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**Rotterdam Convention on the Prior Informed  
Consent Procedure for Certain Hazardous  
Chemicals and Pesticides in International Trade  
Chemical Review Committee**

Fifth meeting

Rome, 23–27 March 2009

Item 4 (b) (viii) of the provisional agenda\*

**Listing of chemicals in Annex III of the Rotterdam Convention:**

**Review of notifications of final regulatory actions to  
ban or severely restrict a chemical: hexachlorobutadiene**

## **Hexachlorobutadiene**

### **Note by the Secretariat**

#### **Addendum**

#### **Supporting documentation provided by Japan**

The Secretariat has the honour to provide, in the annex to the present note, documentation received from Japan to support its notification of final regulatory action on hexachlorobutadiene.

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\* UNEP/FAO/RC/CRC.5/1.  
K0842873 101208

## **Annex**

### **Hexachlorobutadiene: Focused Summary.**

**Biodegradation and bio-accumulation data of existing chemicals (by the  
Chemicals Evaluation and Research Institute, Japan: CERJ)**

**<http://qsar.cerij.or.jp/cgi->**

**[bin/DEGACC/result\\_head.cgi?STRID=01401&LANG=ENG](http://qsar.cerij.or.jp/cgi-bin/DEGACC/result_head.cgi?STRID=01401&LANG=ENG)**

**IPCS/EHC-156**

**<http://www.inchem.org/documents/ehc/ehc/ehc156.htm>**

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The Japanese DNA for the PIC Convention would like to submit the following information on our notification of final regulatory action on Hexachlorobutadiene.

## **Focused Summary Hexachlorobutadiene**

### **1. Introduction**

a) The events that led to the regulatory action

The government of Japan designated Hexachlorobutadiene as Class I Specified Chemical Substances under the Chemical Substances Control Law.

b) Significance of regulatory action, eg one use or many uses, level or degree of exposure

Since Hexachlorobutadiene was classified as Class I Specified Chemical Substance in April 2005, manufacture, import and use of Hexachlorobutadiene have been banned.

c) An overview of the regulatory system of the notifying country if relevant

The government of Japan anticipates that persistent and highly bio-accumulative chemical substances with long-term toxicity (e.g. PCBs) may cause irreversible environmental pollution and have adverse effects on human health or the environment. In order to prevent environmental pollution and adverse effects on human health, the Chemical Substances Control Law stipulates that hazardous properties of chemicals should be checked based on the existing knowledge or by the tests which are consistent with the methods of the OECD Test Guidelines, conducted by the OECD GLP facilities. The government designate the chemical substances that are identified as not-biodegradable (persistent) and bio-accumulative as Type I Monitoring Chemical Substances. Manufacturers and Importers of Type I Monitoring Chemical Substances are required to report the quantities they manufactured and imported. If a Type I Monitoring Chemical Substance is identified as having long-term toxicity for human beings and animals at the top of food chain (higher predators), the government designates that chemical substances as Class I Specified Chemical Substances and are subject to final regulatory action (ban on manufacture, import, and use). The Ministry of Health, Labour and Welfare, the Ministry of Economy, Trade and Industry and the Ministry of the Environment are responsible for these regulations of chemical substances which are persistent, highly bio-accumulative and toxic for long time.

d) Scope of the regulatory action – precise description of the chemicals subject to the regulatory action

Aforementioned in b), it has been banned that manufacture, import and use of Hexachlorobutadiene.

## 2. Risk Evaluation

### a) Key findings of the national risk evaluation

Hexachlorobutadiene is placed in the category of Class I Specified Chemical Substances based on the following judgment.

1. Biodegradation: Based on the report of the biodegradation test with microorganisms, the percentage of biodegradation is 6, 32, 33% and 5, 11, 7%, measured by BOD and by GC, respectively. Thereafter, Hexachlorobutadiene is considered to be very persistent.
2. Bioaccumulation: Based on the report that BCF for fish exposed is 6,280 (high exposure level case) and 7,720 (low exposure level case), Hexachlorobutadiene is considered to be very bio-accumulative.
3. Long-term toxicity: Histological renal abnormalities were observed in rats at 2 mg/kg/day of Hexachlorobutadiene and higher in an approximately two-year study. When they were administered at 20 mg/kg/day, renal tubular adenoma and adenocarcinoma were observed. A reproductive toxicity study on rats suggests that Hexachlorobutadiene decreases the weights of pups at 7.5 mg/kg/day and higher. The same study also indicates that the chemical decreases the conception rate and inhibits implantation at 75 mg/kg/day.

### b) Key data reviews consulted and a brief description

Biodegradation and Bioaccumulation data

[http://www.safe.nite.go.jp/data/hazkizon/pk\\_e\\_kizon\\_disp.html?k\\_no=1637](http://www.safe.nite.go.jp/data/hazkizon/pk_e_kizon_disp.html?k_no=1637)

### c) Reference to national studies, eg toxicological and ecotoxicity studies

No information.

### d) Summary of actual (potential) human exposure and/or environmental fate

Hexachlorobuta-1,3-diene in water and sediment was measured in 1981, but it was not detected in any point. (limit of detection; water...0.02 ug/l, sediment...0.002~2 ug/g-dry)

## 3. Risk Reduction and Relevance to Other States

### a) Estimates of the quantity of chemicals used, or imported/exported at the time of the regulatory action and if possible information on ongoing trade

It was reported that there were neither manufacturing nor import in 2002.

### b) Relevance to other States i.e. those with similar conditions of use

No information.

### c) Comments on the typical use of the chemical within the notifying country, with comments on possible misuse (if appropriate)

No information.

# Biodegradation and Bioconcentration of Existing Chemical Substances under the Chemical Substances Control Law

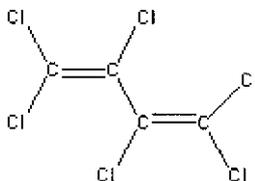
Description Biodegradability Bioconcentration

Information on the chemical published in the Official Bulletin of Economy, Trade and Industry (Former title: The Official Bulletin of the Ministry of International Trade and Industry (published before Jan.6,2001))

| Published Chemical Name  | Published Date | Published result  |
|--|----------------|---|
| perchloro(buta-1,3-diene)<br>[Class-Reference No. : 2-121]<br>[Cas Registry No. : 87-68-3] | 2002/11/8      | Chemical substance<br>determined to be persistent                               |
| perchloro(buta-1,3-diene)<br>[Class-Reference No. : 2-121]<br>[Cas Registry No. : 87-68-3] | 2003/10/14     | Chemical substance<br>determined to be persistent<br>and highly bioaccumulative |

## Chemical Information

### Structural formula



|                                       |  |
|---------------------------------------|--|
| CAS Registry No.                      | 87-68-3  |
| Name of Chemical targeted for testing | hexachlorobutadiene [synonym:hexachloro-1,3-butadiene] |

| Class-Reference No. in The Gazetted List | Existing Chemical Substances Name in The Gazetted List |
|--|--|
| 2-121                                    | Hexachlorobutadiene                                    |

## Biodegradation

|             |                      |
|-------------|----------------------|
| Judgement   | Non-biodegradability |
| Test Method | MITI-I(OECD TG 301C) |

| Test Equipment | Test period | Chemical Concentration | Concentration of Activated |
|----------------|-------------|------------------------|----------------------------|
|                |             |                        |                            |

|  |        |         |               |
|--|--------|---------|---------------|
|  |        |         | <b>Sludge</b> |
| Improved type for a volatile substance | 4weeks | 100mg/L | 30mg/L        |

|                          |            |
|--------------------------|------------|
| <b>Indirect Analysis</b> | <b>BOD</b> |
|                          | 6, 32, 33% |

|                        |           |
|------------------------|-----------|
| <b>Direct Analysis</b> | <b>GC</b> |
|                        | 5, 11, 7% |

**Remarks**

- Adsorption of the test substance on sludge was observed.

**Bioconcentration**

|                    |                       |
|--------------------|-----------------------|
| <b>Judgement</b>   | High bioconcentration |
| <b>Test Method</b> | Bioconcentration test |

|                   |                            |
|-------------------|----------------------------|
| <b>LC50(96hr)</b> | <b>Species</b>             |
| 0.18mg/L          | Rice fish(Oryzias latipes) |

| <b>Test Equipment</b>                  | <b>Test period</b> | <b>Species</b>        | <b>Lipid Content (%)</b>    |                           |
|--|--------------------|-----------------------|-----------------------------|---------------------------|
|  |                    |                       | <b>The start of testing</b> | <b>The end of testing</b> |
| Improved type for a volatile substance | 60days             | Carp(Cyprinus carpio) | 6.2                         | 5.1                       |

|                               | <b>Test Concentration Set</b> | <b>BCF<sub>SS</sub></b> |
|-------------------------------|-------------------------------|-------------------------|
| <b>1st Concentration area</b> | 1microg/L                     | 6280                    |
| <b>2nd Concentration area</b> | 0.1microg/L                   | 7720                    |

Total Search System  
for Chemical Substances  
87-68-3

Return

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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

**ENVIRONMENTAL HEALTH CRITERIA 156**

**HEXACHLOROBUTADIENE**

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organisation, or the World Health Organization.

First draft prepared by Dr. T. Vermeire,  
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Published under the joint sponsorship of  
the United Nations Environment Programme,  
the International Labour Organisation,  
and the World Health Organization

World Health Organization  
Geneva, 1994

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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### WHO TASK GROUP MEETING ON ENVIRONMENTAL HEALTH CRITERIA FOR HEXACHLOROBUTADIENE

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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. 97991111).

\* \* \*

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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. Valic 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. Valic 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<br>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- <i>o</i> -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 µg/m<sup>3</sup> 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  $0.5 \mu\text{g}/\text{m}^3$ . Concentrations in remote areas are less than  $1 \text{ pg}/\text{m}^3$ . In lake and river water in Europe concentrations of up to  $2 \mu\text{g}/\text{litre}$  have been recorded, but mean levels are usually below  $100 \text{ ng}/\text{litre}$ . In the Great Lakes area of Canada, much lower levels (around  $1 \text{ ng}/\text{litre}$ ) were measured. Bottom sediment levels here can be as high as  $120 \mu\text{g}/\text{kg}$  dry weight. Older sediment layers from around 1960 contained higher concentrations (up to  $550 \mu\text{g}/\text{kg}$  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  $1000 \mu\text{g}/\text{kg}$  wet weight have been measured in several species and  $120 \text{ mg}/\text{kg}$  (lipid base) in one species. Present levels generally remain below  $100 \mu\text{g}/\text{kg}$  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  $40 \mu\text{g}/\text{kg}$  and, in one case, over  $1000 \mu\text{g}/\text{kg}$ .

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

### 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<sub>50</sub> 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.

a Based on several long-term tests with algae and fish species, 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<sup>3</sup>, 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<sup>3</sup> 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*.

a In the only long-term (2 years) study, in which rats received 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  $\beta$ -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<sup>3</sup>.

#### 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<sup>3</sup>.

only  
Reduced birth weight and neonatal weight gain was observed  
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.

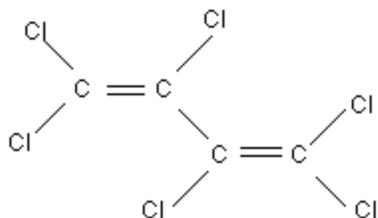
Considering the toxicity of hexachlorobutadiene to mammals, consumption of benthic or aquatic organisms by other species may cause concern.

## 2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

### 2.1 Identity

Chemical formula: C<sub>4</sub>Cl<sub>6</sub>

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<sup>3</sup> (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<sup>a</sup>

|                |                   |
|----------------|-------------------|
| 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 <sup>b</sup>      |
| Log <i>n</i> -octanol-water partition coefficient ( <i>K<sub>ow</sub></i> ) | 4.78 <sup>b</sup> , 4.90 <sup>c</sup>   |
| Density   | 1.68 g/cm <sup>3</sup> at 20 °C         |
| Relative vapour density   | 9.0                                     |
| Vapour pressure   | 20 Pa (0.15 mmHg) at 20 °C <sup>d</sup> |
| Autoignition temperature  | 610 °C                                  |

<sup>a</sup> Unless otherwise stated, the data are selected from secondary sources.

<sup>b</sup> Experimentally derived by Banerjee *et al.* (1980)

<sup>c</sup> Experimentally derived by Chiou (1985)

<sup>d</sup> McConnell *et al.* (1975)

### 2.3 Conversion factors

1 ppm = 10.67 mg/m<sup>3</sup> air at 25 °C and 101.3 kPa (760 mmHg)  
 1 mg/m<sup>3</sup> 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            | gas chromatography              |                    |
| 360 litre            | developed for personal              | Mann <i>et al.</i>              |                    |
| sampling in industry | 101; extraction by hexane (1974)    | with electron capture detection |                    |
| Air                  | adsorption on Amberlite             | gas chromatography              | 10                 |
| µg/m <sup>3</sup>    | 3 litre                             | suitable for personal           | Boyd <i>et al.</i> |
| and area monitoring; | XAD-2; extraction by (1981); Dillon | with electron capture           |                    |

|  |   |   |                 |
|--|---|---|-----------------|
| validation range                           | hexane<br>(1979)  | detection   |                 |
| 10-2000 µg/m <sup>3</sup>                  |   |   |                 |
| Air<br>µg/m <sup>3</sup>                   | adsorption on Tenax-GC;<br>2 litre                                    | gas chromatography<br>suitable for                            | 11<br>Melcher & |
| continuous area                            | purging of water vapour,<br>Caldecourt                                | with flame ionization   |                 |
| monitoring                                 | oxygen, etc., by nitrogen;<br>(1980)                                  | detection   |                 |
|  | desorption by heating   |   |                 |
| Air<br>0.03 µg/m <sup>3</sup> <sup>a</sup> | adsorption on Tenax-GC;<br>desorption by heating<br>(1982); Pellizari | gas chromatography<br>developed for the<br>(capillary column) | Krost et al.    |
| analysis of ambient<br>air                 | under a helium flow;<br>(1982); Barkley                               | with mass   |                 |
| et al. (1980)                              | cryofocussing   | spectro-metric  |                 |
|  |   | detection   |                 |
| Water<br>0.05 µg/litre                     | extraction by hexane;<br>16 litre                                     | gas chromatography<br>developed for the                       | Oliver &        |
| Nicol                                      | concentration; drying with<br>(1982)                                  | (capillary column)  |                 |
| analysis of surface<br>water               | Na <sub>2</sub> SO <sub>4</sub> ; clean-up by silica                  | with electron   |                 |
|  | gel chromatography  | capture detection   |                 |

Table 2 (contd).

| Medium                                | Sampling method                                   | Analytical method |                                    |
|---------------------------------------|---|-------------------|------------------------------------|
| Detection limit                       | Sample size                                       | Comments          | Reference                          |
| Water<br>0.0014 µg/litre              | extraction by<br>0.8-1 litre                      | US EPA Method     | gas chromatography<br>Lopez-Avila  |
| 8120                                  | dichloro-methane-acetone;<br>et al. (1989)        |                   | with electron capture              |
|                                       | drying; concentration<br>by N <sub>2</sub> stream |                   | detection                          |
| Water<br>0.001 µg/litre<br>(1984)     | extraction by<br>12 litre                         | developed for     | gas chromatography<br>Zogorski     |
| monitoring of<br>domestic and process | dichloro-methane;<br>drying; concentration        |                   | with electron capture<br>detection |

waters

|                       |                            |                       |                |
|-----------------------|----------------------------|-----------------------|----------------|
| Water                 | extraction by              | gas chromatography    |                |
| 0.34 µg/litre         | 1 litre                    | US EPA Method 612;    | US EPA         |
| (1984a)               |                            |                       |                |
|                       | dichloro-methane;          | with electron capture |                |
| developed for the     | drying; concentration      | detection             |                |
| analysis of municipal | and exchange to            |                       |                |
| and industrial        | hexane; clean-up by        |                       |                |
| discharges            | fluorisisil chromatography |                       |                |
| Water                 | extraction by              | gas chromatography    | 0.9            |
| µg/litre              | 1 litre                    | US EPA Method 625;    | US EPA (1984b) |
|                       | dichloro-methane at pH     |                       |                |
| developed for the     | >11, then at pH <2;        |                       |                |
| analysis of municipal | drying; concentration      |                       |                |
| and industrial        |                            |                       |                |
| discharges            |                            |                       |                |
| Water                 | purging by helium;         | gas chromatography    | 0.4            |
| µg/litre              | 0.1 litre                  | developed for the     | Otson & Chan   |
|                       | trapping; desorption by    | (capillary column)    |                |
| analysis of volatile  | (1987);                    |                       |                |
| organics in waters    | heating                    | with mass             |                |
|                       | Eichelberger               | spectro-metric        |                |
| et al. (1990)         |                            | detection             |                |

Table 2 (contd).

| Medium            | Sampling method         | Analytical method     |           |
|-------------------|-------------------------|-----------------------|-----------|
| Detection limit   | Sample size             | Comments              | Reference |
| Soil,             | extraction by           | gas chromatography    | 0.7       |
| µg/kg             |                         | Laseter et            |           |
| sediment          | acetone-benzene         | with electron capture | wet       |
| weight            |                         | al. (1976)            |           |
|                   |                         | detection             |           |
| Soil              | add water; adjust to pH | gas chromatography    |           |
| developed for     | Kiang & Grob            | (capillary column)    |           |
| screening of soil | >12; extraction by      |                       |           |
|                   | (1986)                  |                       |           |

|  |   |   |                             |
|--|---|---|-----------------------------|
| for priority pollutants  | dichloromethane; centrifugation; drying; concentration  | with flame ionization and mass spectro-metric detection   |                             |
| Sediment developed for screening of sediment for priority pollutants | add water; adjust to pH<br>Lopez-Avila et al. (1983)<br>≥ 11; extraction by dichloromethane; centrifugation; drying; concentration; clean-up by silica gel chromatography | gas chromatography (capillary column) with flame/electron capture/mass spectro-metric detection |                             |
| Sediment µg/kg <sup>a</sup> weight                                   | extraction by 10-15 g dry 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<br>Oliver & Nicol (1982) |

Table 2 (contd).

| Medium                          | Sampling method   | Analytical method  |                                  |
|---------------------------------|---|--|----------------------------------|
| Detection limit                 | Sample size   | Comments   | Reference                        |
| Biota µg/kg<br>analysis of fish | homogenization; filtration; separation; extraction by al. (1976)<br>hexane; clean-up by fluorisil chromatography                  | gas chromatography method applied to with electron capture detection | 0.7<br>Laseter et                |
| Biota 0.005 mg/kg et<br>weight  | grind and mix edible 25 g (eggs) wet weight<br>tissue; extraction; 50 g (fish) wet weight<br>clean-up by fluorisil chromatography | gas chromatography with electron capture detection                   | Yurawecz<br>wet al. (1976)<br>or |
| 0.04 mg/kg fat                  | 3 g (milk fat)<br>100 g (vegetables)  |  |                                  |

wet weight

|   |   |   |          |
|---|---|---|----------|
| Biota<br>µg/kg <sup>a</sup><br>Nicol  | grinding with Na <sub>2</sub> SO <sub>4</sub> ;<br>15 g   | gas chromatography<br>method applied to<br>Oliver &   | 0.47     |
| analysis of fish  | extraction by<br>(1982)<br>hexane-acetone;<br>back-extraction of acetone<br>by water; concentration;<br>clean-up by silica<br>gel chromatography                              | (capillary column)<br>with electron capture<br>detection  |          |
| Biota<br>µg/kg<br>weight <sup>a</sup><br>chlorinated<br>hydrocarbon residues<br>in human adipose<br>tissue and human milk | extraction by<br>2 g<br>benzene-acetone;<br>filtration; concentration;<br>1986)<br>redissolution in hexane;<br>clean-up including<br>fluorisil-silicic<br>acid chromatography | gas chromatography<br>method applied to<br>Mes et al.<br>with electron capture<br>detection<br>analysis of<br>(1982; 1985;<br>1986) | 1<br>wet |

Table 2 (contd).

| Medium                              | Sampling method   | Analytical method   |           |
|-------------------------------------|---|---|-----------|
| Detection limit                     | Sample size   | Comments  | Reference |
| Biota<br>0.0182 µg/litre<br>Hermann | extraction by hexane<br>100 mg<br>containing an internal<br>(1983)<br>standard; centrifugation;<br>direct injection | gas chromatography<br>method applied to<br>with electron capture<br>detection | Kastl &   |

<sup>a</sup> lowest reported level measured

The method was validated for the concentration range of 10-2000µg/m<sup>3</sup> 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<sup>3</sup>). 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 trichloro-ethene (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 hexachloro-butadiene by recovery from Hex-waste, three pathways for chemical synthesis are known: the chlorination and dehydro-chlorination 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 hexachloro-butadiene 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<sup>3</sup> 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<sup>3</sup>.mol<sup>-1</sup>) 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<sub>ow</sub>) of 4.78-4.90 (Table 1), hexa-chlorobutadiene is expected to adsorb strongly to organic matter. The organic carbon-water partition coefficient (K<sub>oc</sub>) can be estimated to be 25 120 litre/kg on the basis of a log K<sub>ow</sub> of 4.8 using the semi-empirical equation of Karickhoff (1981). Oliver & Charlton (1984) determined a K<sub>oc</sub> 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 hexa-chlorobutadiene 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 hexachloro-butadiene 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 (Gb *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 hexachloro-butadiene, by analogy with other halocarbons. The calculated lifetimes at -8 °C correspond to a pseudo-first order rate constant of  $(2 \pm 1) \times 10^{-14} \text{ cm}^3 \cdot \text{molecules}^{-1} \cdot \text{sec}^{-1}$  at estimated hydroxyl radical concentrations of  $7 \times 10^5 \text{ molecules} \cdot \text{cm}^{-3}$  for the northern hemisphere and  $17 \times 10^5$  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 gamma-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).

When 15 samples of hazardous waste from incineration facilities

in the USA were analysed, 4 sites were found to contain hexa-chlorobutadiene, but the levels were reported to be below 10 mg/kg (Demarini *et al.*, 1987). In sewage sludge, Alberti & Ploger (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<sup>3</sup> 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).

Table 3. Levels of hexachlorobutadiene in environmental air

| Type of Detection<br>air<br>(ng/m <sup>3</sup> ) | Year<br>Levels determined <sup>a</sup><br>(ng/m <sup>3</sup> ) | Location<br>Reference  | limit |
|--|--|--|-------|
| Ambient<br>0.0001-0.0004 (r)                     | 1985   | Atlantic Ocean, lower<br>Class & Ballschmiter                                      |       |
| 0.0003 (m, north)                                | (1987)   | troposphere in a south-north<br>cross section, 8 sites                             |       |
| 0.0001 (m, south)                                |  |  |       |
| Urban<br>nd (n=9)                                | 1978   | USA, Niagara Falls, inside<br>Barkley <i>et al.</i> (1980)<br>homes near dump site |       |
| nd (n=6)   |  | USA, Niagara Falls, outside<br>homes near dump site                                |       |
| trace (n=3)                                      |  |  |       |



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

Table 4 (contd).

| Type of<br>Detection limit<br>water<br>(ng/litre) | Year<br>Levels determined <sup>a</sup><br>(ng/litre) | Location<br>Reference                          |
|---|--|--|
| Surface<br>10                                     | 1976<br>10 (50-percentile)                           | Germany, River Rhine, 865 km<br>Alberti (1983) |
| 180 (90-percentile)                               |  |  |

|                    |                       |                                     |
|--------------------|-----------------------|-------------------------------------|
| 10                 | 1978                  | Germany, idem                       |
|                    | 20 (50-percentile)    |                                     |
| 60 (90-percentile) |                       |                                     |
| 10                 | 1981                  | Germany, idem                       |
|                    | < 10 (50-percentile)  |                                     |
| 40 (90-percentile) |                       |                                     |
| 10                 | 1980-1981             | Germany, 4 River Rhine              |
|                    | nd                    | tributaries                         |
|                    |                       | 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).

| Type of<br>Detection limit<br>water<br>(ng/litre) | Year<br>Levels determined <sup>a</sup><br>(ng/litre) | Location<br>Reference          |
|---|--|--------------------------------|
| Surface   | 1984-1985  | Germany, River Rhine           |
| 10-20   | Petersen (1986)                                      |                                |
|   |  | Germany, River Elbe            |
| 10-150  |  |                                |
| Estuarine   |  | USA, Calcasieu River estuary,  |
| 1298  | Pereira et al. (1988)                                |                                |
|   |  | vicinity of industrial outfall |
| Sea   | 1972-1973  | United Kingdom, Liverpool Bay  |
| 1   | 4 (m, n=150)   | Pearson & McConnell            |
| nd-30 (r)   | (1975)   |                                |
| Sea   | 1977   | USA, Gulf of Mexico,           |
| Sauer (1981)                                      |  | open ocean                     |
| 1   | nd (n=4)   |                                |

1 nd-15 (n=4) coast  
 Ground Switzerland, aquifer contaminated  
 200-300 (r) Giger & Schaffner (1981)  
 water by leachate from a chemical waste  
 disposal site

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

Table 5 (contd).

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

Table 5 (contd).

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

<sup>a</sup> 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

<sup>b</sup> 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.

<sup>c</sup> surficial sediment; the sediment concentration increased with fraction size

<sup>d</sup> surficial sediment

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

| Type of biota<br>Levels determined <sup>a</sup><br>(µg/kg wwt) | Year      | Location                                       |
|--|-----------|--|
|  | Reference |  |
| Detritus (bottom)<br>water 200                                 | 1972-1976 | Netherlands, surface<br>Goldbach et al. (1976) |
| Detritus (floating)<br>220                                     |           |  |

Invertebrates

|                             |           |                            |
|-----------------------------|-----------|----------------------------|
| Plankton                    | 1972-1973 | United Kingdom, sea        |
| water                       | nd-2.0    | Pearson & McConnell (1975) |
| Ragworm,                    |           |                            |
| <i>Nereis diversicolor</i>  |           |                            |
| 0.06                        |           |                            |
| Mussel,                     |           |                            |
| <i>Mytilus edulis</i>       |           |                            |
| nd-3.8                      |           |                            |
| Crab,                       |           |                            |
| <i>Cancer pagarus</i>       |           |                            |
| nd-1.1                      |           |                            |
| Others                      |           |                            |
| nd                          |           |                            |
| <i>Cerastoderma edule</i>   |           |                            |
| <i>Ostrea edulis</i>        |           |                            |
| <i>Buccinum undatum</i>     |           |                            |
| <i>Crepidula fornicata</i>  |           |                            |
| <i>Carcinus maenus</i>      |           |                            |
| <i>Eupagurus bernhardus</i> |           |                            |
| <i>Crangon crangon</i>      |           |                            |
| <i>Asterias rubens</i>      |           |                            |
| <i>Solaster</i> sp.         |           |                            |
| <i>Echinus esculentus</i>   |           |                            |

Table 6 (contd).

| Type of biota                  | Year                   | Location             |
|--------------------------------|------------------------|----------------------|
| Levels determined <sup>a</sup> | Reference              |                      |
| (µg/kg wwt)                    |                        |                      |
| Snail                          | 1972-1976              | Netherlands, surface |
| water                          | Goldbach et al. (1976) |                      |
| <i>Lymnaea peregra</i>         |                        |                      |
| 30, 1670                       |                        |                      |
| Clam,                          |                        |                      |
| <i>Sphaerium</i> 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<br>< 5 (dwt)<br>stomachs of fish   | 1983-1984<br>Malins et al. (1985)       | USA, sea water      |
| Clam,<br>area<br>E. complanatus<br>nd-83 (r, n=34)  | 1982-1983<br>Kauss & Hamdy (1985)       | Canada, Great Lakes |
| Marine algae<br>water<br>Enteromorpha compressa<br>nd<br>Ulva lactuca<br>nd<br>Fucus vesiculosus<br>8.9<br>Fucus serratus<br>0.6<br>Fucus spiralis<br>0.6 | 1972-1973<br>Pearson & McConnell (1975) | United Kingdom, sea |

Table 6 (contd).

| Type of biota<br>Levels determined <sup>a</sup><br><br>(µg/kg wwt)                             | Year<br>Reference                       | Location            |
|--|---|---------------------|
| Fish<br>Ray,<br>water<br>Raja clavata (flesh)<br>0.1-0.4<br>Raja clavata (liver)<br>0.2-1.5    | 1972-1973<br>Pearson & McConnell (1975) | United Kingdom, sea |
| Plaice,<br>Pleuronectes platessa (flesh)<br>nd-0.4<br>Pleuronectes platessa (liver)<br>0.2-1.2 |   |                     |
| Dab,<br>Limanda limanda (flesh)<br>< 0.1<br>Limanda limanda (liver)<br>nd                      |   |                     |
| Mackerel,<br>Scomber scombrus (flesh)<br>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<br>Levels determined <sup>a</sup><br>(µg/kg wwt) | Year<br>Reference      | Location                       |
|--|------------------------|--------------------------------|
| Trout,<br>Lake   | 0.47                   | USA, Niagara River,<br>Ontario |
| Salmo gairdneri  |                        | Canada, Lake Ontario           |
| Trout  | 1981                   |                                |
| 1.3 (dwt)  | Fox et al. (1983)      |                                |
| Catfish (flesh)  | 1973                   | USA, surface water near        |
| trace-4600   | Yurawecz et al. (1976) | chemical plants                |
| Gaspergoo (flesh)  |                        |                                |
| manufacturing  | 200                    | tetrachloroethene              |
| 100  |                        |                                |
| Buffalo fish (flesh)   |                        |                                |
| 100  |                        |                                |
| Mullet (flesh)   |                        |                                |
| trace  |                        |                                |
| Sea trout (flesh)  |                        |                                |
| trace  |                        |                                |
| Sheepshead minnow (flesh)                                      |                        |                                |
| trace  |                        |                                |
| Catfish  | 1973                   | USA, < 40 km from              |
| tetrachloro-   | 10-1200                | Yip (1976)                     |
| Carp   |                        | ethene or                      |
| trichloroethene  | 62                     | manufacturing plants           |
| Gaspergoo  |                        |                                |
| 12-30  |                        |                                |
| Buffalo fish   |                        |                                |
| 120  |                        |                                |
| Whiting  |                        |                                |
| 20   |                        |                                |
| Drum   |                        |                                |
| 10   |                        |                                |

Table 6 (contd).

| Type of biota<br>Levels determined <sup>a</sup><br>(µg/kg wwt)                          | Year<br>Reference | Location                |
|---|-------------------|-------------------------|
| Pike perch,<br>Goldbach et al. (1976)<br>Stizostedion lucioperca<br>(lake) 440 (m, n=8) | 1972-1976         | Netherlands, Ketelmeer  |
| (lake) 23 (m, n=4)<br>Perch,<br>Perca fluviatilis<br>130, 400 (n=2)                     |                   | Netherlands, IJsselmeer |
| Pike,<br>Esox lucius<br>260   |                   | Netherlands, Ketelmeer  |
| Tench,<br>Tinca tinca<br>950  |                   | Netherlands, Ketelmeer  |
| Common bream,<br>Abramis brama<br>1520 (m, n=5)   |                   | Netherlands, Ketelmeer  |
| 33 (m, n=5)<br>White bream<br>Blicca bjoerkna<br>360 (m, n=3)                           |                   | Netherlands, IJsselmeer |
| Roach,<br>Rutilus rutilus<br>910 (m, n=10)  |                   | Netherlands, Ketelmeer  |
| 61 (m, n=4)<br>Eel,<br>Anguilla anguilla<br>33 (m, n=4)                                 |                   | Netherlands, IJsselmeer |
| Smelt<br>Osmerus eperlanus<br>43 (m, n=3)   |                   | Netherlands, IJsselmeer |

Table 6 (contd).

| Type of biota<br>Levels determined <sup>a</sup><br>(µg/kg wwt) | Year<br>Reference | Location |
|--|-------------------|----------|
|--|-------------------|----------|

|                            |                       |                       |
|----------------------------|-----------------------|-----------------------|
| 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                 | 46 000-120 000        | Pereira et al. (1988) |
| River                      | (lipid base)          | outfall in Calcasieu  |
| Atlantic croaker           |                       | estuary               |
| River                      | 41 000 (lipid base)   | idem, in Calcasieu    |
| 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 |
| nd                         | Camanzo et al. (1987) | tributaries and       |
| embayments                 |                       |                       |
| Birds                      |                       |                       |
| Guillemot,                 | 1972-1973             | United Kingdom        |
| Pearson & McConnell (1975) |                       |                       |
| Uria aalge (eggs)          |                       |                       |
| 1.6-9.9                    |                       |                       |

Table 6 (contd).

| Type of biota                  | Year      | Location       |
|--------------------------------|-----------|----------------|
| Levels determined <sup>a</sup> | Reference |                |
| (µg/kg wwt)                    |           |                |
| Swan,                          |           |                |
| Pearson & McConnell (1975)     |           |                |
| Cygnus olor (liver)            |           |                |
| 5.2                            |           |                |
| Cygnus olor (kidney)           |           |                |
| nd                             |           |                |
| Moorhen,                       | 1972-1973 | United Kingdom |
| Pearson & McConnell (1975)     |           |                |

|         |                                  |           |                |
|---------|----------------------------------|-----------|----------------|
| 0.8     | Gallinula chloropus (liver)      |           |                |
|         | 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 platyrhynchos (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      |                                  |           |                |

<sup>a</sup> dwt = dry weight; r = range of individual values; m = mean of individual values; n = number of samples; nd = not detectable; wwt = wet weight

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

| Type of food<br>Levels determined <sup>a</sup><br>or drinking-water<br>(µg/kg wwt or µg/litre) | Year<br>Reference  | Location                |
|--|--------------------|-------------------------|
| Tap water  | 1978               | USA, houses bordering   |
| Old  | nd-trace (r, n=3)  | Barkley et al. (1980)   |
| Falls  | 0.06-0.17 (r, n=6) | Love Canal, Niagara     |
| Well water   | 1978               | USA, Tennessee,         |
| contaminated   | nd-2.53 (r, n=28)  | Clark et al. (1982)     |
| dump   | 0.15 (m, n=22)     | by leachate from waste  |
| Fresh milk   |                    | United Kingdom          |
| 0.08   | Butter             | McConnell et al. (1975) |
| 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  | Year      | Location              |
|-------------------|---|-----------|-----------------------|
|                   | Levels determined <sup>a</sup>                                | Reference |                       |
|                   | or drinking-water   |           |                       |
|                   | ( $\mu\text{g}/\text{kg}$ wwt or $\mu\text{g}/\text{litre}$ ) |           |                       |
|                   | 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

<sup>a</sup> nd = not detected; m = mean of individual values; n = number of samples; r = range; wwt = wet weight

## 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 hexachloro-butadiene 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 activity and gamma-glutamyltranspeptidase. CPB is finally cleaved by renal cysteine conjugate  $\beta$ -lyase to the electrophile trichlorovinyl-chlorothioketene. The renal accumulation of sulfur conjugates and the location of  $\beta$ -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).

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

| Species<br>Metabolite <sup>a</sup><br>dose (%) | Route<br>Fraction of<br>body weight)<br>dosing (h) | Dose (mg/kg)                  | Medium<br>Time after | Reference       |
|--|--|-------------------------------|----------------------|-----------------|
| Rat  | ip   | 0.1                           | urine                | total           |
| 29   | 48   | Davis <i>et al.</i> (1980)    |                      |                 |
| soluble  | 25   |                               | 48                   | water-          |
| 40   | 48   |                               | faeces               | total           |
| 7  | 48   | 300.1                         | urine                | total           |
| soluble  | 6  |                               | 48                   | water-          |
| 7  | 48   |                               | faeces               | total           |
| Rat  | oral   | 200                           | urine                | total           |
| 11   | 120  | Nash <i>et al.</i> (1984)     |                      |                 |
| 1  | 120  |                               |                      | PBSA            |
| ether soluble                                  | 7  |                               | 120                  | non-            |
| 39   | 120  |                               | faeces               | total           |
| Rat  | oral   | 1                             | expired air          | total           |
| 8.9  | 72   | Reichert <i>et al.</i> (1985) |                      |                 |
| 5.3  | 72   |                               |                      | HCBD            |
| 3.6  | 72   |                               |                      | CO <sub>2</sub> |
| 30.6   | 72   |                               | urine                | total           |
| 42.1   | 72   |                               | faeces               | total           |

|     |    |    |             |       |
|-----|----|----|-------------|-------|
| 6.6 | 72 | 50 | expired air | total |
|     |    |    |             | HCBD  |
| 5.4 | 72 |    |             |       |

Table 8 (contd).

| Species<br>Metabolite <sup>a</sup><br>dose (%) | Route<br>dosing (h) | Dose (mg/kg<br>Fraction of<br>body weight) | Medium<br>Time after      | Reference               |
|--|---------------------|--|---------------------------|-------------------------|
| 1.2  | 72                  |  |                           | CO <sub>2</sub>         |
|  |                     |  | urine                     | total                   |
| 11.0   | 72                  |  | faeces                    | total                   |
| 69   | 72                  |  |                           |                         |
| Rat<br>5.4                                     | oral<br>24          | 100<br>Reichert et al. (1985);             | urine                     | total                   |
| containing<br>(1986)                           |                     | ca 4.3                                     | 24                        | Reichert & S-<br>Schutz |
| 0.5  | 24                  |  |                           | ACPB }<br>MTPB }        |
|  |                     |  | faeces                    | CMTPB }<br>total        |
| 60   | 72                  |  |                           |                         |
| 7.45   | oral<br>72          | 1  | expired air               | total                   |
| 2.2  |                     |  |                           | C <sub>2</sub>          |
|  |                     |  | urine                     | total                   |
| 17.5   |                     |  | faeces & git <sup>b</sup> | total                   |
| 61.8   |                     |  | carcass                   | total                   |
| 10.5   |                     |  |                           |                         |
| 7.57   | 72                  | 100  | expired air               | total                   |
| 0.7  |                     |  |                           | CO <sub>2</sub>         |
|  |                     |  | urine                     | total                   |
| 9.0  |                     |  | faeces & git <sup>b</sup> | total                   |
| 72.1   |                     |  | carcass                   | total                   |
| 5.8  |                     |  |                           |                         |

Table 8 (contd).

| Species<br>Metabolite <sup>a</sup><br>dose (%) | Route<br>dosing (h) | Dose (mg/kg<br>Fraction of<br>body weight) | Medium<br>Time after      | Reference       |
|--|---------------------|--|---------------------------|-----------------|
| Rat  | iv                  | 1  | expired air               | total           |
| 8.54   | 72                  | Payan et al. (1991)                        |                           | CO <sub>2</sub> |
| 2.6  |                     |  | urine                     | total           |
| 21.1   |                     |  | faeces & git <sup>b</sup> | total           |
| 59.3   |                     |  | carcass                   | total           |
| 12.9   |                     |  |                           |                 |
| 8.11   | 72                  | 100  | expired air               | total           |
| 0.9  |                     |  |                           | CO <sub>2</sub> |
| 9.2  |                     |  | urine                     | total           |
| 71.5   |                     |  | faeces & git <sup>b</sup> | total           |
| 11.1   |                     |  | carcass                   | total           |
| Mouse  | oral                | 30   | expired air               | total =         |
| HCBD   | 4.5                 | 72   | Dekant et al. (1988a)     | total           |
| 7.2  | 72                  |  | urine                     | total           |
| 72.0   | 72                  |  | faeces                    | total           |
| > 57   | 72                  |  |                           | HCBD            |
| 7.2  | 72                  |  |                           | GPB             |

<sup>a</sup> For abbreviations see Fig. 1; "total" indicates that no individual chemicals were specified

<sup>b</sup> git = gastrointestinal tract

#### 6.2.1 In vitro studies

Incubation of hexachlorobutadiene with rat or mouse liver or kidney subcellular fractions caused a depletion of non-protein sulphhydryl 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  $\beta$ -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). Trichloro-vinyl-chlorothioketene has been identified as the ultimate reactive intermediate in hexachlorobutadiene metabolism catalysed by  $\beta$ -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

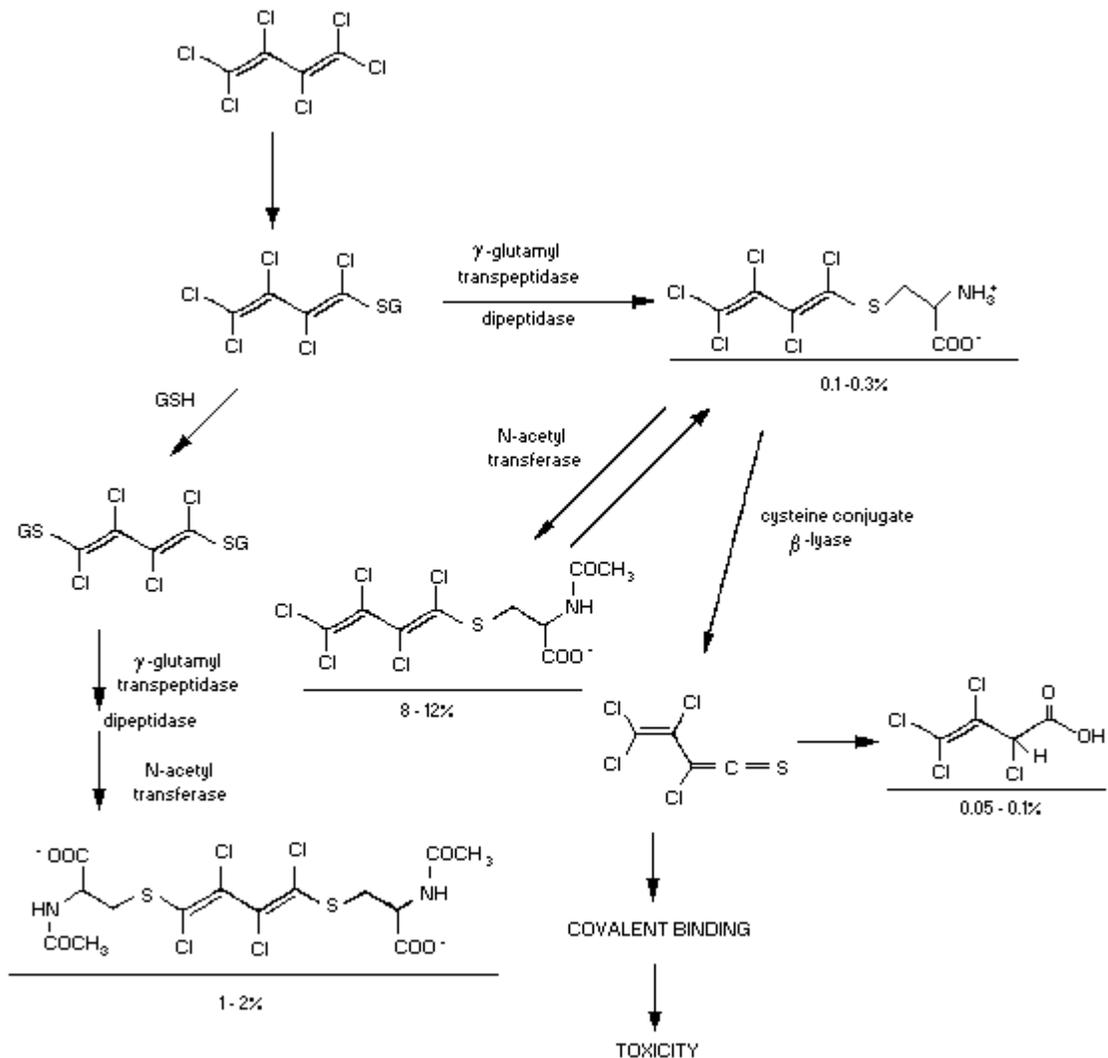
In *in vivo* studies, hexachlorobutadiene caused a marked, dose-related depletion of renal nonprotein sulfhydryl (NP-SH) in mice at single intraperitoneal doses of 33-50 mg/kg body weight but little or no decrease in hepatic NP-SH (Kluwe *et al.*, 1981; Lock *et al.*, 1984). This pattern was also observed 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 0.071 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 hexachloro-butadiene 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 hexachloro-butadiene 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 [<sup>14</sup>C] hexachlorobutadiene (200 mg/kg) in female rats (Reichert & Schütz, 1986). The same authors also identified 1-carboxymethylthio-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-tetra-chlorobutenic acid were identified as urinary metabolites (Dekant *et al.*, 1988a).

It is probable that 2,3,4,4-tetrachlorobutenic 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 [<sup>14</sup>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 [<sup>14</sup>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 hexachloro-butadiene 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 & Scultz, 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 hexachloro-butadiene 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 hexachloro-butadiene 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 hexachloro-butadiene 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<sub>2</sub> and P<sub>3</sub> renal epithelial cells exhibited marked vacuolation and necrosis, which persisted up to day 7. Increased gamma-glutamyl-transferase (EC 2.3.2.2) staining was seen in P<sub>2</sub> and P<sub>3</sub> 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                   | pH            | Dissolved  | Reference        |
|-------------------|------------------------|-------------------------------|---------------|------------|------------------|
| Hardness          | Stat/flow <sup>a</sup> | Parameter                     | Concentration | oxygen     | (mg              |
| CaCO <sub>3</sub> | open/closed            | (°C)                          | (mg/litre)    | (mg/litre) | per              |
| litre)            | period                 |                               |               |            | litre)           |
|                   | (h)                    |                               |               |            |                  |
| Fresh water       |                        |                               |               |            |                  |
| Algae             | Haematococcus          | 20                            |               |            |                  |
| stat, closed      | 24                     | EC <sub>10</sub> <sup>b</sup> | > 2           |            | Knie et al.      |
| (1983)            | pluvialis              |                               |               |            |                  |
| Bacteria          | Pseudomonas putida     | 25                            |               | 7.0        |                  |
| stat, closed      | 16                     | TT <sup>c</sup>               | > 25          |            | Bringmann &      |
| Kuhn (1977)       |                        |                               |               |            |                  |
| Bacteria          | Pseudomonas putida     | 20                            |               | 7.2        |                  |
| stat, open        | 0.5                    | EC <sub>10</sub> <sup>b</sup> | > 0.9         |            | Knie et al.      |
| (1983)            |                        |                               |               |            |                  |
| Protozoans        | Chilomonas             | 20                            |               | 6.9        |                  |
| stat, closed      | 48                     | TT <sup>c</sup>               | > 10          |            | Bringmann et al. |
| (1980)            | paramecium             |                               |               |            |                  |

|                           |                          |      |                         |              |             |
|---------------------------|--------------------------|------|-------------------------|--------------|-------------|
| Molluscs                  | great pond snail,        | 19   |                         |              |             |
| stat, <sup>d</sup> closed | 24 LC <sub>50</sub>      | 0.21 |                         | Leeuwangh et |             |
| 96                        | LC <sub>50</sub>         | 0.21 | al. (1975) <sup>e</sup> |              |             |
|                           | <i>Lymnaea stagnalis</i> |      |                         |              |             |
| Crustaceans               | water flea,              | 20   |                         | 7            |             |
| 250                       | stat, open               | 24   | EC <sub>50</sub>        | 0.5          | Knie et al. |
| (1983)                    | <i>Daphnia magna</i>     |      |                         |              |             |
|                           | aquatic sowbug,          | 19   |                         |              |             |
| stat,d closed             | 96 LC <sub>50</sub>      | 0.13 |                         | Leeuwangh et |             |
| al. (1975) <sup>e</sup>   | <i>Asellus aquaticus</i> |      |                         |              |             |

Table 9 (contd).

| Organisms               | Species                      | Temperature | pH               | Dissolved    | Reference     |
|-------------------------|------------------------------|-------------|------------------|--------------|---------------|
| Hardness                | Stat/flow <sup>a</sup>       | Parameter   | Concentration    | oxygen       | (mg           |
| CaCO <sub>3</sub>       | open/closed                  | (°C)        | (mg/litre)       | (mg/litre)   | per           |
| litre)                  | period                       |             |                  |              | litre)        |
|                         | (h)                          |             |                  |              |               |
| Fish                    | goldfish,                    | 17.5        |                  |              |               |
| stat, <sup>d</sup> open | 96 LC <sub>50</sub>          | 0.09        |                  | Leeuwangh et |               |
| al. (1975) <sup>e</sup> | <i>Carassius auratus</i>     |             |                  |              |               |
| Fish                    | zebrafish,                   | 20          |                  | 8.0          | 9.0           |
| 180                     | flow, closed                 | 48          | LC <sub>50</sub> | 1            | Slooff (1979) |
|                         | <i>Brachydanio rerio</i>     |             |                  |              |               |
| Fish                    | rainbow trout,               |             |                  |              |               |
| LC <sub>50</sub>        | 0.320 US EPA (1980)          |             |                  |              |               |
|                         | <i>Salmo gairdnerii</i>      |             |                  |              |               |
| Fish                    | bluegill sunfish,            |             |                  |              |               |
| LC <sub>50</sub>        | 0.326 US EPA (1980)          |             |                  |              |               |
|                         | <i>Lepomis macrochirus</i>   |             |                  |              |               |
| Fish                    | sheepshead minnow,           |             |                  |              |               |
| LC <sub>50</sub>        | 0.557 US EPA (1980)          |             |                  |              |               |
|                         | <i>Cyprinodon variegatus</i> |             |                  |              |               |
| Fish                    | golden orfe,                 | 20          |                  | 8            |               |
| 270                     | open                         | 48          | LC <sub>50</sub> | 3            | Knie et al.   |
| (1983) <sup>e</sup>     | <i>Leuciscus idus</i>        |             |                  |              |               |
| Fish                    | fathead minnow,              | 25          |                  | 6.7-7.6      | 8.0           |
| 45                      | flow, open                   | 96          | LC <sub>50</sub> | 0.10         | Walbridge et  |

Pimephales promelas

al. (1983)<sup>e</sup>

Table 9 (contd).

| Organisms         | Species                | Temperature | pH                        | Dissolved  | Reference |
|-------------------|------------------------|-------------|---------------------------|------------|-----------|
| Hardness          | Stat/flow <sup>a</sup> | Parameter   | Concentration             | oxygen     | (mg       |
| CaCO <sub>3</sub> | open/closed            | (°C)        | (mg/litre)                | (mg/litre) | per       |
| litre)            | period                 |             |                           |            | litre)    |
|                   | (h)                    |             |                           |            |           |
| Marine            |                        |             |                           |            |           |
| Crustaceans       | harpacticoid           | 21          | 7.9                       | ≥ 5        |           |
| stat, open        | 96 LC <sub>50</sub>    | 1.2         | Bengtsson &               |            |           |
|                   | copepod                |             |                           |            |           |
| Tarkpea (1983)    |                        |             |                           |            |           |
| LC <sub>50</sub>  | 0.032                  |             |                           |            |           |
|                   | grass shrimp,          |             |                           |            |           |
|                   | US EPA (1980)          |             |                           |            |           |
|                   | Palaemonetes pugio     |             |                           |            |           |
| LC <sub>50</sub>  | 0.059                  |             |                           |            |           |
|                   | Mysid shrimp,          |             |                           |            |           |
|                   | US EPA (1980)          |             |                           |            |           |
|                   | Mysidopsis bahia       |             |                           |            |           |
| Fish              | sailfin molly,         | 22-24       | 6.6-7.9                   | 8-9        |           |
| flow, open        | 26 LC <sub>50</sub>    | 4.2         | Laseter et                |            |           |
|                   | Poecilia latipinna     |             |                           |            |           |
| 30                | LC <sub>50</sub>       | 4.5         | al. (1976) <sup>e,f</sup> |            |           |
| 77                | LC <sub>50</sub>       | 1.4-1.9     |                           |            |           |
| 115               | LC <sub>50</sub>       | 1.7         |                           |            |           |
| 138               | LC <sub>50</sub>       | 1.2         |                           |            |           |
| LC <sub>50</sub>  | 0.399                  |             |                           |            |           |
|                   | pinfish, Lagodon       |             |                           |            |           |
|                   | US EPA (1980)          |             |                           |            |           |
|                   | rhomboides             |             |                           |            |           |

<sup>a</sup> static or flow-through test, open or closed system semi-static (daily renewal) test d

<sup>b</sup> effect is 10% reduction in oxygen consumption analysis for hexachlorobutadiene was reported e

<sup>c</sup> TT = toxic threshold for inhibition of cell multiplication salinity was 0.25%, 96-h LC<sub>50</sub> was calculated to be 1.6 mg/litre f

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<sub>50</sub> 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<sub>3</sub>/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<sub>50</sub> 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<sub>3</sub>/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 hexachloro-butadiene 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 hexachloro-butadiene following inhalation, groups of 20 SPF mice of the OF<sub>1</sub> 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 OF<sub>1</sub> mice was measured following exposure for 15 min to hexachlorobutadiene vapour at concentrations between 886 and 2625 mg/m<sup>3</sup>. 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<sup>3</sup> (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<sub>50</sub> 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 biochemical findings are discussed extensively in sections 8.8.2 (liver) and 8.8.3 (kidney). In oral LD<sub>50</sub> studies on rats and mice, Gradiski *et al.* (1975) observed hyper-reactivity just after exposure, followed by decreased activity and staggering.

Table 10. Mortality of hexachlorobutadiene from single exposure

| Species/strain<br>Observation | Age<br>LD <sub>50</sub> (mg/kg<br>body weight) | Sex  | Route of exposure<br>Reference |
|-------------------------------|--|--|--------------------------------|
| Mouse                         |  |  | oral                           |
| 87 (78.1-95.9)                | Murzakayev (1963) <sup>b</sup>                 |  |                                |
| OF <sub>1</sub>               | adult  | female                                     | inhalation (6 h)               |
| 14                            | 107 (102-113)                                  | Gage (1970)                                |                                |
| OF <sub>1</sub>               | adult  | female                                     | oral                           |
| 14                            | 65 (60-70)                                     | Gradiski <i>et al.</i> (1975) <sup>c</sup> |                                |
| OF <sub>1</sub>               | adult  | male                                       | oral                           |
| 14                            | 80 (75-85)                                     | Gradiski <i>et al.</i> (1975) <sup>c</sup> |                                |
| Alderley Park                 | adult  | male                                       | intraperitoneal                |
| 14                            | 67 (53-85)                                     | Lock <i>et al.</i> (1984) <sup>e</sup>     |                                |
| Alderley Park                 | adult  | female                                     | intraperitoneal                |
| 14                            | 85 (65-111)                                    |  |                                |
| C <sub>57</sub> BL/10J        | adult  | male                                       | intraperitoneal                |
| 14                            | 57 (41-81)                                     |  |                                |
| C <sub>3</sub> H              | adult  | male                                       | intraperitoneal                |
| 14                            | 25-75  |  |                                |
| BALB/c                        | adult  | male                                       | intraperitoneal                |
| 14                            | 32-40  |  |                                |
| DBA/2J                        | adult  | male                                       | intraperitoneal                |
| 14                            | 53 (36-76)                                     |  |                                |

period (days)  
or LC<sub>50</sub> (mg/m<sup>3</sup>)<sup>a</sup>

|               |                     |                                     |        |      |
|---------------|---------------------|-------------------------------------|--------|------|
|               | Rat                 |                                     |        | oral |
| 350           | (323-377)           | Murzakayev (1963) <sup>b</sup>      |        |      |
|               | OF <sub>2</sub> rat | adult                               | female | oral |
| 14            | 270 (250-290)       | Gradiski et al. (1975) <sup>c</sup> |        |      |
|               | OF <sub>2</sub> rat | adult                               | male   | oral |
| 14            | 250 (220-280)       | Gradiski et al. (1975) <sup>c</sup> |        |      |
|               | Sprague-Dawley      | adult                               | female | oral |
| 200-400       |                     | Kociba et al. (1977a) <sup>d</sup>  |        |      |
|               | Sprague-Dawley      | adult                               | male   | oral |
| 580 (504-667) |                     | Kociba et al. (1977a) <sup>d</sup>  |        |      |
|               | Sprague-Dawley      | weanling                            | female | oral |
| 46 (26-81)    |                     | Kociba et al. (1977a) <sup>d</sup>  |        |      |
|               | Sprague-Dawley      | weanling                            | male   | oral |
| 65 (46-91)    |                     | Kociba et al. (1977a) <sup>d</sup>  |        |      |

Table 10 (contd).

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

<sup>a</sup> When available, 95% confidence limits are reported between brackets.

<sup>b</sup> Observation period, strain, sex, age (or body weight) and vehicle were not reported.

<sup>c</sup> Vehicle was olive oil.

<sup>d</sup> Confidence limits not calculable; observation until no toxicity was observed any longer; vehicle was corn oil.

<sup>e</sup> Vehicle was corn oil.

<sup>f</sup> Application undiluted using glass vials (3.6 cm<sup>2</sup>).

### 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 hexa-chlorobutadiene 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<sup>3</sup> 6 h/day for 15 days, 1067 mg/m<sup>3</sup> 6 h/day for 12 days, or 2668 mg/m<sup>3</sup> 4 h/day for 2 days. Petroleum ether was used as a solvent for concentrations below 1067 mg/m<sup>3</sup>. 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<sup>3</sup> 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<sup>3</sup> and the weight gain of females was reduced at 107 and 267 mg/m<sup>3</sup>. Irritation of eyes and nose was observed at the two highest levels. At 267 and 1067 mg/m<sup>3</sup> rats were in a poor condition, females being more affected than males. Respiratory difficulties were seen at and above 267 mg/m<sup>3</sup>. Haematological examination at the end of the exposure period showed slight anaemia

in females at 1067 mg/m<sup>3</sup>. Urinalysis did not reveal abnormalities at any of the exposure levels. Macroscopically enlarged, pale kidneys were found at 267 and 1067 mg/m<sup>3</sup> and enlarged adrenals at 1067 mg/m<sup>3</sup>. Histopathological investigations revealed proximal tubular degeneration in the kidneys and cortical degeneration in adrenals at concentrations of 267 mg/m<sup>3</sup> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> mice<sup>a</sup>

| Concentration<br>(mg/kg feed) | Dose (mg/kg body<br>weight per day) |        | Number of mice with<br>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  |

<sup>a</sup> 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 conjunctival 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<sup>3</sup> 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<sup>3</sup>. 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<sup>3</sup>. 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  $\beta$ -lyase inhibitor, AOAA (Vamvakas *et al.*, 1988a, 1989a) and the gamma-glutamyltranspeptidase inhibitor, acivicin (Vamvakas *et al.*, 1989a).

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  $\beta$ -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).

Table 12. Studies on mutagenicity of hexachlorobutadiene

| Test description<br>Conditions <sup>a</sup>                        | Species/strain/cell type<br>Result <sup>c</sup> Reference                                      |              |
|--|--|--------------|
| Reverse mutations<br>rat liver S9, purity 98%,<br>incorporation    | Salmonella typhimurium TA98,<br>- De Meester et al.<br>TA100, TA1530, TA1535, TA1538<br>(1981) | +/-<br>plate |
| rat liver S9, purity > 99%,<br>incorporation                       | S. typhimurium TA100<br>- Stott et al. (1981)  | +/-<br>plate |
| rat liver S9, purity not<br>reported, suspension test <sup>b</sup> | S. typhimurium TA98, TA100<br>- Reichert et al. (1983)   | +/-          |
| rat liver S9, purity not<br>reported, preincubation test           | S. typhimurium TA98, TA100,<br>- Haworth et al. (1983)<br>TA1535, TA1537                       | +/-          |
| liver S9*, purity > 99.5%,<br>preincubation test                   | +  | + rat        |
| rat liver S9, purity not<br>reported, plate incorporation          | S. typhimurium TA100, TA1535<br>- Chudin et al. (1985)<br>TA1538                               | +/-          |
| rat liver S9,<br>liver S9*   | S. typhimurium TA100<br>- Reichert et al. (1984)   | +/-<br>- rat |
| liver S9*, purity >99.5%,<br>preincubation test                    | S. typhimurium TA100<br>+ <sup>d</sup> Wild et al. (1986)                                      | + rat        |
| activation, purity 98%   | S. typhimurium TA100<br>+ Vamvakas et al. (1988a)  | no           |
| activation, purity > 99.5%,<br>preincubation test                  | -  | no           |

Table 12 (contd).

| Test description<br>Conditions <sup>a</sup>                              | Species/strain/cell type<br>Result <sup>c</sup> Reference |                |
|--|---|----------------|
| liver microsomes<br>without additional GSH                               | - S. typhimurium TA100<br>Vamvakas et al. (1988a)         | + rat          |
| liver microsomes and<br>additional GSH, purity > 99.5%,<br>incorporation | + <sup>e</sup>  | + rat<br>plate |
| Sex-linked lethals<br>feeding or injection                               | Drosophila melanogaster<br>- Woodruff et al. (1985)       |                |
| Chromosome aberrations<br>rat liver S9                                   | CHO cells<br>- Galloway et al. (1987)                     | +/-            |
| Chromosome aberrations<br>liver S9                                       | human lymphocytes<br>- German (1988)                      | - rat          |
| Sister chromatid exchanges<br>rat liver S9                               | CHO cells<br>+ Galloway et al. (1987)                     | +/-            |
| Chromosome aberrations<br>inhalation, 4 h                                | mouse bone marrow cells<br>+ German (1988)                |                |
| Chromosome aberrations<br>gavage   | mouse bone marrow cells<br>+ German (1988)                | oral           |

<sup>a</sup> S9\* = a fortified S9 mix containing 3 times the normal protein concentration; GSH = reduced glutathione

<sup>b</sup> The extreme toxicity of the compound without S9 was supposed to exclude testing in this system

<sup>c</sup> + =  $\geq$  twice the background rate or, in the case of bacterial studies, a reproducible dose-related increase in the number of revertants per plate; - = negative

<sup>d</sup> 0.23 revertants per nmol

<sup>e</sup> Addition of rat kidney microsomes further increased the number of revertants; positive results were inhibited by the  $\beta$ -lyase inhibitor aminooxyacetic acid

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 *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 hexachloro-butadiene 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<sup>3</sup> 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, dimethyl-nitrosamine, 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 <sup>14</sup>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

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

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

Table 13 (contd).

| Metabolite and abbreviation <sup>a</sup>                | Conditions <sup>b</sup> | Result <sup>c</sup> | 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-butenic acid (PBA)              | +/- rat liver S9        | +                   | Reichert et al. (1984) |
| 2,2,3,4,4-pentachloro-3-butenic acid chloride (PBAC)    | +/- rat liver S9        | +                   | Reichert et al. (1984) |

- <sup>a</sup> See also Figure 1
- <sup>b</sup> 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
- <sup>c</sup> + =  $\geq$  twice background rate; - = negative
- <sup>d</sup> Mutagenic potency enhanced by rat kidney microsomes or mitochondria and less so by cytosol; positive results were inhibited by the  $\beta$ -lyase inhibitor aminooxyacetic acid
- <sup>e</sup> Mutagenic potency enhanced by rat kidney S9
- <sup>f</sup> Positive results were inhibited by the  $\beta$ -lyase inhibitor aminooxyacetic acid
- <sup>g</sup> 18.7 revertants per nmol; mutagenic potency decreased by addition of pyridoxal phosphate; activation by cytosol, with and without cofactors, had the same results as S9 mix; microsome mix was inactive

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 hexa-chlorobutadiene in acetone to the dorsal skin. After 14 days, the mice similarly received 5 µg of the tumour promoter 12- *o*-tetra-decanoylphorbol-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<sup>a</sup>

| Dose (mg/kg<br>body weight<br>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                                 | 3/40b          | 6/40 (P < 0.05) |

<sup>a</sup> From: Kociba *et al.* (1977a)

<sup>b</sup> 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 tricapyrylin, 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 tricapyrylin 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 demyelination 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<sub>1</sub> mice 24 h after whole-body inhalation exposure for 4 h to hexachloro-butadiene at measured concentrations of 29.3, 53.4, 106.7 or 266.8 mg/m<sup>3</sup>. 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<sub>50</sub> was calculated to be 76.8 mg/m<sup>3</sup> (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- $\beta$ -D-glucosaminidase, gamma-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<sub>3</sub>-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- $\beta$ -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,  $\beta$ -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 hexachloro-butadiene in weanling rats (Hook *et al.*, 1983).

#### 8.9.1.3 Inhibitors of gamma-glutamyltranspeptidase (EC 2.3.2.2)

Male rats pretreated with Acivicin (L-(alphaS, 5S)-alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), an inhibitor of gamma-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 gamma-glutamyltranspeptidase inhibition did not limit the formation of nephrotoxic metabolites (Davis, 1988).

Male Swiss OF<sub>1</sub> 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.

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  |
|------------------|--------------|---------------|------------|
| Duration of      | Dose (mg/kg) | NOAEL (mg/kg) | References |
| study            | body weight  | body weight   | animals    |

| day) | per day)   |                   |                                     | per group                        | per     |
|------|--|-------------------|-------------------------------------|----------------------------------|---------|
|      | Rat (Wistar-derived)<br>7.3, 18.2, 44.7<br>(1979)                            | weanling<br>< 7.3 | male<br>Harleman & Seinen<br>female | 6<br>6                           | 2 weeks |
|      | Rat (Sprague-Dawley)<br>0.2, 20  | adult<br>0.2      | male<br>Stott et al. (1981)         | 5                                | 3 weeks |
|      | Rat (Sprague-Dawley)<br>1, 3, 10, 30, 65, 100                                | adult<br>3        | female<br>Kociba et al. (1971)      | 4                                | 30 days |
|      | Rat (Sprague-Dawley)<br>months 0.2, 2.0, 20<br>(1977)                        | adult             | male<br>0.2<br>female               | 10-12<br>Schwetz et al.<br>20-24 | 3       |
|      | Rat (Sprague-Dawley)<br>months 0.2, 2.0, 20<br>(1977a,b)                     | adult             | male<br>0.2<br>female               | 39-40<br>Kociba et al.<br>39-40  | 24      |
|      | Mouse (B6C3F1)<br>4.3, 14.3, 43, 143, 430<br>(1989); Yang (1991)             | adult<br>< 4.3    | male<br>Yang et al.<br>female       | 5<br>5                           | 2 weeks |
|      | Mouse (B6C3F1)<br>weeks 0.1, 0.4, 1.5, 4.9, 18.8<br>0.2, 0.5, 1.8, 4.5, 19.2 | adult<br>< 0.2    | male<br>1.5<br>female               | 10<br>Yang et al.<br>10          | 13      |
|      |  |                   | (1989); Yang (1991)                 |                                  |         |

#### 8.9.1.4 Inhibitors of cysteine conjugate $\beta$ -lyase

Male Swiss OF1 mice, pretreated with the two  $\beta$ -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-(dipropyl-amino)sulfonyl)] benzoic acid (105  $\mu$ mol/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  $\mu$ mol/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 gamma-glutamyl-transpeptidase (EC 2.3.2.2) or amino-oxyacetic acid (AOAA), an inhibitor of renal cysteine conjugate  $\beta$ -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 nephro-toxicity in the isolated perfused rat kidney, as indicated by the appearance in the urine of alkaline phosphatase, gamma-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  $\text{Ca}^{2+}$ , 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  $\geq$  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 hexachloro-butadiene is dependent on the biosynthesis of the toxic sulfur conjugate GPB. This conjugate is mainly synthesized in the liver and further metabolized in the bile, gut, and kidneys to the CPB. Cysteine conjugate  $\beta$ -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 aminoxyacetic acid, specific inhibitors of gamma-glutamyltranspeptidase and cysteine conjugate  $\beta$ -lyase protected against the cytotoxicity of hexachloro-butadiene-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  $\beta$ -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 hexachloro-butadiene, an increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes was observed (German, 1986). The workers were exposed to hexachloro-butadiene concentrations that ranged from 1.6 to 16.9 mg/m<sup>3</sup>. 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  $\beta$ -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  $\beta$ -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:

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

## 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 monogluthathione conjugate of hexachloro-butadiene is processed to the cysteine- S-conjugate, which is then a substrate for renal cysteine conjugate  $\beta$ -lyase. Hexachloro-butadiene 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<sup>3</sup>.

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 hexachloro-butadiene 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 hexachloro-butadiene 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<sub>50</sub> 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.

NOAEL Considering the toxicity of the substance to mammals (the 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 µg/m<sup>3</sup> 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 calo-porteur, 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-compartmental s'effectue principalement par volatilisation, adsorption sur les matières particulaires puis dépôt ou sédimentation. L'hexachloro-butadiène 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 µg/m<sup>3</sup>. Dans les régions écartées, les concentrations sont inférieures à 1 µg/m<sup>3</sup>. 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 sédiments 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<sup>3</sup> 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<sub>50</sub> 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'hexa-chlorobutadiène égale à 267 mg/m<sup>3</sup>; cette concentration a également entraîné des difficultés respiratoires, ainsi qu'une dégénérescence des corticosurrénales.

## 7.2 Reproduction, embryotoxicité et teratogénicité

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<sup>3</sup>, 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- $\beta$  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<sup>3</sup>.

#### 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'hexachloro-butadiène 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<sup>3</sup> 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 µg/m<sup>3</sup> 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 hexaclorobutadieno 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 hexaclorobutadieno en el aire

urbano; en todos los casos, los niveles eran inferiores a  $0,5 \mu\text{g}/\text{m}^3$ . Las concentraciones en lugares aislados son inferiores a  $7 \text{ pg}/\text{m}^3$ . En las aguas de lagos y ríos de Europa se han registrado concentraciones de hasta  $2 \mu\text{g}/\text{litro}$ , pero los niveles medios son generalmente inferiores a  $100 \text{ ng}/\text{litro}$ . En la región de los Grandes Lagos del Canadá se han detectado niveles muy inferiores

(de aproximadamente  $1 \text{ ng}/\text{litro}$ ). Allí los niveles en el sedimento del fondo pueden ser de  $120 \mu\text{g}/\text{kg}$  de peso en seco. En capas más antiguas de sedimento, de 1960 aproximadamente, se encontraron concentraciones más elevadas (de hasta  $550 \mu\text{g}/\text{kg}$  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  $1000 \mu\text{g}/\text{kg}$  de peso húmedo en varias especies y de  $120 \text{ mg}/\text{kg}$  (base grasa) en una especie. Lejos de los efluentes industriales, los niveles actuales se mantienen en general por debajo de  $100 \mu\text{g}/\text{kg}$  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  $40 \mu\text{g}/\text{kg}$  y, en un caso, más de  $1000 \mu\text{g}/\text{kg}$ .

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

#### 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 hexacloro-butadieno 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

en 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 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.

Los 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.

con En una prueba de inhalación de corto plazo (6 horas por día durante 12 días) se observaron efectos semejantes en los riñones una concentración de vapor nominal de 267 mg/m<sup>3</sup>, 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 día, 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 anomalías 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<sup>3</sup> 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 glutati6n. En un estudio *in vivo* se observ6 que habia inducido aberraciones cromos6micas, pero no se observaron tales aberraciones en dos estudios *in vitro*. En una prueba *in vitro* se observ6 que la frecuencia de los intercambios entre crom6tidas hermanas habia aumentado en las c6lulas ov6ricas de h6msters de China. Se ha se6alado el gran potencial mutag6nico de los metabolitos sulfurosos del hexaclorobutadieno. En estudios *in vitro*, el compuesto indujo s6ntesis imprevistas de ADN en cultivos de fibroblastos de embriones de h6msters de Siria, pero no en cultivos de hepatocitos. Indujo s6ntesis imprevistas de ADN en ratas *in vivo*, pero no indujo mutaciones letales recesivas ligadas al sexo en *Drosophila melanogaster*.

En el 6nico estudio de largo plazo (dos a6os), en el cual las ratas recibieron una dieta que contenia hexaclorobutadieno en dosis de 0,2, 2 6 20 mg/kg de peso corporal por d6a, se observ6 una mayor incidencia de neoplasias de los tubos renales 6nicamente con la dosis m6s elevada.

### 7.4 Mecanismos de toxicidad

La nefrotoxicidad, mutagenicidad y carcinogenicidad del hexaclorobutadieno depende de la bios6ntesis del conjugado sulfuroso t6xico 1-glutati6n- S-yl-1,2,3,4,4-pentaclorobutadieno. Este conjugado se sintetiza principalmente en el h6gado y se metaboliza luego en la bilis, el intestino y los ri6ones convirti6ndose en 1-ciste6na- S-yl-1,2,3,4,4-pentaclorobutadieno (CPB). La activaci6n de CPB, que depende del conjugado de ciste6na beta-lyasa, en una tiocetena reactiva en las c6lulas de los tubos proximales finalmente da lugar a un enlace covalente con macromol6culas celulares.

## 8. Efectos en el ser humano

No se han descrito efectos patog6nicos en la poblaci6n 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<sup>3</sup> 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<sup>3</sup>.

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 hexaclorobutadieno, las observaciones realizadas en animales son limitadas y las efectuadas en el ser humano insuficientes.

Tras la administración oral a largo plazo del hexacloro-butadieno 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 alimentos con elevado contenido de lípidos.

## 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.

See Also:

[Toxicological Abbreviations](#)  
[Hexachlorobutadiene \(HSG 84, 1993\)](#)  
[Hexachlorobutadiene \(ICSC\)](#)  
[Hexachlorobutadiene \(IARC Summary & Evaluation, Volume 20, 1979\)](#)  
[Hexachlorobutadiene \(IARC Summary & Evaluation, Volume 73, 1999\)](#)