The MAK-Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Hexachlorobutadiene

MAK Value Documentation, supplement – Translation of the German version from 2016

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Keywords

hexachlorobutadiene; kidney; nephrotoxicity; carcinogenicity; maximum workplace concentration; MAK value; peak limitation; developmental toxicity; skin absorption

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated hexachlorobutadiene [87-68-3], to derive a maximum concentration at the workplace (MAK value), considering all toxicity end points. Available unpublished study reports and publications are described in detail. The critical effect of hexachlorobutadiene is its toxicity and carcinogenicity in the kidney. In a 2-year feeding study in rats, hexachlorobutadiene caused significantly increased incidences of adenomas and carcinomas of the kidneys only at the highest dose of 20 mg/kg body weight, whereas nephrotoxicity was observed from 2 mg/kg body weight. From the NOAEL of 0.2 mg/kg body weight in this study, a MAK value of 0.02 ml/m^3 is derived. As the critical effect is systemic, Peak Limitation Category II is assigned. The default excursion factor of 2 is set as no half-life is known. Concerning the carcinogenicity of hexachlorobutadiene, the Commission concluded that a non-genotoxic mode of action is of prime importance and genotoxic effects play at most a minor part provided the MAK value is observed. Therefore, hexachlorobutadiene was assigned to Category 4 of carcinogenic substances. As the possible genotoxicity of hexachlorobutadiene is specific for the kidney and it has not been shown to reach the germ cells in an active form, hexachlorobutadiene is not classified in any of the categories for germ cell mutagens. The difference between the NOAEC for developmental toxicity in rats of 10 ml/m^3 and the MAK value is sufficiently large, so that damage to the embryo or foetus is unlikely when the MAK value is observed. Thus, the substance is classified in Pregnancy Risk Group C. Skin contact may contribute significantly to systemic toxicity and hexachlorobutadiene continues to be designated with an "H" notation. Sensitization is not expected from the limited data.

Citation Note:
Hartwig A, MAK Commission. Hexachlorobutadiene. MAK Value Documentation, supplement – Translation of the German version from 2016. MAK Collect Occup Health Saf. 2022 Jun;7(2):Doc030. https://doi.org/10.34865/mb8768e7_2ad

Manuscript completed:
24 Jul 2014

Publication date:
29 Jun 2022

MAK value (2015)	**0.02 ml/m³ (ppm) ≙ 0.22 mg/m³**
Peak limitation (2015)	**Category II, excursion factor 2**
Absorption through the skin (1983)	**H**
Sensitization	**–**
Carcinogenicity (2015)	**Category 4**
Prenatal toxicity (2015)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	HCBD 1,3-hexachlorobutadiene hexachlorobuta-1,3-diene perchlorobutadiene
Chemical name	1,1,2,3,4,4-hexachloro-1,3-butadiene
CAS number	87-68-3
Structural formula	$Cl_2C{=}CCl{-}CCl{=}CCl_2$
Molecular formula	C_4Cl_6
Molar mass	260.76 g/mol
Melting point	–21 °C (IFA 2013)
Boiling point at 1013 hPa	215 °C (IFA 2013)
Density at 20 °C	1.55 g/cm³ (ATSDR 1994)
Vapour pressure at 25 °C	0.29 hPa (IFA 2013)
log K_{OW}	4.78 (IFA 2013)
Solubility	2–4 mg/l water (20 °C) (BUA 1991)
1 ml/m³ (ppm) ≙ 10.82 mg/m³	**1 mg/m³ ≙ 0.092 ml/m³ (ppm)**

For hexachlorobutadiene (HCBD), there is documentation available from 1983 (Henschler 1983, available in German only). As new data have become available, this supplement has become necessary. It is based mainly on a BUA substance report and an ATSDR report (ATSDR 1994, 2012; BUA 1991).

1 Toxic Effects and Mode of Action

Hexachlorobutadiene is moderately irritating to the skin. Earlier studies in rats, guinea pigs and rabbits with repeated inhalation exposure to hexachlorobutadiene, revealed histological damage to the kidneys and liver at concentrations of 3 ml/m³ and above. In rats, also respiratory disturbances occurred at higher concentrations of 25 ml/m³ and above, histological lung damage at 30 ml/m³ and above, and irritation of the eyes, nose and the respiratory tract at 100 ml/m³ and above.

The main target organ of toxicity in rats and mice after ingestion of hexachlorobutadiene is the kidney, where degeneration, necrosis and regeneration of tubular epithelial cells occurred. In addition, effects on the liver and lymphatic organs were found at higher doses.

In a 2-year feeding study in rats, significantly increased incidences of renal tumours were described at the nephrotoxic dose of 20 mg hexachlorobutadiene/kg body weight. An initiation–promotion experiment in rats confirmed a tumour-promoting effect of hexachlorobutadiene on the kidneys.

In in vitro genotoxicity studies, positive results were obtained usually only under conditions necessary for the activation of hexachlorobutadiene to the reactive thioketene or if the respective metabolites were tested. In vivo studies revealed the increased binding of hexachlorobutadiene to mitochondrial DNA, especially of the kidneys, but also of the liver. In addition, DNA synthesis and DNA repair synthesis were induced in the kidneys. In a study of limited validity, chromosomal aberrations were observed after inhalation exposure of mice to hexachlorobutadiene. A dominant lethal test in rats yielded negative results.

In rats, effects on the body weights of the foetuses and the dams were observed at the highest concentration tested of 15 ml/m^3 after inhalation exposure from gestation days 6 to 20. After dietary administration of hexachlorobutadiene from gestation day 17 to postnatal day 10 or during mating, gestation and lactation, the body weights of the offspring of the rats were reduced at 17 and 14 mg/kg body weight, respectively, on postnatal day 10, while at the same time marked maternal toxicity in the form of reduced body weights and feed consumption and nephrotoxicity occurred.

A maximization test for skin sensitization in guinea pigs yielded a positive result with and without the use of an adjuvant. Due to the shortcomings of this study, for example incomplete documentation and the lack of control groups, the suspected skin-sensitizing effect could not be verified.

2 Mechanism of Action

2.1 Nephrotoxicity

The cysteine derivative of hexachlorobutadiene formed after glutathione conjugation of hexachlorobutadiene in the liver and after enzymatic degradation by γ-glutamyl transferase (GGT) and dipeptidase can be metabolized to a thioketene by renal β-lyase activity (see also Section 3.2). The covalent binding of the thioketene to DNA, proteins and other macromolecules is believed to be responsible for the cytotoxic and genotoxic effects of hexachlorobutadiene and its metabolites. The limitation of these effects to the proximal tubule can probably be attributed on the one hand to uptake processes leading to a concentration of the cysteine conjugate or its precursors in the epithelial cells, and on the other hand to the localization of GGT, dipeptidase and β-lyase activity in this renal segment (Anders and Dekant 1998; US EPA 2003).

In vitro studies indicate that the mitochondria of the renal tubular epithelial cells are the main targets of the toxicity of hexachlorobutadiene or its metabolites. The reactive metabolites are thought to interact with the components of the inner mitochondrial membrane. This initially leads to an uncoupling of oxidative phosphorylation and to the inhibition of ATP formation. The decrease in the ATP concentration inhibits ATP-dependent reabsorption processes in the tubule. The damage to the mitochondria also leads to the inhibition of cytochrome C oxidase and to the disruption of electron transport and, in the proximal tubule, to cell death (US EPA 2003). Further investigations indicate a direct effect of the reactive hexachlorobutadiene metabolites on mitochondrial DNA (mtDNA) (Schrenk and Dekant 1989). Renal mtDNA may be the preferential target due to the high concentration of β-lyase in the mitochondrial membrane, the lack of protective histones associated with mitochondrial DNA, and an inadequate repair function. Mutations in the mtDNA can lead to a respiratory chain deficiency and cell dysfunction (US EPA 2003).

It has been demonstrated that the excretion of hexachlorobutadiene with the urine in male rats is associated with the binding of hexachlorobutadiene to α_{2u}-globulin. Its binding to α_{2u}-globulin may be involved in the renal toxicity of hexachlorobutadiene in male rats (Pähler et al. 1997).

In addition, the more pronounced renal toxicity in males compared with in female Wistar rats after single oral doses of hexachlorobutadiene has been attributed to the formation of the sulfoxide of *N*-acetylpentachlorobutadienylcysteine (Birner et al. 1995, 1998). Similarly, in Sprague Dawley and NBR rats, acute renal toxicity is more pronounced in males than in females (Pähler et al. 1997). In other studies, however, after a single intraperitoneal injection in Alderley Park rats (Wistar-derived) (Hook et al. 1983) or after repeated oral administration of hexachlorobutadiene to Wistar-derived rats, the females have been shown to be more sensitive regarding histopathological changes in the kidneys (Harleman and Seinen 1979; Jonker et al. 1993). The reasons for these different observations and thus the relevance of the sulfoxide for renal toxicity are not known. However, the increase in renal toxicity in male rats after induction with phenobarbital (Hook et al. 1983) suggests that this is related to increased sulfoxidation after the induction of enzymes of the cytochrome P450 (CYP) 3A family and that, in rats, the sulfoxide is important for the renal effects. The absence of an effect on the induction or inhibition of CYP enzymes in male mice compared with the renal toxicity of hexachlorobutadiene (Lock et al. 1984) suggests that the β-lyase pathway plays the major role in this species and that sulfoxidation is only of minor importance.

2.2 β-Lyase activity

At least 11 enzymes with β-lyase activity have now been identified in mammalian tissues. The most important enzymes are glutamine transaminase K (GTK) and mitochondrial aspartate aminotransferase (AST). These enzymes are widely distributed in mammalian tissues. In rats, GTK was detected in almost all tissues investigated (Cooper et al. 2010; Cooper and Pinto 2006).

The specific GTK activity was demonstrated in rats not only in the kidneys and liver but also in the testes and seven other tissues. The β-lyase activity measured by the conversion of dichlorovinylcysteine (DCVC), a metabolite of trichloroethylene, was 3.05 and 1.32 in the kidneys, 0.84 and 0.22 in the liver and 0.40 and 0.07 nmol/min/mg protein in the testes, respectively, in the presence and absence of an activating α-keto-acid (Jones et al. 1988). The β-lyase activity in the kidneys is thus 8 and 19 times as high, respectively, as that in the testes.

From a study with trichloroethylene in rats, the authors conclude that there is little evidence for the existence of β-lyase activity in the epididymis and the efferent ducts, which connect the testis with the epididymis (DuTeaux et al. 2003).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

3.1.1 Absorption

Hexachlorobutadiene can be absorbed following inhalation and oral or dermal exposure (BUA 1991).

There are no studies available for the absorption of hexachlorobutadiene after inhalation exposure. However, since effects after inhalation exposure have been described, absorption can be assumed (ATSDR 1994).

Information on absorption can be derived from several studies investigating the distribution and excretion of hexachlorobutadiene after oral administration (see Section 3.1.3). In female Wistar rats, 72 hours after a single oral dose of 1 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight (as a suspension in glycerol trioctanoate), 5.3% was exhaled unchanged, 42.1% was excreted predominantly unchanged with the faeces and 30.6% after metabolism with the urine, and 3.6% was exhaled as CO_2. After administration of 50 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight, 5.4% was exhaled unchanged, 69% was excreted unchanged with the faeces and 11% after metabolism with the urine, and

1.2% was exhaled as CO_2. From the results it can be concluded that, at the high dose, absorption was limited and the metabolism saturated (Reichert et al. 1985).

Almost complete absorption was reported 16 hours after oral administration of 200 mg ^{14}C-labelled hexachlorobutadiene/kg body weight (in corn oil) to male Wistar rats (Nash et al. 1984).

After oral doses of 1 or 100 mg ^{14}C-labelled hexachlorobutadiene/kg body weight in aqueous polyethylene glycol to male Sprague Dawley rats, 18% and 9%, respectively, of the label was excreted with the urine within 72 hours (Payan et al. 1991). Compared with the results obtained by Reichert et al. (1985), it is clear that gastrointestinal absorption may be improved by administration in a lipophilic vehicle (glycerol trioctanoate) (US EPA 2003).

Undiluted hexachlorobutadiene (388 to 1550 mg/kg body weight) was completely absorbed within 8 hours after application to rabbit skin (ATSDR 1994; Duprat and Gradiski 1978).

Using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), absorbed amounts of 75, 0.9 and 0.3 mg hexachlorobutadiene, respectively, are calculated for a saturated aqueous solution (4 mg/l), assuming the exposure of 2000 cm^2 of skin for 1 hour.

3.1.2 Distribution

After single oral or intravenous doses of ^{14}C-labelled hexachlorobutadiene of 0.58 to 166 mg/kg body weight, about 10% of the radioactivity was detected in the body fat of F344 rats after 72 hours. At this time 0.5% to 2% of the administered dose was found in muscle, the kidneys and skin. The levels in muscle, body fat and the kidneys decreased to 0.3% to 0.4% of the dose after 240 hours. The highest concentrations occurred in the kidneys of all animals except at 166 mg/kg body weight (tissue/blood ratios of 42–74:1). The concentrations in plasma after intravenous administration were 2.4 to 3.8 times as high as those after oral administration of similar doses (NTP 1985).

In B6C3F1 mice, the highest concentrations in the kidneys occurred 10 days after oral administration of 2 mg ^{14}C-labelled hexachlorobutadiene/kg body weight. The tissue/blood ratio was 17:1 (NTP 1985).

After oral administration of ^{14}C-labelled hexachlorobutadiene to rats or mice, 6% to 14% of the label was detected in the tissues and carcass 72 hours later (Dekant et al. 1988 a; Reichert et al. 1985).

After single gavage doses of up to 200 mg ^{14}C-labelled hexachlorobutadiene/kg body weight to rats, the radioactivity accumulated in the kidneys (outer medulla), liver and adipose tissue (Dekant et al. 1988 a; Nash et al. 1984; Reichert et al. 1985).

72 hours after oral administration of ^{14}C-labelled hexachlorobutadiene to rats, covalent binding of the radioactivity to proteins was higher in the kidneys than in the liver (Reichert et al. 1985).

After bile duct-cannulated donor rats had been given a gavage dose of ^{14}C-labelled hexachlorobutadiene of 100 mg/kg body weight, their bile was passed directly into the duodenum of untreated recipient rats. The level of radioactivity was examined in the liver, kidneys and plasma of donor and recipient rats. In the donor rats, after 30 hours 0.26% of the dose was detected in the kidneys, 0.11% in the liver and 0.013% in the blood plasma. In the recipient rats the kidneys contained 0.15%, the liver 0.07% and the plasma 0.009% of the dose. In all tissues examined, the level of radioactivity from the absorbed metabolites was about two thirds of the original dose. In both cases, the kidneys contained higher levels of the label than the liver, indicating that the kidney is a target organ (Payan et al. 1991).

After intraperitoneal injection of 0.1 and 300 mg ^{14}C-labelled hexachlorobutadiene/kg body weight in rats, the label was found 48 hours later in the liver (2.6% and 2.3% of the dose, respectively), kidneys (2.5% and 0.5% of the dose) and adipose tissue (0.3 µg/g and 856 µg/g). Very small amounts were found in the lungs and heart (0.1% to 0.2% of the dose), brain (0.05% to 0.1% of dose) and muscle tissue (200-fold lower concentration than in adipose tissue) (Davis et al. 1980).

3.1.3 Elimination

After oral administration of 200 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight to male Alderley Park rats, 35% of the radioactivity was excreted within 48 hours with the bile. During the same period only 5% and, within 5 days, 39% of the radioactivity was excreted with the faeces and a maximum of 3.5% per day with the urine (Nash et al. 1984).

Male F344 rats were given single intravenous injections of ^{14}C-labelled hexachlorobutadiene at dose levels of 0.58, 1.8 or 27 mg/kg body weight in propylene glycol or Carbowax 200 and single oral doses of 0.7, 2.1, 5.8, 34 or 166 mg/kg body weight in corn oil. The excreta were collected for 72 or 240 hours. After administration of doses up to and including 34 mg/kg body weight, 47% to 63% of the radioactivity was found in the faeces and 15% to 26% in the urine within 3 days. After an oral dose of 166 mg/kg body weight, only 5% to 20% was excreted with the faeces and 5% to 6% with the urine within 72 hours; after 72 hours, however, about 10% of the radioactivity was found in the stomach, 34% in the caecum, 3% in the small intestine and 4% in the large intestine (NTP 1985).

Male B6C3F1 mice were given single gavage doses of ^{14}C-labelled hexachlorobutadiene of 2, 24 and 50 mg/kg body weight in corn oil or single intravenous injections of 19 mg/kg body weight. After 10 days, 51%, 53% and 35% of the dose, respectively, was excreted with the faeces after oral administration and 52% after intravenous administration. Most of this (80% to 95%) was excreted within the first 48 hours. After oral administration of 24 or 50 mg/kg body weight, the total proportion of radioactivity excreted was 83% and 51%, respectively, and that excreted with the urine was 20% and 5%, respectively. Both levels were significantly lower than those excreted after a dose of 2 mg/kg body weight (total percentages excreted in the urine of 93% and 31%). Up to 4% of the dose was excreted as unchanged hexachlorobutadiene with the urine within 12 hours after the administration. In the exhaled air 8% to 10% of the radioactivity was detected within 48 hours. After oral administration of 24 mg/kg body weight, about 4% of the dose was found in the faeces within 24 hours as volatile compounds, presumably hexachlorobutadiene (NTP 1985).

After female rats were given single oral doses of ^{14}C-labelled hexachlorobutadiene of 1 and 50 mg/kg body weight, 42% and 69% of the radioactivity was detected within 72 hours in the faeces, 31% and 11% in the urine and 5% each in the exhaled air as unchanged hexachlorobutadiene and 3.6% and 1% as $^{14}CO_2$, respectively (Reichert et al. 1985).

In rats, after a single oral dose of 100 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight, 60% of the radioactivity was excreted with the faeces within 72 hours and 5.4% of the radioactivity with the urine within 24 hours (Reichert and Schütz 1986).

After a single oral dose of 30 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight, 67% to 77% of the radioactivity was excreted with the faeces of mice within 72 hours, while excretion with the urine was about 7%. In the exhaled air, 4% to 5% of unchanged hexachlorobutadiene was found; $^{14}CO_2$ was not detected (Dekant et al. 1988 a).

Some of the radioactive label excreted with the urine comes from the biliary metabolites, which are reabsorbed in the intestine and further metabolized in the kidneys for excretion. The proportion of reabsorbed biliary metabolites excreted in the urine was shown in experiments in rats with and without bile duct cannulation (ATSDR 1994).

After the administration of oral doses of ^{14}C-labelled hexachlorobutadiene of 1 or 100 mg/kg body weight in polyethylene glycol to male Sprague Dawley rats, the faeces and intestinal contents contained 62% and 72%, respectively, of the radioactivity after 72 hours. With the urine, 18% and 9% of the label was excreted within 72 hours, respectively. At both dose levels, about 7.5% of the radioactivity was determined in the exhaled air; 2.2% and 0.7%, respectively, of the radioactivity was present as CO_2 (Payan et al. 1991).

In another study with oral administration, 67% of the radioactivity was detected within 72 hours in the bile of bile duct-cannulated rats after a dose of 1 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight, compared with 58% at a dose of 100 mg/kg body weight. In the faeces, 3% and 16% of the radioactive label, respectively, was found. The urine collected over the same period contained 11% of the label at the low dose, and 7% at the high dose (Payan et al. 1991).

After the oral administration of 100 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight, the bile of cannulated rats was passed directly into the duodenum of animals not exposed. Radioactivity levels were determined 30 hours later in the urine, bile and faeces of both groups. The radioactivity in the urine and bile of the recipient rats represented the

portion reabsorbed from the gastrointestinal tract. It was observed that 80% of the biliary metabolites were reabsorbed and only 20% remained in the faeces and gastrointestinal tract (Payan et al. 1991).

After the administration of a single gavage dose of 200 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight to male and female Wistar rats, 16% and 11% of the label was found within 48 hours in the faeces, and 3% and 4.5% in the urine, respectively. In the same period of time, 1% and 2% were detected as hexachlorobutadiene and 0.02% and 0.03% as $^{14}CO_2$ in the exhaled air, respectively (Birner et al. 1995).

In male and female Sprague Dawley and NBR rats, 6% to 12% of the label was found in the faeces and 3% to 4% in the urine within 48 hours after a single gavage dose of 200 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight. In the same period of time, 1% to 3% was detected as hexachlorobutadiene and 0.1% to 0.3% as $^{14}CO_2$ in the exhaled air. Unlike in female animals, the excretion of unmetabolized hexachlorobutadiene was detected in the urine of male Sprague Dawley rats (up to 10% of the metabolites in the urine). No hexachlorobutadiene was found in the urine of NBR rats, which do not produce α_{2u}-globulin. Thus, it could be shown that the excretion of hexachlorobutadiene in the urine of male rats is related to the binding of hexachlorobutadiene to α_{2u}-globulin (Pähler et al. 1997).

After intravenous injection of 1 or 100 mg ^{14}C-labelled hexachlorobutadiene/kg body weight in rats, the excretion of the radioactive label was followed for 72 hours. At both doses, about 8% of the radioactivity was detected in the exhaled air; 2.6% and 0.9% of the radioactivity was present as CO_2. In the urine, 21% of the label was found at the low dose and 9% at the high dose. In the faeces, the percentages were 59% and 72%, respectively. In a parallel study with bile duct-cannulated rats, excretion of the label was determined in the faeces, urine and bile. The urine contained 6% to 7% and the faeces less than 0.5% at both dose levels. In the bile, 89% was detected in the 1 mg/kg group and 72% in the 100 mg/kg group (Payan et al. 1991).

3.1.4 Summary

Hexachlorobutadiene is absorbed after inhalation, oral or dermal exposure. It accumulates in the kidneys, liver and adipose tissue. In animal experiments, hexachlorobutadiene and its metabolites were found to be excreted in the faeces, urine and with the exhaled air. After a single oral dose of 1 mg/kg body weight, up to 30% of the dose of hexachlorobutadiene and its metabolites could be found in the urine. At high oral doses of up to 200 mg/kg body weight, this proportion dropped to 5% to 10%. After a single oral dose of 200 mg hexachlorobutadiene/kg body weight, 39% of the dose was found in the faeces collected over 5 days. The faeces contained unmetabolized, unabsorbed hexachlorobutadiene and some of the metabolites excreted with the bile. At the lower doses, almost all the radioactive label was from the metabolites, while at the high doses, the faeces contained also unabsorbed hexachlorobutadiene (ATSDR 1994). In rats given single oral doses of 1 to 100 mg of ^{14}C-labelled hexachlorobutadiene/kg body weight, up to 7.5% was exhaled within 72 hours as unchanged hexachlorobutadiene and 0.7% to 3.6% as carbon dioxide.

3.2 Metabolism

A diagram of the metabolism of hexachlorobutadiene is shown in Figure 1.

For trichloroethylene and tetrachloroethylene, oxidative metabolism in the liver is of particular importance (Greim 1998; Hartwig 2014); in contrast, this metabolic pathway could not be demonstrated in several studies with hexachlorobutadiene in vitro with rat and mouse liver microsomes (Dekant et al. 1988 a; Wolf et al. 1984) and in vivo in rats (Nash et al. 1984; Reichert et al. 1985; Reichert and Schütz 1986) and mice (Dekant et al. 1988 a). The release of $^{14}CO_2$ in the exhaled air (up to 0.3% of the radioactivity after 48 hours and up to 3.6% after 72 hours) shown in some studies with rats (Birner et al. 1995; Pähler et al. 1997; Payan et al. 1991; Reichert et al. 1985), but not with mice (Dekant et al. 1988 a) after the administration of ^{14}C-labelled hexachlorobutadiene could also have been caused by decarboxylation of a metabolite derived from thioketene (No. 6 in Figure 1). However, no other experimental data are available.

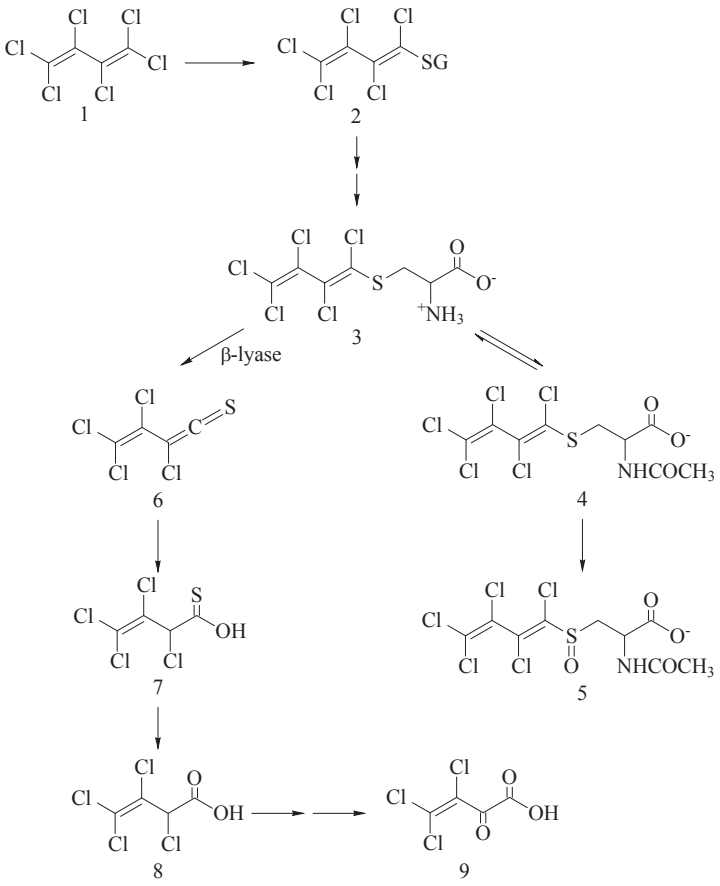

Fig. 1 Metabolism of hexachlorobutadiene (Dekant 2014)

Hexachlorobutadiene (No. 1 in Figure 1) is conjugated with glutathione in the liver. In rats both mono-substituted and di-substituted conjugates have been identified, whereas in mice probably only mono-substituted glutathione conjugates are formed (ATSDR 1994).

Metabolites detected in the urine of rats and mice include *S*-(1,1,2,3,4-pentachlorobutadienyl)glutathione (No. 2 in Figure 1); *S*-(1,1,2,3,4-pentachlorobutadienyl)cysteine (No. 3 in Figure 1); 1,1,2,3,-tetrachlorobutenoic acid (No. 8 in Figure 1); 1,1,2,3,4-pentachlorobutadienyl sulfenic acid; *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-*L*-cysteine (No. 4 in Figure 1); *S*-pentachlorobutadienylmercaptoacetic acid; 1,1,2,3,4-pentachlorobutadienylmethyl thioether and 1,1,2,3,4-pentachlorobutadienylcarboxymethyl thioether (Dekant et al. 1988 b; Nash et al. 1984; Reichert et al. 1985; Reichert and Schütz 1986).

The only metabolite identified in the faeces of mice was *S*-(1,1,2,3,4-pentachlorobutadienyl)glutathione (No. 2 in Figure 1), with other unidentified metabolites present which were probably cysteine derivatives (Dekant et al. 1988 a).

A major metabolite of hexachlorobutadiene in the urine of male Wistar rats is the sulfoxide of *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-*L*-cysteine (No. 5 in Figure 1). In a study with human liver microsomes from male and female donors, sulfoxidation of *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-*L*-cysteine (No. 4 in Figure 1) was demonstrated. Experiments with CYP inhibitors and with recombinant CYP enzymes have shown that enzymes of the CYP3A family are responsible for the sulfoxidation. Since enzymes of the CYP3A family represent an important fraction of CYP enzymes in the human liver and occur also in the human kidney, it can be assumed that the sulfoxidation of *N*-acetylpentachlorobutadienylcysteine occurs also in humans (Birner et al. 1995; Werner et al. 1995).

A comparison of the kinetic parameters of the enzymes involved in the sulfoxidation of *N*-acetylpentachlorobutadienylcysteine determined in liver microsomes from rats (Birner et al. 1995) and humans (Werner et al. 1995) shows that the reaction rate (V_{max}) in humans is about 5 times as high as that in rats, while the value for K_m in humans is about 2.5 times as high. Overall, the activity as a ratio of V_{max}/K_m in the rat is 52% of the value in humans.

The possible significance of sulfoxidation for the renal toxicity of hexachlorobutadiene in male rats was demonstrated by increased toxicity in a study after the induction of CYP enzymes by pretreatment with phenobarbital. Female animals were not examined (Hook et al. 1983; see Section 5.1.4). In male mice, however, the induction (phenobarbital, β-naphthoflavone) or inhibition (piperonyl butoxide) of CYP enzymes had no effect on the renal toxicity of hexachlorobutadiene. Female animals were not studied (Lock et al. 1984; see Section 5.1.4). This suggests that sulfoxidation is probably of minor importance in mice.

The activities of liver and kidney enzymes relevant for the β-lyase-dependent toxicity of hexachlorobutadiene were compared in an in vitro study in rats and humans (see Table 1). Tissue samples from the liver and kidneys of male Alderley Park rats and human liver (3 male donors) and kidney tissue (4 male and 2 female donors) were examined. In the liver microsomes, the activity of glutathione *S*-transferase was determined via the formation of pentachlorobutadienylglutathione. The reaction rate (V_{max}) was about 5 times as high in the rat liver microsomes as that in the human samples. The affinity constant (K_m) was about 1.3 times as high in the rats compared with that in the human samples. In vitro studies with rat and human liver showed that the conjugation of hexachlorobutadiene with glutathione was faster with microsomal glutathione *S*-transferases than with the cytosolic enzymes (Dekant et al. 1988 b; Oesch and Wolf 1989; Wallin et al. 1988; Wolf et al. 1984). The β-lyase activity of the kidneys was determined on the basis of the cleavage of pentachlorobutadienylcysteine. In human renal cytosol, the reaction rate could not be determined. The reaction rate in rats was about 23 times as high as the detection limit. In the mitochondrial fraction of the kidney, the activity was higher; here, V_{max} was 3 times as high in the rat samples as in the corresponding human samples. The K_m values of the mitochondrial fractions of humans and rats were similar. To determine the *N*-acetyltransferase activity in renal microsomes, the formation of *N*-acetyl-*S*-(1,1,2,3,4-pentachlorobutadienyl)-*L*-cysteine was determined. The reaction rate in rats was about 4 times as high as that in human samples. Compared with the competing β-lyase reaction, V_{max} was 27 times as high in rats and 21 times as high in humans. The acylase activity in the renal cytosol was determined on the basis of the formation of pentachlorobutadienylcysteine. No activity was found in the human samples. The activity in rats was 74 times as high as the detection limit of the system. Using a physiologically-based toxicokinetic (PBTK) model, the metabolism of the substance via the β-lyase pathway after inhalation exposure of rats was estimated to be approximately 20 times as high as that in humans. The authors state that for the rat an oral dose of 0.2 mg/kg body weight and day corresponds to an inhaled concentration of 0.07 ml/m^3 after exposure for 24 hours (Green et al. 2003). In their modelling, the authors assume an alveolar ventilation rate of 0.35 m^3 per hour for humans, which corresponds to a respiratory volume of 2.8 m^3 within 8 hours. Assuming a respiratory volume of 10 m^3 within 8 hours, which is usual for the assessment of the workplace situation, the difference between humans and rats is reduced to a factor of 5. One limitation is that the calculations were not validated by measured values. In addition, there are deficiencies in the documentation of the modelling; this may be incorrect because changes in glutathione concentration were not taken into account. Overall, however, it is nevertheless probable that humans are less exposed than rats to the critical metabolites of the β-lyase pathway due to the marked differences in enzyme activity. Furthermore, it is known from other haloalkenes, such as tetrachloroethylene and the sevoflurane metabolite compound A, that humans are less exposed to the metabolites of the β-lyase pathway than rats (Kharasch and Jubert 1999; Pähler et al. 1999 a, b; Völkel et al. 1998).

Tab. 1 In vitro comparative study of the metabolism of hexachlorobutadiene (Green et al. 2003)

Reaction, species	K_m (mM)	V_{max} (nmol/min/mg)
glutathione conjugation (liver microsomes)		
humans (mean value)	0.16	0.25
rats	0.21	1.23

Tab. 1 *(continued)*

Reaction, species	K_m (mM)	V_{max} (nmol/min/mg)
β-lyase activity (kidney cytosol)		
humans (mean value)	–	<0.05
rats	0.30	1.13
β-lyase activity (kidney mitochondria)		
humans (mean value)	0.25	1.76
rats	0.12	4.17
N-acetyltransferase activity (kidney microsomes)		
humans (mean value)	0.19	37.6
rats	0.39	144.9
acylase activity (kidney cytosol)		
humans (mean value)	–	<0.10
rats	1.43	7.36

4 Effects in Humans

There are no data available in humans concerning single exposures, effects on skin and mucous membranes, allergenic effects, reproductive toxicity or carcinogenicity.

4.1 Repeated exposure

In a group of 153 vineyard workers seasonally exposed to hexachlorobutadiene and polychlorobutane-80 (1.2 to 10 mg/m^3 in the inhaled air over gas-treated areas), increased hypotension, heart disease, gastrointestinal complaints, blood count changes, upper respiratory tract changes, liver disease and nervous complaints were observed compared with the findings in a control group of 52 persons (BUA 1991).

In workers, after long-term exposure to estimated hexachlorobutadiene concentrations of 0.005 to 0.02 ml/m^3, serum concentrations of bile acids (deoxycholic acid, glycine deoxycholic acid, taurine-chenodeoxycholic acid and total deoxycholates) were increased. The workers may have been exposed to other solvents (carbon tetrachloride and tetrachloroethylene). Due to exposure to a mixture of substances and the lack of liver parameters, the study is of limited use for evaluation (ATSDR 1994).

A longitudinal prevalence study was conducted with residents of houses in England where hexachlorobutadiene concentrations in the air in the range of 0.0006 up to 0.0068 ml/m^3 (in one house up to 1 ml/m^3) were found. The subjects had left their homes within 2 months before the start of the study. Specific urinary markers for renal tubular and glomerular function were used (albumin, transferrin, retinol binding protein (RBP), *N*-acetyl-β-glucosaminidase (NAG), GGT, leucine aminopeptidase (LAP), α-glutathione *S*-transferase (α-GST), π-glutathione *S*-transferase (π-GST)). The comparison of the results from 37 participants aged 16 to 65 years with the reference values from healthy workers without exposure to nephrotoxic substances revealed increased prevalences of abnormal values for α-GST (22%), GGT (22%), LAP (19%) and π-GST (22%). When the 37 participants were re-examined 10 months after they left their home, a significant decrease in elevated biomarkers was observed. At 14%, increased GGT activity was the only marker with a prevalence exceeding 10% (Staples et al. 2003). It is unclear whether the measured exposure to hexachlorobutadiene alone is responsible for the increased biomarkers.

4.2 Genotoxicity

Due to qualitative shortcomings, a study from 1986 of the frequency of chromosomal aberrations in exposed workers cannot be included in the evaluation of hexachlorobutadiene (Hughes et al. 2001).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

In rats, the first deaths occurred after 2 hours of exposure to 200 ml/m^3 (2160 mg/m^3) (BUA 1991).

All rats survived inhalation exposure to hexachlorobutadiene concentrations of 161 ml/m^3 for 0.88 hours and to 34 ml/m^3 for 3.5 hours (NTP 1991). Some or all rats died after exposure to 133 to 500 ml/m^3 for 4 to 7 hours (NTP 1991). Most guinea pigs and cats died after exposure to 161 ml/m^3 for 0.88 hours or to 34 ml/m^3 for 7.5 hours (NTP 1991).

In mice, exposure to hexachlorobutadiene concentrations of 155 ml/m^3 or higher for 15 minutes resulted in reduced respiratory rates. This observation is attributed by the authors to nasal irritation (de Ceaurriz et al. 1988).

After exposure of mice to hexachlorobutadiene concentrations of 2.75 to 25 ml/m^3 for 4 hours, an increase in the number of damaged renal tubules to between 4% and 91% (controls: 0.2% to 1.5%) was observed (de Ceaurriz et al. 1988).

5.1.2 Oral administration

In rats, the oral LD_{50} of hexachlorobutadiene was between 200 and 580 mg/kg body weight; in mice it was in the range of 65 to 116 mg/kg body weight. Hamsters were less sensitive with an LD_{50} of 960 to 1920 mg/kg body weight, whereas guinea pigs were the most sensitive species with an LD_{50} of 90 mg/kg body weight (BUA 1991).

Groups of 4 male and 4 female Wistar rats were given single gavage doses of ^{14}C-labelled hexachlorobutadiene of 200 mg/kg body weight. After 48 hours, histopathological examination of the kidneys revealed pronounced necrosis of the proximal tubular epithelium, which was more severe in males than in females. The increase in the urinary activity of GGT as a marker of tubular damage was also more pronounced in the male rats. In addition, slight liver damage was observed in the male rats, while the histopathological examination of the liver in the female animals did not yield unusual findings (Birner et al. 1995).

In Swiss mice given single oral hexachlorobutadiene doses of 80 mg/kg body weight, damage to about 50% of the proximal renal tubules was observed after 8 hours. Pre-treatment of the animals with a GGT inhibitor reduced the number of damaged tubules by 59%. Pre-treatment with β-lyase inhibitors reduced the damage by 46% to 59%. The authors concluded that both enzymes play an important role in the nephrotoxicity of hexachlorobutadiene (de Ceaurriz and Ban 1990).

5.1.3 Dermal application

Dermal application of 126 mg hexachlorobutadiene/kg body weight for 24 hours was lethal to all 4 rabbits used. Application of the same dose to 2 rabbits for only 7 hours was lethal in 1 animal. The animals survived dermal exposure to 126 mg hexachlorobutadiene/kg body weight for 4 hours or exposure to 63 mg/kg body weight for 24 hours (BUA 1991).

Dermal application of undiluted hexachlorobutadiene to the shaved dorsal skin of 10 female rabbits in doses of 0, 0.25, 0.5, 0.75 or 1.0 ml/kg body weight (0, 388, 775, 1163 or 1550 mg/kg body weight at a density of 1.55 g/cm^3) for 8 hours caused drowsiness in the animals. The application was carried out with vials, which were attached to the animals' backs. The hexachlorobutadiene was completely absorbed at all dose levels. At the end of the exposure period, bleeding and necrosis of the skin were observed, which increased with time. In some animals of both high dose groups dyspnoea and cyanosis were observed; they died within 24 hours. In these animals, the gross pathological examination revealed congestion in the lungs, liver and kidneys. Histopathological examination revealed degenerative changes in the epidermis, oedema and leukocyte infiltration in the dermis and subcutaneous tissue, fat deposits in the liver and degenerative changes in renal tubules. Other animals from the 0.5 ml/kg group and higher died from day 2 to day 9 of the study, exhibiting weakness and anorexia. Scabbing on the skin was observed during this period. Histo-

pathological examination revealed increasing epidermal and dermal necrosis with partial destruction of hair follicles, fatty degeneration of the liver and epithelial necrotizing nephritis. The authors calculated an LD_{50} of 0.72 ml/kg body weight (1116 mg/kg body weight at a density of 1.55 g/cm^3). The surviving animals were killed after 2 and 5 weeks and examined histopathologically. After 2 weeks the skin lesions healed with scar formation. In the liver, distinct fatty degeneration was still observed. This was reversible and disappeared after 5 weeks. After 2 and after 5 weeks, there were signs of renal regeneration in the low dose groups, although damage to the renal epithelium was still present, especially in the high dose group (Duprat and Gradiski 1978).

5.1.4 Intraperitoneal injection

In male and female rats, an LD_{50} of 175 to 216 mg/kg body weight was determined after intraperitoneal injection of hexachlorobutadiene. In male and female mice, the LD_{50} of hexachlorobutadiene after intraperitoneal administration was 67 to 105 mg/kg body weight (BUA 1991).

In adult female rats, a single intraperitoneal injection of 50 mg hexachlorobutadiene/kg body weight caused marked renal tubular necrosis 24 hours after the treatment. In young male rats, similar effects were observed even at 25 mg/kg body weight, whereas in adult male rats, these effects were not observed until 200 mg/kg body weight. Plasma urea levels were significantly increased in adult females at 50 mg/kg body weight and above and in adult males at 100 mg/kg body weight and above. Depending on age, the LD_{50} after intraperitoneal injection in male rats was 57, 96 and 360 mg/kg body weight for 22, 29 and 49-day-old animals, respectively. After the 7-day treatment of young male rats with phenobarbital in their drinking water, the renal toxicity after intraperitoneal administration of hexachlorobutadiene was significantly increased compared with that in animals not subjected to the induction treatment (Hook et al. 1983).

Intraperitoneal injection of a single dose of hexachlorobutadiene of 170 mg/kg body weight in male Wistar rats caused necrosis of the proximal renal tubules. The urine volume was increased 10-fold, urinary protein 5-fold, urinary glucose 175-fold and brush border enzymes 10 to 600-fold, which is evidence of severe proximal tubular damage. These effects were reversible 6 days after the treatment. Histopathological examination revealed signs of regeneration and repair of the lesions 7 days after the treatment, which were completed after 21 days (Kirby and Bach 1995).

After intraperitoneal injection of a single dose of hexachlorobutadiene of 150 mg/kg body weight in male Wistar rats, the urine volume and the urinary brush border enzymes alkaline phosphatase (AP), GGT and alanine aminopeptidase as well as the chloride concentration were increased after 3 and after 4 days (Delacruz et al. 1997).

In male Wistar rats aged 1, 3, 6, 9 or 12 months given a single intraperitoneal injection of a hexachlorobutadiene dose of 100 mg/kg body weight, moderate to marked necrosis was observed in the S3 segment of the proximal tubule of the kidneys, regardless of the age of the animals. These lesions were also related to changes in biomarkers in urine and plasma and gene expression in the renal cortex, which likewise occurred without age-dependency (Zanetti et al. 2010).

After intraperitoneal injection of a single dose of hexachlorobutadiene of 45 mg/kg body weight in male Wistar rats, the animals were killed 1 to 28 days after the treatment and examined. Histopathological examination of the kidneys revealed tubular degeneration from day 1 until day 3 after treatment. This correlated with increased urinary levels of α-glutathione *S*-transferase, albumin, glucose and KIM-1 (kidney injury molecule-1). In the kidneys, gene expression of KIM-1, NAD(P)H dehydrogenase and haem oxygenase-1 was increased. As from the second day after treatment, histopathological examination revealed evidence of tubular regeneration, which correlated with increased urinary levels of KIM-1 and osteopontin and increased gene expression of KIM-1 and annexin A7 (Maguire et al. 2013).

In male and female Alderley Park mice, the intraperitoneal administration of single hexachlorobutadiene doses of 48 µmol/kg body weight (about 12.5 mg/kg body weight) and above led within 24 hours to necrosis of the proximal tubular epithelium and doses of 96 µmol/kg body weight (about 25 mg/kg body weight) and above to increased plasma urea levels. There was no difference between the sexes regarding these findings. The extent of renal damage in the male animals was independent of pretreatment with inducers (phenobarbital, β-naphtoflavone) or inhibitors (piperonyl butoxide) of the CYP-dependent monooxygenases (Lock et al. 1984).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

The studies with inhalation exposure to hexachlorobutadiene are summarized in Table 2. All are earlier studies that do not meet the current requirements in all respects. In particular, the scope of the investigations and the documentation of the studies are limited.

In studies in rats, guinea pigs and rabbits with repeated inhalation exposure to hexachlorobutadiene, histological damage to the kidneys and liver was reported at concentrations of 3 ml/m³ and above (Dow Chemical Company 1962). In rats, higher concentrations of 25 ml/m³ and above additionally caused respiratory difficulty, concentrations at and above 30 ml/m³ histological lung damage and concentrations at and above 100 ml/m³ irritation of the eyes, nose and respiratory tract (Dow Chemical Company 1962; Gage 1970). In a study in rats, no organ changes were observed after 184 days (about 26 weeks) at 1 ml/m³ (Dow Chemical Company 1962).

Tab. 2 Toxicity studies after repeated inhalation exposure to hexachlorobutadiene

Species, strain, number per group	Exposure	Findings	References
rat, Alderley Park, 4 ♂, 4 ♀	**2 days**, 0, 250 ml/m³ 4 hours/day	**250 ml/m³**: eye and nose irritation, respiratory difficulty, kidneys: tubular degeneration; adrenal glands: degeneration of cortex	Gage 1970
rat, CD, 10 ♂, 10 ♀	**5 days**, 0, 10, 50 ml/m³ 7 hours/day	**10 ml/m³**: **NOAEC**; **50 ml/m³**: dizziness, body weights ↓ (♂: –39%, ♀: –16%)	NIOSH 1981
rat, Alderley Park, 4 ♂, 4 ♀	**12 days**, 0, 100 ml/m³ 6 hours/day	**100 ml/m³**: mortality 2/4 ♀, eye and nose irritation, respiratory difficulty, body weights ↓, slight anaemia (♀), kidneys: enlarged, degeneration of cortical tubules with epithelial regeneration; adrenal glands: enlarged	Gage 1970
rat, Alderley Park, 4 ♂, 4 ♀	**15 days**, 0, 25 ml/m³ 6 hours/day	**25 ml/m³**: respiratory difficulty, body weight gains ↓ (♀), kidneys: enlarged, histologically detectable damage to proximal tubules	Gage 1970
rat, Alderley Park, 4 ♂, 4 ♀	**15 days**, 0, 10 ml/m³ 6 hours/day	**10 ml/m³**: body weight gains ↓ (♀)	Gage 1970
rat, Alderley Park, 4 ♂, 4 ♀	**15 days**, 0, 5 ml/m³ 6 hours/day	**5 ml/m³**: no histologically detectable organ changes	Gage 1970
rat, no other details, 5 ♂, 5 ♀	**14 days**, 0, 30 ml/m³ 7 hours/day, 5 days/week (10 exposures)	**30 ml/m³**: mortality 1/10, histological examination: severe damage to the lungs, liver and kidneys in all surviving animals	Dow Chemical Company 1962
rat, no other details, 5 ♂, 5 ♀	**27 days**, 0, 8 ml/m³ 7 hours/day, 5 days/week (19 exposures)	**8 ml/m³**: no mortality, histological examination: pronounced damage to the liver (1/5 ♂; 5/5 ♀) and kidneys (5/5 ♂; 5/5 ♀)	Dow Chemical Company 1962
rat, no other details, 10 ♂, 10 ♀	**143 days**, 0, 3 ml/m³ 7 hours/day, 5 days/week (100 exposures)	**3 ml/m³**: liver: moderate degenerative changes, centrilobular vacuolization; kidneys: glomerulonephritis, degenerative changes of the tubules, interstitial proliferation; ♀: kidney and spleen weights ↑; no incidences given; lungs, heart, spleen, adrenal glands, pancreas and testes: no histopathological findings	Dow Chemical Company 1962

Tab. 2 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, no other details, 10 ♂, 10 ♀	**184 days**, 0, 1 ml/m³ 7 hours/day, 5 days/week (129 exposures)	**1 ml/m³**: **NOAEC**; clinical observations, growth, mortality, body weights, organ weights, blood urea nitrogen, histopathology of lungs, heart, liver, kidneys, spleen, adrenal glands, pancreas and testes: no findings	Dow Chemical Company 1962
rat, no other details	**7 months**, 0, 2.2 ml/m³ no other details	**2.2 ml/m³**: no effects	Gulko et al. 1964
mouse, B6C3F1, 10 ♂	**5 days**, 0, 10, 50 ml/m³ 7 hours/day	**10 ml/m³**: mild dizziness; **50 ml/m³**: mortality 10/10	NIOSH 1981
mouse, no other details	**7 months**, 0, 2.2 ml/m³ no other details	**2.2 ml/m³**: no effects	Gulko et al. 1964
guinea pig, no other details, 5 ♂, 5 ♀	**14 days**, 0, 30 ml/m³ 7 hours/day, 5 days/week	**30 ml/m³**: mortality 10/10 after 4 exposures	Dow Chemical Company 1962
guinea pig, no other details, 2 ♂, 3 ♀	**27 days**, 0, 8 ml/m³ 7 hours/day, 5 days/week (19 exposures)	**8 ml/m³**: mortality 1/5, necrosis of liver and kidneys in 3/3 ♀, no findings in ♂	Dow Chemical Company 1962
guinea pig, no other details, 10 ♂, 10 ♀	**143 days**, 0, 3 ml/m³ 7 hours/day, 5 days/week (100 exposures)	**3 ml/m³**: body weights ↓ (♀); liver: moderate degenerative changes, centrilobular vacuolization; kidneys: changes in the interstitium, mild opaque swelling of tubules; no incidences given; lungs, heart, spleen, adrenal glands, pancreas and testes: no histopathological findings	Dow Chemical Company 1962
guinea pig, no other details, 10 ♂, 10 ♀	**184 days**, 0, 1 ml/m³ 7 hours/day, 5 days/week (129 exposures)	**1 ml/m³**: **NOAEC**; clinical observations, growth, mortality, body weights, organ weights, blood urea nitrogen, histopathology of lungs, heart, liver, kidneys, spleen, adrenal glands, pancreas and testes: no findings	Dow Chemical Company 1962
rabbit, no other details, 2 ♂, 2 ♀	**143 days**, 0, 3 ml/m³ 7 hours/day, 5 days/week (100 exposures)	**3 ml/m³**: liver: moderate degenerative changes, centrilobular vacuolization; no incidences given; lungs, heart, kidneys, spleen, adrenal glands, pancreas and testes: no histopathological findings	Dow Chemical Company 1962
rabbit, no other details, 2 ♂, 2 ♀	**184 days**, 0, 1 ml/m³ 7 hours/day, 5 days/week (129 exposures)	**1 ml/m³**: **NOAEC**; clinical observations, growth, mortality, body weights, organ weights, blood urea nitrogen, histopathology of lungs, heart, liver, kidneys, spleen, adrenal glands, pancreas and testes: no findings	Dow Chemical Company 1962

NOAEC: no observed adverse effect concentration

5.2.2 Oral administration

The studies with oral exposure to hexachlorobutadiene are summarized in Table 3.

The target organ of the toxicity of hexachlorobutadiene in rats and mice after repeated ingestion was likewise the kidneys, where degeneration, necrosis and the regeneration of tubular epithelial cells were observed. In addition, effects on the liver and lymphatic organs were seen at higher doses. From a 2-year feeding study in rats, a NOAEL (no observed adverse effect level) for renal toxicity of 0.2 mg hexachlorobutadiene/kg body weight and day is available. The LOAEL (lowest observed adverse effect level) was 2 mg/kg body weight and day (Kociba et al. 1977 a, b). In a 13-week feeding study in mice (NTP 1991), in 1 female animal of the low dose group of 0.2 mg hexachlorobutadiene/kg body

weight and day, renal toxicity was found in the form of tubular cell regeneration. For this effect, a benchmark dose (BMD) of 0.2 mg/kg body weight and day, based on a benchmark response (BMR) of 10%, was calculated with a lower confidence limit (BMDL) of 0.1 mg/kg body weight and day (US EPA 2003).

Tab. 3 Toxicity studies with repeated oral administration of hexachlorobutadiene

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 6 ♂, 6 ♀	**2 weeks,** 0, 50, 150, 450 mg/kg diet (about 0, 6, 18, 54 mg/kg body weight and day[a]), purity > 95%	**about 6 mg/kg body weight and above**: body weights ↓, kidneys: tubular epithelial cell degeneration; **about 18 mg/kg body weight and above**: feed consumption ↓, relative kidney weights ↑	Harleman and Seinen 1979
rat, Sprague Dawley, 5 ♂	**3 weeks,** 0, 0.2, 20 mg/kg body weight and day, 7 days/week, gavage, purity > 99%	**0.2 mg/kg body weight**: **NOAEL**; **20 mg/kg body weight**: body weights ↓, kidneys: relative weights ↑, histopathological changes (loss of cytoplasm, nuclear pyknosis, basophilia ↑, mitotic activity ↑, cellular debris in the tubules ↑, DNA synthesis 1.8-fold ↑)	Stott et al. 1981
rat, Wistar, 3 ♂	**3 weeks,** 0, 0.008, 0.04, 0.2% in the diet (about 0, 10, 48, 240 mg/kg body weight and day[a]), purity > 99%	**about 10 mg/kg body weight**: **NOAEL**; **about 48 mg/kg body weight**: body weights ↓; **about 240 mg/kg body weight**: body weights ↓, kidneys: extensive regeneration of the renal tubules, PCNA-positive cells in the outer stripe of the medulla	Nakagawa et al. 1998
rat, Wistar, 5 ♂, 5 ♀	**4 weeks,** 0, 25, 100, 400 mg/kg diet (about 0, 3, 12, 48 mg/kg body weight and day[a]), purity > 98%	**about 3 mg/kg body weight and above**: ♀: blood plasma: urea ↓; **about 12 mg/kg body weight and above**: body weights ↓, urine: number of epithelial cells ↑; kidneys: relative weights ↑; ♀: blood plasma: creatinine ↓; tubular cytomegaly; liver: absolute weights ↓; adrenal glands: absolute weights ↓; **about 48 mg/kg body weight**: urine: ketones ↑; blood plasma: AST ↑, bilirubin ↑; kidneys: tubular cytomegaly; ♂: blood plasma: total protein ↓, albumin ↓, calcium ↓, urea ↓; adrenal glands: relative weights ↑; liver: absolute weights ↓; ♀: mortality 1/5	Jonker et al. 1993
rat, Wistar, 5 ♂, 5 ♀	**4 weeks,** 0, 20, 100 mg/kg diet (about 0, 2, 12 mg/kg body weight and day[a]), purity > 98%	**about 2 mg/kg body weight**: **NOAEL**; **about 12 mg/kg body weight**: body weights ↓, ♂: kidneys: relative weights ↑; ♀: urine: volume ↑, number of epithelial cells ↑; blood plasma: AST and ALT ↑, albumin ↓, protein and calcium ↓; kidneys: absolute weights ↓, inner renal cortex: necrosis, karyomegaly, hypercellularity and variable nucleus sizes in 5/5; adrenal glands: absolute weights ↓; liver: absolute weights ↓	Jonker et al. 1993
rat, Sprague Dawley, 4 ♀	**30 days**, 0, 1, 3, 10, 30, 65, 100 mg/kg body weight and day, in the diet, purity > 99%	**1 mg/kg body weight**: **NOAEL**; **3 mg/kg body weight**: kidneys: absolute and relative weights ↑; **10 mg/kg body weight and above**: body weights ↓, blood: haemoglobin and erythrocyte concentration ↑; brain: relative weights ↑; **30 mg/kg body weight and above**: feed consumption ↓, kidneys: weights ↑, tubular degeneration, regeneration and necrosis ↑; **65 mg/kg body weight and above**: abdominal fat ↓; **100 mg/kg body weight**: liver: minimal cellular enlargement	Occidental Chemical Corporation 1992

Tab. 3 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 10 ♂, 10 ♀	**13 weeks,** 0, 0.4, 1.0, 2.5, 6.3, 15.6 mg/kg body weight and day, gavage, purity > 95%	**0.4 mg/kg body weight and above**: ♂: relative kidney weights ↑; **2.5 mg/kg body weight and above**: ♀: kidneys: concentration-dependent proximal tubular degeneration; urine: osmolarity ↓ (reduced concentrating of urine); **6.3 mg/kg body weight**: body weight gains ↓ (♂: 13%; ♀: 30%), ♂: kidneys: concentration-dependent proximal tubular degeneration; relative liver and testis weights ↑; ♀: relative weights of brain, spleen and kidneys ↑; **15.6 mg/kg body weight**: body weight gains ↓ (about 40%), ♂: urine: osmolarity ↓; relative weights of liver, testis, brain and spleen ↑; liver: flocky granulation in the cytoplasm ↑ (increase in rough ER); ♀: relative weights of liver, brain, spleen and kidney ↑; haematology and liver enzymes (AP, AST, GGT): no unusual findings	Harleman and Seinen 1979
rat, Wistar, 6 ♀	**18 weeks,** 0, 150 or 1500 mg/kg diet (about 0, 14 or 135 mg/kg body weight and day[b]), purity > 95%	**about 14 mg/kg body weight**: body weights ↓, kidneys: relative weights ↑, hypercellularity of epithelial cells, single cell degeneration and necrosis of the proximal tubule; **about 135 mg/kg body weight**: body weights ↓, weakness and ataxia of hindlimbs, all animals killed after 10 weeks due to their poor condition; kidneys: enlarged, tubular degeneration; liver: slight proliferation of the epithelial cells of bile ducts; femur: fragmentation and demyelination of nerve fibres; no accumulation of porphyrin in the liver or kidneys	Harleman and Seinen 1979
rat, Wistar, 21 ♂	**30 weeks,** 0, 0.1% in the diet (about 0, 90 mg/kg body weight and day[b]), purity > 99%	**about 90 mg/kg body weight**: body weights ↓, kidneys: relative weights ↑, simple tubular hyperplasia (10/21; controls 8/21), multiplicity of hyperplastic foci ↑, BrdU-labelled cells (cell proliferation) in outer stripe of medulla and cortex ↑	Nakagawa et al. 1998
rat, Sprague Dawley, 10–12 ♂, 20–24 ♀, controls: 17 ♂, 34 ♀	**148 days**, 0, 0.2, 2.0, 20 mg/kg body weight and day, in the diet, purity 99%	**0.2 mg/kg body weight**: **NOAEL**; **2 mg/kg body weight and above**: ♂: kidneys: mottled renal cortex; **20 mg/kg body weight**: feed consumption and body weight gains ↓, kidneys: tubular dilation, hypertrophy, tubular epithelial degeneration and regeneration	Schwetz et al. 1977
rat, Sprague Dawley, 40 ♂, 40 ♀	**2 years** (♂: 22 months, ♀: 24 months), 0, 0.2, 2.0, 20 mg/kg body weight and day, in the diet, purity 99%	**0.2 mg/kg body weight**: **NOAEL**; **2 mg/kg body weight**: kidneys: slight toxicity (hyperplastic tubular epithelium, no other details); ♀: urine: excretion of coproporphyrin ↑ (only after 14 months); **20 mg/kg body weight**: body weight gains ↓, kidney weights ↑, tubular epithelial hyperplasia, adenomas and adenocarcinomas (see Section 5.7); urine: excretion of coproporphyrin ↑ (♂ only after 12 months, ♀ only after 24 months); ♂: erythrocyte count slightly ↓ (after 22 months); ♂: mortality ↑ (from month 20 onwards); liver weights, liver enzymes (AP, ALT) and histopathological examination of liver and other organs: no unusual findings	Kociba et al. 1977 a, b
mouse, B6C3F1, 5 ♂, 5 ♀	**15 days**, 0, 30, 100, 300, 1000, 3000 mg/kg diet (♂: 0, 3, 12, 40, 19, 24 mg/kg body weight and day; ♀: 0, 5, 16, 49, 30, 36 mg/kg body weight and day), purity about 98%	**3/5 mg/kg body weight and above**: kidneys: tubular cell necrosis and regeneration; **12/16 mg/kg body weight and above**: body weights ↓; **40/49 mg/kg body weight and above**: lethargy, rough coats, hunched position and incoordination, thymus and heart weights ↓; **19/30 mg/kg body weight and above**: mortality 10/10 up to day 7, kidneys: necrosis of cortex and outer medulla; liver: necrosis, cytoplasmic vacuolization; lymphoid tissues: necrosis and atrophy (lymph nodes, spleen, thymus); testes: giant cells in seminiferous tubules	NTP 1991

Tab. 3 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 10 ♂, 10 ♀	**13 weeks,** 0, 1, 3, 10, 30, 100 mg/kg diet (♂: 0, 0.1, 0.4, 1.5, 4.9, 16.8 mg/kg body weight and day; ♀: 0, 0.2, 0.5, 1.8, 4.5, 19.2 mg/kg body weight and day), purity about 98%	**0.1/0.2 mg/kg body weight**: ♂: mortality 1/10; ♀: kidneys: tubular regeneration (1/10); **0.4/0.5 mg/kg body weight**: ♀: kidneys: tubular regeneration (9/10); **1.5/1.8 mg/kg body weight**: ♂: kidneys: relative weights ↓; spleen: relative weights ↑; ♀: kidneys: tubular regeneration (10/10); **4.9/4.5 mg/kg body weight**: kidneys: tubular regeneration (♂ and ♀: 10/10); ♂: body weight gains ↓ (–49%), kidneys: absolute and relative weights ↓; spleen : relative weights ↑; **16.8/19.2 mg/kg body weight**: body weight gains ↓ (♂: –56%, ♀: –47%); kidneys: absolute weights ↓, tubular regeneration (♂ and ♀: 10/10); brain: relative weights ↑; ♂: kidneys: relative weights ↓; liver: relative weights ↑; heart: absolute weights ↓; spleen: relative weights ↑	NTP 1991

[a] conversion factor 0.12 according to EFSA (2012)
[b] conversion factor 0.09 according to EFSA (2012)
ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; BrdU: bromodeoxyuridine; GGT: γ-glutamyl transferase; NOAEL: no observed adverse effect level; PCNA: proliferating cell nuclear antigen

5.2.3 Dermal application

There are no data available.

5.2.4 Intraperitoneal injection

Male Sprague Dawley rats were given intraperitoneal injections of hexachlorobutadiene of 0, 0.04, 0.2 or 0.4 mmol/kg body weight (corresponding to doses of 0, 10.4, 52.1 and 104.3 mg/kg body weight) on 3 consecutive days. The animals were examined 4 hours after the last treatment. In the serum, at the two higher doses, AP and bilirubin were significantly increased, whereas AST was significantly decreased only at the highest dose tested. The concentration of bile acids in the serum was significantly increased only at the highest dose. This was attributed to increased levels of cholic acid and taurocholic acid. Histopathological examination of the liver was not carried out (Bai et al. 1992).

Male Wistar rats were given daily intraperitoneal injections of hexachlorobutadiene of 25 mg/kg body weight for 2, 3, 4 or 7 days. After 2 and 3 days of treatment, substantial necrosis in the straight portion (pars recta) of the proximal tubule was found. After 4 days of treatment, regeneration in the affected section was evident, and after 7 days of exposure, the appearance of the kidneys was normal and the organ had acquired resistance to further hexachlorobutadiene treatments. In further investigations, the animals were given 2 intraperitoneal injections of hexachlorobutadiene of 25 mg/kg body weight and day. After a subsequent recovery period of 14 or 21 days, the animals were again given 2 hexachlorobutadiene doses of 25 mg/kg body weight or a single dose of 100 mg/kg body weight. Treatment of the rats with 25 mg/kg body weight after recovery phases of 14 or 21 days led to significantly less severe effects on the kidneys compared with those after the 2-day treatment without prior resistance formation. However, no resistance effects were observed in the animals exposed to the high hexachlorobutadiene dose of 100 mg/kg body weight (Boroushaki 2003).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

Amounts of 0.78 g (0.5 ml) of undiluted hexachlorobutadiene (purity > 95%) were applied to the intact or abraded skin (method of application not specified) of groups of 6 rabbits for 24 hours. Hexachlorobutadiene was found to be moderately irritating to the skin, the primary dermal irritation index was 4 on a scale up to 8 (Duprat et al. 1976; WHO 1994).

Eight-hour occlusive dermal application of undiluted hexachlorobutadiene in doses of 0, 0.25, 0.5, 0.75 or 1.0 ml/kg body weight (0, 388, 775, 1163 and 1550 mg/kg body weight at a density of 1.55 g/cm^3) induced bleeding and necrosis of the skin of rabbits at the site of application (Duprat and Gradiski 1978; see also Section 5.1.3).

Single exposures of the skin to hexachlorobutadiene for 3 or 4 hours caused redness of the skin, oedema and slight necrosis in rabbits. Data for the method used are not available (BUA 1991).

5.3.2 Eyes

After repeated inhalation exposure of rats to hexachlorobutadiene, eye irritation was observed (Gage 1970; see Section 5.2.1).

In groups of 6 rabbits, amounts of 0.15 g (0.1 ml) of undiluted hexachlorobutadiene (purity >95%) were instilled into the conjunctival sac of the left eye. The substance was hardly irritating to the eyes; the primary irritation index was 1.5 on a scale up to 110 (Duprat et al. 1976; WHO 1994).

5.4 Allergenic effects

In a maximization test, 25 Hartley guinea pigs (the number of animals used differs in the text (15) and in a table (25)) were given injections of 5% hexachlorobutadiene in arachis oil for intradermal induction. Topical induction treatment was performed with a 25% preparation in petrolatum after prior non-occlusive application of a sodium lauryl sulfate preparation (no other details). Seven days later, the challenge treatment was carried out in 20 animals using a 20% hexachlorobutadiene preparation in petrolatum, and 5 animals were treated with petrolatum only. All 20 animals challenged with the test substance and none of the vehicle controls produced a reaction (7× weak, 8× moderate and 5× strong reactions (no other details)). In a supplementary experiment, 5 animals were subjected to an analogous induction treatment, but without the use of FCA (Freund's complete adjuvant). These 5 animals likewise produced a reaction to the challenge treatment. However, a negative result was reported from a Draize test without further details being given (Gradiski et al. 1975).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

Lifelong exposure to hexachlorobutadiene doses of up to 20 mg/kg body weight and day with the diet did not produce any treatment-related changes in the reproductive organs in rats (Kociba et al. 1977 a, b; Schwetz et al. 1977; see Section 5.2.2).

Sperm motility was significantly reduced, but not in a dose-dependent fashion, in male mice after the administration of hexachlorobutadiene with the diet (0, 1, 3, 10, 30, 100 mg/kg diet; ♂: 0, 0.1, 0.4, 1.5, 4.9, 16.8 mg/kg body weight and day; ♀: 0, 0.2, 0.5, 1.8, 4.5, 19.2 mg/kg body weight and day; see Section 5.2.2) for 13 weeks. The sperm count, the incidence of abnormal sperm and the oestrous cycle in females were unaffected (NTP 1991).

In mice, repeated inhalation exposure to hexachlorobutadiene concentrations of 10 ml/m^3 did not produce any significant effects on sperm morphology (NIOSH 1981).

Rats were given hexachlorobutadiene at dose levels of 0, 0.2, 2.0 or 20 mg/kg body weight and day (purity 99%) for 90 days prior to mating, 15 days during mating and during gestation and lactation. Ten males and 20 females were used in the lower dose groups, and 12 males and 24 females in the high dose group. At both high dose levels feed consumption and body weight gains were decreased and there were effects on the kidneys. No effects on pregnancy and survival of the offspring and their development were observed. In the highest dose group, after weaning on postnatal day 21, the body weights of the offspring were slightly but significantly reduced compared with those of the control

litter. The NOAEL was 0.2 mg/kg body weight and day for the parent animals, and 2 mg/kg body weight and day for the offspring (Kociba et al. 1977 b; Schwetz et al. 1977).

Groups of 6 female Wistar rats were given hexachlorobutadiene doses of 0, 150 or 1500 mg/kg diet (about 0, 14 and 135 mg/kg body weight and day; purity > 95%) for up to 18 weeks. After the first 3 weeks, the females were mated with two untreated males per group. No conception took place in the high dose group. The animals lost weight and displayed weakness in the hind legs and an unsteady gait. From week 6 onwards, ataxia developed, and from week 8 onwards, the condition of the animals deteriorated markedly, so that these animals were killed in week 10. In the enlarged kidneys tubular degeneration was found. Slight proliferation of the epithelial cells of the bile ducts was observed in the liver. In the femoral nerve fibres fragmentation and demyelination were observed. At the low dose, 5 of 6 animals became pregnant, in 1 animal the litter consisted of 3 animals only. The body weights of the offspring were significantly reduced on the day of birth and on postnatal days 10 and 20. The resorption rate was low, as in the control group. No gross malformations were observed. The dams were killed after 18 weeks and examined. Compared with those of the control group, the body weights were significantly reduced and the relative kidney weights were significantly increased. In the kidneys, hypercellularity of the epithelial cells and degeneration and necrosis of single cells of the proximal tubule occurred. A LOAEL for developmental and maternal toxicity of 14 mg/kg body weight and day and a NOAEL for fertility of 14 mg/kg body weight and day were obtained (Harleman and Seinen 1979).

In dominant lethal tests in rats, no significant differences in the fertility index, the number of corpora lutea or implantations, and the frequency of early deaths were observed, compared with the values for the controls, after inhalation exposure to hexachlorobutadiene concentrations of 10 or 50 ml/m^3 (NIOSH 1981).

5.5.2 Developmental toxicity

Inhalation

In an inhalation study, 24 pregnant rats per group were exposed to hexachlorobutadiene concentrations of 0, 2, 5, 10 or 15 ml/m^3 (0, 22, 54, 108 and 162 mg/m^3, purity 99%) for 6 hours daily from gestation days 6 to 20. The body weight gains of the dams and the body weights of the foetuses were significantly reduced in the high dose group. The number of implantations, foetal losses, resorptions, the number of live foetuses and the sex ratio were unaffected by the treatment. No treatment-related morphological or histological changes or skeletal abnormalities were observed in the foetuses (Saillenfait et al. 1989). The NOAEC (no observed adverse effect concentration) for developmental and maternal toxicity was 10 ml/m^3.

Oral administration

After the treatment of rats with hexachlorobutadiene doses of 0, 0.2, 2.0 or 20 mg/kg body weight and day via the diet (see Section 5.5.1), the body weights of the offspring in the high dose group were significantly reduced on postnatal day 21. Other signs of foetotoxicity were not observed and teratogenic effects did not occur (Kociba et al. 1977 b; Schwetz et al. 1977).

Groups of 6 female Wistar rats received hexachlorobutadiene doses of 0, 150 or 1500 mg/kg feed (about 0, 14 and 135 mg/kg body weight and day; purity > 95%) for up to 18 weeks. After 3 weeks, the females were mated with 2 untreated males per group. No conception took place in the high dose group. At 14 mg/kg body weight and day and above, the body weights of the dams were reduced, and toxic effects on the kidneys and nerves were observed (see Section 5.5.1). Five of 6 rats became pregnant, in 1 animal the litter consisted of 3 offspring only. The body weights of the offspring on the day of birth and on postnatal day 10 and 20 were significantly reduced. The resorption rate was low, as in the control group. No gross malformations could be detected. The LOAEL for developmental and maternal toxicity was 14 mg/kg body weight and day (Harleman and Seinen 1979).

Groups of 8 to 9 female Sprague Dawley rats were fed hexachlorobutadiene (purity about 98%) in concentrations of 0, 100, 200, 400, 750, 1100 or 1500 mg/kg diet from gestation day 17 to postnatal day 10. On postnatal day 4, the litters

were standardized to 10 animals. In addition to the determination of the body weights of the dams and offspring at different points in time, urine, serum and milk samples were examined, the organ weights of the liver and kidneys were determined and histopathological examinations were performed. All dams of the high dose group and 1 animal of the 200 mg/kg group were killed in extremis in the period between gestation day 20 and postnatal day 1, the dams and offspring of the 1100 mg/kg group from postnatal days 1 to 3. The body weights of the dams were reduced in a dose-dependent fashion at 100 mg/kg and above during pregnancy and lactation. Feed consumption of the dams was reduced at 200 mg/kg and above during the whole exposure period, water consumption only during lactation at 400 mg/kg and above. The daily intake of hexachlorobutadiene was estimated to be 5.4, 4.5, 10.5 and 9.5 mg/kg body weight, respectively, for the 100 to 750 mg/kg groups on gestation day 20. On postnatal day 9, the hexachlorobutadiene doses ingested daily by the dams were 17, 37, 53 and 81 mg/kg body weight, respectively. Dose-dependent clinical findings were weight loss, excessive urination, weakness of the hindlimbs, tremor and lethargy. The kidney weights of the dams were increased at 200 mg/kg and above. Histopathological examination revealed tubular regeneration with a dose-dependent increase in severity in all treated groups and tubular distention at the higher doses. Reduced osmolality was observed in the urine on postnatal day 10. In the offspring, the effects were in some cases obscured by the pronounced toxicity in the dams. In the high dose group, mortality occurred during pregnancy and in the 1100 mg/kg group in the early postnatal phase. Three litters of the 750 mg/kg group were all stillbirths. Postnatal mortality of the offspring was increased in the 750 mg/kg group up to postnatal day 10. The body weights of the offspring were reduced on postnatal days 1, 4 and 10 in all dose groups, whereas the animals of the 400 and 750 mg/kg groups were emaciated and dehydrated on postnatal day 10. Estimates of the hexachlorobutadiene intake of the offspring, including the hexachlorobutadiene content in the milk, showed that the offspring absorbed about 3% to 7% of the hexachlorobutadiene doses of the dams. The histopathological examination of the kidneys revealed a reduced size and retention of the subcapsular metanephric blastemal zone in the offspring of the 750 mg/kg group, an effect that was considered to indicate a delay in postnatal development. In the serum, a slight dose-dependent increase in glucose and urea nitrogen was determined. As maternal toxicity in the form of reduced feed consumption, tubular regeneration and reduced body weights in the offspring occurred even at the lowest dose tested of 100 mg/kg feed, a NOAEL for maternal or developmental toxicity cannot be derived (NTP 1990).

Intraperitoneal injection

After pregnant rats had been given daily intraperitoneal injections of hexachlorobutadiene of 10 mg/kg body weight from gestation days 1 to 15, the incidence of soft tissue abnormalities (no other details) in the offspring was 3 times as high as that in the controls. Hydrocephalus externus was found in 1 animal (BUA 1991).

No teratogenic effects were observed in Sprague Dawley rats after intraperitoneal injection of a daily hexachlorobutadiene dose of 10 mg/kg body weight from days 1 to 15 of gestation. However, the organ weights of the dams were reduced and the development of the foetuses delayed (BUA 1991).

5.6 Genotoxicity

5.6.1 In vitro

The results of genotoxicity studies in vitro are summarized in Table 4.

Bacteria

The results of a series of bacterial mutagenicity tests with and without the addition of metabolic activation using the Salmonella typhimurium strains TA98, TA100, TA1530, TA1535, TA1537, TA1538 were negative (BUA 1991).

In a Salmonella mutagenicity test with the strain TA100, hexachlorobutadiene was only mutagenic when the protein concentration in the S9 mix was 3 times as high as the standard concentration (Reichert et al. 1984).

The cysteine conjugate of hexachlorobutadiene was mutagenic in the bacterial test system only after activation with rat kidney S9 mix (Green and Odum 1985).

In a bacterial mutagenicity test with the Salmonella typhimurium strain TA100, the *N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine conjugate (mercapturic acid) of hexachlorobutadiene was mutagenic in the presence of metabolic activation. The effect of the conjugate was 80 times as strong as that of hexachlorobutadiene (when hexachlorobutadiene was tested with 3 times the protein concentration in the S9 mix) (Reichert and Schütz 1986; Wild et al. 1986).

In other investigations, a mutagenic effect was observed in Salmonella typhimurium after activation of the mercapturic acid of hexachlorobutadiene with rat kidney cytosol (BUA 1991; Vamvakas et al. 1987).

The mutagenicity of pentachlorobutadienylcysteine was demonstrated in the Salmonella typhimurium strains TA98, TA100 and TA2638 without metabolic activation. A β-lyase inhibitor reduced the mutagenic activity (Dekant et al. 1986).

Purified hexachlorobutadiene (purity > 99.5%) was not mutagenic without metabolic activation or after the addition of rat liver microsomes with NADPH. After pre-incubation of hexachlorobutadiene with glutathione and liver microsomes containing GST, a mutagenic effect was detected in the Salmonella mutagenicity test. The mutagenic potency of the GSH–hexachlorobutadiene conjugate was significantly increased after metabolic activation with renal microsomes or mitochondria (high GGT activity), and was decreased in the presence of a β-lyase inhibitor (Vamvakas et al. 1988).

The bis-glutathione conjugate and the bis-cysteine conjugate of hexachlorobutadiene were not mutagenic in the Salmonella typhimurium strains TA98, TA100 and TA2638 with and without the addition of subcellular rat kidney fractions (microsomes, mitochondria, cytosol) (Vamvakas et al. 1988).

Mammalian cells

The results of a UDS (DNA repair synthesis) test with human fibroblasts were negative in the concentration range from 8 to 250 µg hexachlorobutadiene/ml (NIOSH 1981).

UDS was not observed in primary rat hepatocytes with hexachlorobutadiene in concentrations of 0.0078 to 7823 µg/ml (BUA 1991; Stott et al. 1981).

Hexachlorobutadiene and pentachlorobutenoic acid, which was suspected to be a metabolite of hexachlorobutadiene, induced UDS with and without metabolic activation in fibroblasts from Syrian hamster embryos. The lowest effective doses were 1 µg pentachlorobutenoic acid/ml and 2 µg hexachlorobutadiene/ml (Schiffmann et al. 1984).

The genotoxicity of the hexachlorobutadiene metabolite *S*-(1,1,2,3,4-pentachlorobutadienyl)glutathione was investigated in cultured porcine kidney cells (LLC-PK). Incubation of confluent monolayers of these cells with the metabolite led to the dose-dependent induction of DNA repair. The addition of a GGT inhibitor or a β-lyase inhibitor prevented the genotoxic effects (Vamvakas et al. 1989 a, b).

In studies with CHO (Chinese hamster ovary cell line) cells, hexachlorobutadiene in the concentration range from 1.4 to 14 µg/ml increased the incidence of sister chromatid exchange in the presence and absence of metabolic activation (Aroclor 1254-induced rat S9 mix) (Galloway et al. 1987).

In a chromosomal aberration test according to OECD test guideline 473 with V79 cells, hexachlorobutadiene (purity 98%) in the concentration range of 3.1 to 25 µg/ml with metabolic activation did not cause a statistically significant increase in the frequency of chromosomal aberrations. The proportion of aberrant cells of 5.5% and 5.0% (control 4%) at 6.3 and 12.5 µg/ml, respectively, was above the range of the historical controls (no other details), and was considered biologically relevant by the authors. Cytotoxicity in the form of a reduced cell count and reduced mitotic index was not observed up to the highest concentration tested. In the study without metabolic activation (concentration range 3.1 to 50 µg/ml), the increase in the frequency of chromosomal aberrations (without gaps; control 3%) was statistically significant at 12.5 and 25 µg/ml (8% and 13%, respectively). The mitotic index was 103% and 99% of the control, respectively. At the highest concentration tested, the increase in the frequency of chromosomal aberrations was not statistically

significant (4.8%), but was above the range of the historical controls (no other details). The mitotic index was 36.5% of the control. This suggests cytotoxicity, as the cell count likewise decreased in a dose-dependent fashion to 50% of the control (Brüschweiler et al. 2010). Unlike the authors, the Commission does not consider the results of the studies with metabolic activation to be positive, as they were not statistically significant and the proportion of aberrant cells with a maximum of 5.5% was only slightly above that of the concurrent control (4%).

In human peripheral lymphocytes, hexachlorobutadiene in concentrations of 0.001 to 0.01 µg/ml did not cause chromosomal aberrations (BUA 1991; German 1988).

In a chromosomal aberration test with CHO cells, negative results were obtained for hexachlorobutadiene in the concentration range from 5.3 to 35 µg/ml in the presence and absence of metabolic activation (Galloway et al. 1987).

In a chromosomal aberration test with CHL (Chinese hamster lung cell line) cells, the results for hexachlorobutadiene were positive in the absence of metabolic activation; structural and numerical aberrations were increased (no other details). Data for the cytotoxicity of the substance are not available (Matsushima et al. 1999).

In a micronucleus test with CHL cells, hexachlorobutadiene yielded positive results in the absence of metabolic activation, but negative results in the presence of metabolic activation. Data for the cytotoxicity of the substance are not available (Matsushima et al. 1999).

Tab. 4 Genotoxicity of hexachlorobutadiene and its metabolites in vitro

End point	Test system	Concentration [µg/ml]	Effective concentration [µg/ml]	Cytotoxicity	Results		References
					–m. a.	+m. a.	
gene mutation	Salmonella typhimurium TA98, TA100, TA1530, TA1535, TA1537, TA1538	HCBD (no other details)	–	no data	–	–	BUA 1991
	Salmonella typhimurium TA100	HCBD 0.001–3 µg/plate	0.1–1.0 µg/plate	no data	–	+[a]	Reichert et al. 1984
	Salmonella typhimurium TA100	PCBD-GSH 1–20 µg/plate	+m. a.: 1–20 µg/plate	no data	–	+[b]	Green and Odum 1985
	Salmonella typhimurium TA100	PCBD-Cys 1–20 µg/plate	–m. a.: 1–10 µg/plate +m. a.: 1–20 µg/plate	no data	+	+[b]	Green and Odum 1985
	Salmonella typhimurium TA98, TA100, TA2638	PCBD-Cys 1–20 nmol/plate	TA98: no other details TA100: 1–5 nmol/plate TA2638: 2–10 nmol/plate	no data	+[c]	n. t.	Dekant et al. 1986
	Salmonella typhimurium TA100	*N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine[d] –m. a.: up to 400 µg/plate +m. a.: 2–20 µg *N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine[d]/plate	2–20 µg/plate	no data	–	+	Wild et al. 1986
	Salmonella typhimurium TA100	*N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine[d] –m. a.: 5–200 µg/plate +m. a.: 10–50 µg/plate	+m. a.: 10–50 µg/plate	no data	–	+	Reichert and Schütz 1986
	Salmonella typhimurium TA100	*N*-acetyl-*S*-pentachlorobutadienyl-L-cysteine[d] 5–100 µg/plate	+m. a.: 5–20 µg/plate	no data	–	+[e]	Vamvakas et al. 1987
	Salmonella typhimurium TA100	HCBD –m. a.: 2–2000 nmol/plate +m. a.: 2–50 nmol/plate	+m. a.: 5–50 nmol/plate	no data	–	+[f]	Vamvakas et al. 1988

Tab. 4 *(continued)*

End point	Test system	Concentration [µg/ml]	Effective concentration [µg/ml]	Cytotoxicity	Results		References
					–m. a.	+m. a.	
	Salmonella typhimurium TA100	PCBD-GSH –m. a.: 1–1200 nmol/plate +m. a.: 1–20 nmol/plate	–m. a.: 100–700 nmol/plate +m. a.: 3–15 nmol/plate	–m. a.: > 700 nmol/plate	+	+[g]	
	Salmonella typhimurium TA98, TA100, TA2638	BGTB and BCTB (no other details)	–	no data	–	–	
UDS	human fibroblasts	HCBD 8–250	–	> 63 µg/ml	–	n. t.	NIOSH 1981
	rat hepatocytes	HCBD 0.0078–7823	–	no data	–	n. t.	Stott et al. 1981
	SHE fibroblasts	HCBD 1–10	at 2 and above	no data	+	+	Schiffmann et al. 1984
	LLC-PK1 (porcine kidney cells)	PCBD-GSH 0.75–100 µM	0.75–2.5 µM	100 µM: 50% LDH release	+	n. t.	Vamvakas et al. 1989 a, b
SCE	CHO cells	HCBD 1.4–14	–m. a.: 1.4–14 +m. a.: 4.2–14	no data	+	+	Galloway et al. 1987
CA	CHO cells	HCBD 5.3–35	–	no data	–	–	Galloway et al. 1987
	human lymphocytes	HCBD 0.001–0.01	–	no data	–[h]		German 1988; WHO 1994
	CHL cells	HCBD 3.1–50	no data	no data	+	n. t.	Matsushima et al. 1999
	V79 cells	HCBD –m. a.: 3.1–50 +m. a.: 3.1–25	–m. a.: 12.5 and 25	–m. a., 50 µg/ml: MI 36.5% +m. a., 25 µg/ml: MI 83.2%	+	–	Brüschweiler et al. 2010
MN	CHL cells	HCBD 3.1–50	no data	no data	+	–	Matsushima et al. 1999

[a] positive only when protein content in the S9 mix is increased 3-fold
[b] with rat kidney S9 mix
[c] the strains used have a high level of β-lyase activity
[d] HCBD-mercapturic acid conjugate
[e] with rat kidney cytosol
[f] pre-incubation with GSH and liver or kidney microsomes
[g] pre-incubation with rat kidney mitochondria or microsomes
[h] no information on metabolic activation
BCTB: bis-cysteine tetrachlorobutadiene; BGTB: bis-glutathione tetrachlorobutadiene; CA: chromosomal aberrations; CHL: Chinese hamster lung cell line; CHO: Chinese hamster ovary cell line; HCBD: hexachlorobutadiene; m. a.: metabolic activation; MI: mitotic index; MN: Micronuclei; n. t.: not tested; PCBD-GSH: pentachlorobutadienylglutathione; PCBD-Cys: pentachlorobutadienylcysteine; SCE: sister chromatid exchanges; SHE: Syrian hamster embryo; UDS: DNA repair synthesis

5.6.2 In vivo

The genotoxicity studies in vivo are summarized in Table 5.

In a sex-linked recessive lethal test in Drosophila (SLRL test), hexachlorobutadiene was not genotoxic after inhalation exposure, after feeding or after the injection of solutions containing hexachlorobutadiene in male animals (NIOSH 1981; Woodruff et al. 1985).

The covalent binding of hexachlorobutadiene metabolites to renal and hepatic DNA was investigated after single oral doses of 30 mg/kg body weight in NMRI mice. A low level of binding with a covalent binding index (CBI) of 27 was detected in the nuclear DNA of the kidney, while no binding was detected in the nuclear DNA of the liver. The level of

covalent binding to mitochondrial DNA from the liver and the kidneys was significantly higher with CBIs of 513 and 7506, respectively. On further analysis of the mitochondrial DNA of the kidney, three ^{14}C-labelled compounds were identified. These were regarded as DNA bases modified by hexachlorobutadiene metabolites (Schrenk and Dekant 1989).

Oral administration of hexachlorobutadiene doses of 0.2 or 20 mg/kg body weight to groups of 5 male Sprague Dawley rats for 3 weeks led at the high dose to a 1.8-fold renal DNA synthesis, compared with that in the control group, that was not statistically significant. Single oral doses at the same levels in two independent experiments resulted in a statistically significant 1.5-fold renal DNA synthesis in only one investigation at the high dose (Stott et al. 1981).

After the administration of a single oral hexachlorobutadiene dose of 20 mg/kg body weight to groups of 5 male Sprague Dawley rats in two independent experiments, a 1.27-fold and a 1.54-fold renal UDS compared with that in the control group was found. DNA alkylation in the kidneys was likewise slightly increased (Stott et al. 1981).

In rats, the frequency of chromosomal aberrations in the bone marrow was not increased after single or 5-day inhalation exposures to hexachlorobutadiene concentrations of 10 or 50 ml/m^3 (NIOSH 1981).

The administration of hexachlorobutadiene in the diet in concentrations of 0, 0.2, 2.0 or 20 mg/kg body weight and day (see Section 5.5.1) for about 130 days did not lead to increased frequencies of chromosomal aberrations in the bone marrow of rats (Schwetz et al. 1977).

After the administration of single oral doses of hexachlorobutadiene of 2 or 10 mg/kg body weight and inhalation exposure to 10 mg/m^3 (about 1 ml/m^3) for 4 hours, a statistically significant increase in chromosomal aberrations in the bone marrow cells of mice, compared with that in untreated control animals, was observed. After an oral dose of 0.4 mg/kg body weight the results were negative (BUA 1991; German 1988; WHO 1994). No positive control was included in this study, and the evaluation is ambiguous in parts. Therefore, the study can only be regarded as of limited validity.

After inhalation exposure of rats to hexachlorobutadiene concentrations of 10 or 50 ml/m^3 for 5 days, dominant lethal mutations were not induced (NIOSH 1981).

Tab. 5 In vivo studies of the genotoxicity of hexachlorobutadiene

Test system		Exposure	Results	References
SLRL test	Drosophila, ♂	1 hour, 0, 25 ml HCBD/m^3	–	NIOSH 1981
	Drosophila, ♂	0, 15 mg HCBD/l (feeding or injection)	–	Woodruff et al. 1985
DNA binding, liver and kidneys (bound radioactivity)	mouse, NMRI, 12 ♀	1×, 0, 30 mg HCBD/kg body weight, oral	+ (binding to mtDNA, kidneys > liver)	Schrenk and Dekant 1989
DNA synthesis, kidneys	rat, Sprague Dawley, 5 ♂	3 weeks, 0, 0.2, 20 mg HCBD/kg body weight and day, oral	20 mg/kg body weight: renal DNA synthesis 1.8-fold of control (not statistically significant)	Stott et al. 1981
	rat, Sprague Dawley, 5 ♂, 2 independent tests	1×, 0, 0.2, 20 mg HCBD/kg body weight, oral	test 1: – test 2: 20 mg/kg body weight: renal DNA synthesis 1.5-fold of control	
UDS test, kidneys	rat, Sprague Dawley, 5 ♂, 2 independent tests	1×, 0; 20 mg HCBD/kg body weight, oral	DNA repair synthesis 1.27-fold or 1.54-fold of control	Stott et al. 1981
CA, bone marrow	rat, 10 ♂, 10 ♀	5 days, 7 hours/day, 0, 10, 50 ml HCBD/m^3, examination after 6 hours	–	NIOSH 1981
	rat, 10 ♂, 10 ♀	7 hours, 0, 10, 50 ml HCBD/m^3, examination after 6, 24, 48 hours	–	

Tab. 5 *(continued)*

Test system		Exposure	Results	References
CA, bone marrow	rat, Sprague Dawley, 4 ♂, 4 ♀ F0 and F1 animals	about 130 days, 0, 0.2, 2.0, 20 mg HCBD/kg body weight and day, diet	–	Schwetz et al. 1977
CA, bone marrow	mouse	1×, 0, 0.4, 2, 10 mg HCBD/kg body weight, oral	+ starting at 2 mg/kg body weight	German 1988; WHO 1994
	mouse	4 hours, 0, 10 mg HCBD/m^3 (about 1 ml/m^3)	+	
DLT	rat, ♂	5 days, 7 hours/day, 0, 10, 50 ml HCBD/m^3	–	NIOSH 1981

CA: test for structural chromosomal aberrations; DLT: dominant lethal test; HCBD: hexachlorobutadiene; mtDNA: mitochondrial DNA; SLRL: sex-linked recessive lethal mutations in Drosophila; UDS: DNA repair synthesis

5.6.3 Summary

Several bacterial mutagenicity tests with hexachlorobutadiene, the glutathione conjugate and the mercapturic acid derivative of hexachlorobutadiene yielded positive results in the presence of metabolic activation. The cysteine conjugate of hexachlorobutadiene was mutagenic in bacterial test systems in the presence of β-lyase activity.

In mammalian cells, hexachlorobutadiene induced UDS and sister chromatid exchange as well as significantly increased frequencies of chromosomal aberrations.

After single oral doses of hexachlorobutadiene, UDS in the kidneys was increased in rats and the covalent binding to mitochondrial DNA of the liver and kidneys in mice was significantly increased.

In mice, a significant increase in chromosomal aberrations was reported in studies with limited validity after single oral doses or inhalation exposure to hexachlorobutadiene for 4 hours. In two other valid studies in rats, the frequency of chromosomal aberrations in the bone marrow was not increased after inhalation or oral exposure to hexachlorobutadiene. No dominant lethal mutations were observed in rats after inhalation exposure to hexachlorobutadiene.

Overall, hexachlorobutadiene induced a tissue-specific genotoxic effect in the kidneys, which requires high β-lyase activity.

5.7 Carcinogenicity

5.7.1 Short-term studies

Hexachlorobutadiene and pentachlorobutenoic acid, which was suspected to be a metabolite of hexachlorobutadiene, induced morphological transformations in Syrian hamster embryo fibroblasts in a cell transformation test. The lowest effective doses were 0.8 µg pentachlorobutenoic acid/ml and 10 µg hexachlorobutadiene/ml (BUA 1991; Schiffmann et al. 1984).

Groups of 20 male A/St mice (sensitive for the development of lung tumours) were given intraperitoneal injections of hexachlorobutadiene of 0, 4 or 8 mg/kg body weight 3 times a week. After a total of 12 to 13 injections, the surviving animals were killed 24 weeks after the start of treatment and examined for lung tumours. The tumour incidences were similar between the groups (Theiss et al. 1977).

Initiation–promotion studies

Male Wistar rats (n = 21, 6 weeks old) were given 0.1% *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) with their drinking water for 2 weeks and then 0.1% hexachlorobutadiene (purity > 99%; about 90 mg hexachlorobutadiene/kg body weight, conversion factor 0.09 according to EFSA 2012) in their diet for 30 weeks. Three further groups of 21 animals

received either only EHEN for 2 weeks, only hexachlorobutadiene or basal diet according to the same scheme. All animals survived. In the group that received EHEN and hexachlorobutadiene, the incidence of renal cell tumours was significantly higher with 15 of 21 than in the group that were given EHEN alone (5 of 21). The incidence of preneoplastic adenomatous hyperplasia was likewise significantly higher with 21 of 21 compared with 4 of 21. Neither adenomatous hyperplasia nor renal tumours occurred in the group that were given hexachlorobutadiene alone or in the control group. In contrast, hexachlorobutadiene increased the multiplicity of simple hyperplastic foci and cell proliferation in the outer stripe of the medulla and cortex (Nakagawa et al. 1998). Thus, hexachlorobutadiene has a tumour–promoting effect in the kidneys of rats.

Groups of 30 female Swiss mice were treated once with 15 mg hexachlorobutadiene in 0.2 ml acetone applied to the dorsal skin. After 14 days, 5 µg phorbol-12-myristate-13-acetate in 0.2 ml acetone was applied as a tumour promoter 3 times a week for 428 to 576 days. Survival of the animals was described as "excellent". Hexachlorobutadiene did not lead to the initiation of skin tumours (IARC 1999).

5.7.2 Long-term studies

In a study from 1985, rats were given daily oral hexachlorobutadiene doses of 0.6, 5.8 or 37 mg/kg body weight for 1 year. Examination of the animals revealed merely some benign tumours of the kidneys and liver (BUA 1991). This insufficiently described study cannot be included in the evaluation.

In a 2-year study, groups of 40 male and 40 female Sprague Dawley rats (controls 90 animals per sex) were given hexachlorobutadiene doses of 0, 0.2, 2.0 or 20 mg/kg body weight and day with the diet. The surviving males were killed after 22 months, the females after 24 months. In the high dose group, increased mortality was observed in the males and decreased body weight gains, increased coproporphyrinuria and increased kidney weights in both males and females. Hyperplasia and proliferation as well as adenomas and adenocarcinomas (see Table 6) occurred in the renal tubular epithelium. The tumour incidence was not increased in the middle dose group. In addition to increased coproporphyrinuria, increased hyperplasia of the tubular epithelium was observed in the female animals. No pathological changes were diagnosed in the 0.2 mg/kg group (Kociba et al. 1977 a, b). More detailed information on the occurrence of the tumours or the time of death of the animals were not given in the publications.

After dermal exposure of groups of 30 female Swiss mice to doses of 2 or 6 mg hexachlorobutadiene per animal in 0.2 ml acetone, 3 times a week, for 440 to 594 days, no skin papillomas or carcinomas were observed. Lung tumours and tumours in the forestomach occurred in the treated animals and in the controls. The authors concluded that hexachlorobutadiene was not carcinogenic after dermal application in mice (ATSDR 1994; IARC 1999). The results of this study are not sufficient to rule out a carcinogenic effect after dermal exposure.

Tab. 6 Studies of the carcinogenicity of hexachlorobutadiene

Author:	Kociba et al. 1977 a, b
Substance:	hexachlorobutadiene (purity: 99%)
Species:	**rat,** Sprague Dawley, 40 ♂, 40 ♀ (controls: 90 ♂, 90 ♀)
Administration route:	diet
Dose:	0, 0.2, 2.0, 20 mg/kg body weight and day
Duration:	2 years (♂: 22 months, ♀: 24 months)
Toxicity:	**2 mg/kg body weight and above**: kidneys: tubular epithelial hyperplasia (no data for incidence); ♀: urine: excretion of coproporphyrin ↑; **20 mg/kg body weight**: body weight gains ↓, kidneys: relative weights ↑; ♂: urine: excretion of coproporphyrin ↑; mortality ↑ (from month 20 onwards)

Tab. 6 *(continued)*

		dose (mg/kg body weight and day)			
		0	**0.2**	**2.0**	**20**
Survivors[a)]	♂	about 25%	about 25%	about 25%	about 10%
	♀	about 25%	about 25%	about 25%	about 40%
Tumours and preneoplasms					
Kidneys:					
tubular adenomas	♂	1/90 (1.1%)	0/40 (0%)	0/40 (0%)	2/39 (5.1%)
	♀	0/90 (0%)	0/40 (0%)	0/40 (0%)	3/40 (7.5%)*
tubular carcinomas	♂	0/90 (0%)	0/40 (0%)	0/40 (0%)	7/39 (18%)**
	♀	0/90 (0%)	0/40 (0%)	0/40 (0%)	3/40 (7.5%)*
tubular adenomas and carcinomas	♂	1/90 (1.1%)	0/40 (0%)	0/40 (0%)	9/39 (23%)**
	♀	0/90 (0%)	0/40 (0%)	0/40 (0%)	6/40 (15%)**

*$p \leq 0.05$; **$p \leq 0.01$
[a)] Information taken from a figure
historical controls: no data

6 Manifesto (MAK value/classification)

The critical effect of hexachlorobutadiene is its nephrotoxicity and nephrocarcinogenicity. The chronic NOAEL for other effects is considerably higher.

MAK value. Data sufficient to derive a MAK value in humans are not available.

Hexachlorobutadiene is moderately irritating to the skin. In the studies with repeated inhalation exposure, rats exposed to concentrations of 25 ml/m^3 and more had respiratory difficulties and, at 100 ml/m^3 and above, suffered additionally from irritation of the eyes, nose and respiratory tract (Gage 1970). In an earlier study, which does not meet the current requirements in all respects, a NOAEC of 1 ml hexachlorobutadiene/m^3 was reported after inhalation exposure of rats for 26 weeks (Dow Chemical Company 1962).

From the only long-term study, a NOAEL of 0.2 mg/kg body weight and day was obtained in rats after dietary administration of hexachlorobutadiene for 2 years (Kociba et al. 1977 a, b). In a 13-week feeding study in mice (NTP 1991), nephrotoxicity in the form of tubular regeneration (after previous degeneration) still occurred in 1 female animal at the lowest dose tested of 0.2 mg hexachlorobutadiene/kg body weight and day, for which a BMDL of 0.1 mg/kg body weight and day was derived from a benchmark calculation (US EPA 2003).

According to the studies of the metabolism of hexachlorobutadiene and the comparison with other haloalkenes such as tetrachloroethylene and compound A (see Section 3.2), it is very likely that humans are less exposed to the metabolites resulting from the β-lyase pathway than rats. If the β-lyase pathway alone were to play a role, humans would most likely be less burdened than rats due to the lower enzyme activity. However, it cannot be excluded that the sulfoxidation of the mercapturic acid of hexachlorobutadiene contributes to the nephrotoxicity in humans. The activity of the sulfoxidation of *N*-acetylpentachlorobutadienylcysteine as a ratio of V_{max}/K_m in rats, determined in in vitro studies with liver microsomes of rats (Birner et al. 1995) and humans (Werner et al. 1995), is 52% of the value in humans. There are no other data available. In rats, sulfoxidation is likely to contribute to the nephrotoxicity whereas, in mice, only the β-lyase pathway is likely to contribute to the nephrotoxicity (see Section 5.1.4). Therefore, the NOAEL from the rat study is used to derive the MAK value in order to include the contribution of sulfoxidation to the nephrotoxicity, which may also be relevant for humans.

The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL of 0.2 mg/kg body weight to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the species-specific correction value for the rat (1:4), the assumed oral absorption

(100%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentration calculated from this is 0.49 mg/m^3 or 0.045 ml/m^3. As this value comes from a NOAEL from experimental studies with animals, a MAK value of 0.02 ml hexachlorobutadiene/m^3 can be derived according to the procedure of the Commission (see List of MAK and BAT Values, Section I).

A MAK value based on the study in mice (NTP 1991) would lead to an overestimation of the risks due to the lower relevance of the β-lyase pathway in humans (even compared with rats), although it would be of the same order of magnitude when the lower relevance of the β-lyase pathway is considered.

Peak limitation. As the MAK value is based on a systemic effect, the substance has been assigned to Peak Limitation Category II. Since no information on half-lives is available, the default excursion factor of 2 has been set. According to the available data, irritant effects are not to be expected.

Prenatal toxicity. In two 1-generation studies with dietary administration of hexachlorobutadiene from gestation day 17 to postnatal day 10 or during mating, gestation and lactation, effects on the offspring in the form of reduced body weights on postnatal days 1, 10 and 20 were observed in rats at 150 mg/kg body weight (corresponds to about 14 mg/kg body weight; Harleman and Seinen 1979) and on postnatal days 1, 4 and 10 at 100 mg/kg body weight (about 17 mg/kg body weight on PND 9; NTP 1990). However, simultaneous severe maternal toxicity (reduced body weights and food consumption, and nephrotoxicity) was observed at these dose levels. These postnatal effects after oral administration are considered by the Commission to be caused by the severe maternal toxicity and are therefore not used to evaluate prenatal developmental toxicity.

In a prenatal developmental toxicity study, effects on maternal and foetal body weights were observed in rats after whole-body exposure from gestation days 6 to 20 at the highest concentration tested of 15 ml/m^3. No teratogenicity was observed. The NOAEC for developmental and maternal toxicity was 10 ml/m^3 (Saillenfait et al. 1989). Since no embryotoxic and teratogenic effects occurred after inhalation and the 500-fold margin between this NOAEC and the MAK value of 0.02 ml/m^3 is sufficiently large, hexachlorobutadiene has been assigned to Pregnancy Risk Group C.

Carcinogenicity. Based on the available data for the mechanism of action, metabolism and genotoxicity of the substance, a genotoxic effect of hexachlorobutadiene in the kidneys after metabolic activation is possible. However, from the available information on the metabolism of hexachlorobutadiene and the comparison with other haloalkenes such as tetrachloroethylene and the sevoflurane metabolite compound A, it can be concluded that humans are less exposed to the critical metabolites of the β-lyase pathway than rats (see Section 3.2). In a 2-year feeding study with hexachlorobutadiene, significantly increased incidences of renal adenomas and carcinomas in male and female rats were observed only at the highest dose tested of 20 mg/kg body weight. Nephrotoxicity in the form of hyperplasia of the tubular epithelium and increased coproporphyrin excretion occurred even at 2 mg/kg body weight (Kociba et al. 1977 a, b). Genotoxic effects in the kidneys (binding to mitochondrial DNA (Schrenk and Dekant 1989) and increased UDS (Stott et al. 1981)) were observed in rats and mice in vivo at doses of 20 mg/kg body weight and above, but not at 0.2 mg/kg body weight. An initiation–promotion experiment in rats confirmed a tumour-promoting effect of hexachlorobutadiene in the kidneys (Nakagawa et al. 1998). Preneoplastic hyperplasia or tumours did not occur after administration of hexachlorobutadiene alone, but increased proliferation of the tubular cells was observed. Nephrotoxicity or cell proliferation can therefore be considered a prerequisite for the carcinogenicity of hexachlorobutadiene. If the MAK value is observed, which protects against the nephrotoxicity and tubular epithelial hyperplasia and thus also against the carcinogenicity of the substance, genotoxic effects play only a minor role. Hexachlorobutadiene has therefore been assigned to Carcinogen Category 4.

Germ cell mutagenicity. In vitro, in the presence of β-lyase activity, hexachlorobutadiene or its cysteine conjugate are mutagenic in bacteria and mammalian cells and clastogenic in mammalian cells. In vivo studies showed hexachlorobutadiene to cause the tissue-specific induction of UDS in the kidneys. No chromosomal aberrations or dominant lethal mutations were detected in rats after oral or inhalation exposure. It could not be demonstrated that the reactive metabolites of hexachlorobutadiene reach the germ cells. Due to the tissue-specific genotoxic effect in the kidneys and the quantitatively low formation of genotoxic metabolites outside the kidneys (see Section 2), the

available data are not sufficient to justify the classification of hexachlorobutadiene in one of the categories for germ cell mutagens.

Absorption through the skin. There are no human data available for potential absorption through the skin. Animal experiments to determine the dermal LD_{50} provide evidence for the complete absorption of hexachlorobutadiene after dermal application. Systemic effects, including those in the kidneys, have been observed in rabbits after a single application of < 500 mg/kg body weight. With inhalation exposure at the level of the MAK value for 8 hours, an absorbed amount of 22 mg is to be expected. This amount corresponds to a volume of 14 µl liquid hexachlorobutadiene which would have to be absorbed through the skin. In view of the good dermal absorption and, at the same time, the small amounts required to produce a relevant internal burden, the potential contribution of dermal absorption to systemic toxicity does not appear negligible. Using the models given, under standard conditions the maximum amount absorbed was calculated to be 75 mg. Hexachlorobutadiene therefore remains designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There are no data available for a skin sensitizing effect of hexachlorobutadiene in humans due to the lack of exposure. The positive results in a maximization test in guinea pigs, with and without the use of adjuvants, provide evidence of a contact sensitizing potential of hexachlorobutadiene. However, due to incomplete documentation of the study and the absence of an adequate control group, a contact sensitizing potential cannot be concluded from these findings with sufficient certainty. Hexachlorobutadiene has therefore not been designated with "Sh" (for substances which cause sensitization of the skin). There are no findings available for a sensitizing effect on the airways. The substance has therefore not been designated with "Sa" (for substances which cause sensitization of the airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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