*-yl-1,2,3,4,4-pentaclorobutadieno. Este conjugado se sintetiza principalmente en el hígado y se metaboliza luego en la bilis, el intestino y los riñones convirtiéndose en 1-cisteína-*S*-yl-1,2,3,4,4-pentaclorobutadieno (CPB). La activación de CPB, que depende del conjugado de cisteína beta-lyasa, en una tiocetena reactiva en las células de los tubos proximales finalmente da lugar a un enlace covalente con macromoléculas celulares.

8. Efectos en el ser humano

No se han descrito efectos patogénicos en la población en general.

Se conocen dos casos de trastornos padecidos por trabajadores agrícolas que utilizaban el hexaclorobutadieno como fumigante, pero esas personas también habían estado expuestas a otras sustancias. En los linfocitos de la sangre periférica de operarios que trabajaban en la producción de hexaclorobutadieno y estaban expuestos, según se informa, a concentraciones de 1,6 a 12,2 mg/m^3 se observó una frecuencia mayor de aberraciones cromosómicas.

9. Evaluación de los riesgos para la salud humana y de los efectos en el medio ambiente

9.1 Evaluación de los riesgos para la salud humana

Como se han hecho muy pocos estudios en el ser humano, la evaluación se basa principalmente en estudios efectuados en animales de laboratorio. Sin embargo, los limitados datos

existentes sobre el ser humano, obtenidos *in vitro*, sugieren que el metabolismo del hexaclorobutadieno en el ser humano es semejante al observado en animales.

Se considera que el vapor de hexaclorobutadieno irrita las membranas mucosas del ser humano y que en estado líquido es corrosivo. El compuesto también debe considerarse como un agente sensibilizador.

Los principales órganos afectados por la toxicidad son los riñones y, en mucho menor grado, el hígado. En base a estudios de corto y largo plazo de ingestión por vía oral por ratas y ratones, se determinó un nivel sin efectos adversos observados de 0,2 mg/kg de peso corporal por día. En un estudio de inhalación en el corto plazo en ratas (12 días, a razón de 6 horas por día), el nivel sin efectos adversos observados fue de 53 mg/m^3.

Se observaron una reducción del peso al nacer y un aumento de peso neonatal únicamente con dosis tóxicas para la madre; lo mismo puede decirse de los efectos tóxicos para el desarrollo.

Se ha observado que el hexaclorobutadieno induce mutaciones genéticas, aberraciones cromosómicas, aumento de los intercambios entre cromátidas hermanas y síntesis imprevistas de ADN, aunque algunos estudios han dado resultados negativos. Con respecto a la genotoxicidad del hexaclorubutadieno, las observaciones realizadas en animales son limitadas y las efectuadas en el ser humano insuficientes.

Tras la administración oral a largo plazo del hexaclorobutadieno a ratas, se ha observado una mayor frecuencia de neoplasias de los tubos renales, pero solamente con dosis elevadas causantes de nefrotoxicidad notable. Hay indicios limitados de carcinogenicidad en animales e indicios insuficientes en el ser humano.

En base al nivel sin efectos adversos observados en ratones y ratas, que es de 0,2 mg/kg de peso corporal por día, se ha estimado un nivel sin efectos adversos observados en el ser humano, que es de 0,03 a 0,05 mg/kg de peso corporal por día. Hay un margen de seguridad de 150 entre el nivel sin efectos adversos observados estimado y la ingesta diaria total máxima estimada, suponiendo que el compuesto se absorba a través del agua de bebida contaminada y de alimentos con elevado contenido de lípidos.

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9.2 Evaluación de los efectos en el medio ambiente

El hexaclorobutadieno es de moderadamente a muy tóxico para los organismos acuáticos; los crustáceos y peces son los más vulnerables. Se ha establecido un nivel de riesgo para el medio ambiente de 0,1 μg/litro. Se estima que la concentración ambiental prevista máxima lejos de las fuentes equivale al nivel de riesgo ambiental extrapolado multiplicado por dos y, por consiguiente, los organismos acuáticos tal vez estén en peligro en las aguas de superficie contaminadas. No pueden excluirse efectos adversos en organismos bentónicos.

En vista de la toxicidad del hexaclorobutadieno para los mamíferos, el consumo de organismos bentónicos o acuáticos por otras especies tal vez sea motivo de inquietud.

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