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Hexachloroethane

MAK Value Documentation, supplement – Translation of the German version from 2020

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Keywords

hexachloroethane; kidney; irritation; maximum workplace concentration; MAK value; toxicity; hazardous substance; carcinogenicity; developmental toxicity; skin absorption

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated hexachloroethane [67-72-1]. The critical effect of hexachloroethane is kidney toxicity in rats and mice. Male rats accumulate hexachloroethane in their kidneys possibly due to its binding to alpha 2u-globulin, a mechanism that is specific for the male rat. Therefore, they are considered to be especially sensitive for kidney toxicity. A subchronic study with oral application via the feed resulted in a LOAEL for nephrotoxicity in female rats of 62 mg/kg body weight and day. Based on the NOAEL of 15 mg/kg body weight and day in female rats, a maximum concentration at the workplace (MAK value) of 1 ml/m^3 has been set. According to inhalation studies in dogs and rats, irritation of the nose and neurotoxicity can be ruled out at 1 ml/m^3. As the critical effect is systemic, hexachloroethane remains assigned to Peak Limitation Category II. An excursion factor of 8 would have been possible because of the long half-life; however, to avoid local irritation, an excursion factor of 2 has been established. The NOAELs for developmental toxicity in rats were 100 and 167 mg/kg body weight and day after oral application and in another inhalation study a NOAEC of 260 ml/m^3 was obtained. After toxicokinetic scaling to concentrations at the workplace, damage to the embryo or foetus is unlikely when the MAK value is not exceeded. Hexachloroethane is therefore classified in Pregnancy Risk Group C. Hexachloroethane is not genotoxic in vitro and not a clastogen in vivo. In carcinogenicity studies in rats and mice, it induces kidney tumours only in male rats which can be explained by an alpha 2u-globulin mechanism that is not relevant to humans. Model calculations predict that hexachloroethane can be taken up via the skin in toxicologically relevant amounts and the substance is therefore designated with "H". There are no data that show that hexachloroethane is a skin or airway sensitizer.

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MAK value (1969)	**1 ml/m³ ≙ 9.8 mg/m³**
Peak limitation (2002)	**Category II, excursion factor 2**
Absorption through the skin (2019)	**H**
Sensitization	**–**
Carcinogenicity (2019)	**Category 3 B**
Prenatal toxicity (2019)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	perchloroethane
Chemical name (IUPAC)	1,1,1,2,2,2-hexachloroethane
CAS number	67-72-1
Structural formula	CCl_3–CCl_3
Molecular formula	C_2Cl_6
Molar mass	236.74 g/mol
Melting point	sublimates (US EPA 2011)
Boiling point	186.8 °C (US EPA 2011)
Vapour pressure at 20 °C	0.28 hPa (NLM 2018)
log K_{OW}	4.14 (NLM 2018)
Solubility at 25 °C	50 mg/l water (NLM 2018)
1 ml/m³ (ppm) ≙ 9.823 mg/m³	**1 mg/m³ ≙ 0.102 ml/m³ (ppm)**

Note: The substance can occur simultaneously as vapour and aerosol.

Documentation for hexachloroethane was published in 1974 (Henschler 1974, available in German only), followed by a supplement on peak limitation in 2002 (Greim 2002, available in German only).

Hexachloroethane is used in the manufacture of smoke grenades and pyrotechnic devices for military purposes. It has been used also as a polymer additive, a moth repellent, a plasticizer for cellulose ethers, in formulations for insecticides and for refining aluminium alloys (US EPA 2011). In the EU, its use in the manufacturing and processing of non-ferrous metals has been banned (ECHA n.d.). A REACH dossier is not available (status as of 2018), and there is no harmonized EU classification for hexachloroethane.

Studies that investigated exposure to hexachloroethane in the smoke from smoke grenades or pyrotechnic devices have not been included in the evaluation of hexachloroethane because there was simultaneous exposure to titanium dioxide or zinc oxide/zinc chloride and other substances (US EPA 2011).

1 Toxic Effects and Mode of Action

After oral exposure of rats, hexachloroethane is almost completely absorbed and accumulates in the adipose tissue. In addition, the substance accumulates in the kidneys of male rats. The half-life in the adipose tissue, liver and blood of rats is 2.5 days. Exhalation is the main route of elimination, but the exhaled metabolite has not been identified. Hexachloroethane caused irritation of the eyes of rabbits in the Draize test and caused irritation of the eyes of dogs after repeated exposure to a concentration of 260 ml/m^3. At this concentration, central nervous effects were observed in rats and dogs, and the body weight gains were reduced in guinea pigs and rats. Hexachloroethane was nephrotoxic after doses of 590 mg/kg body weight and day and above in mice and increased the rat typical nephropathy after doses of 10 mg/kg body weight and day and above in male rats and at 80 mg/kg body weight and day and above in female rats. Because hexachloroethane binds presumably to the species and sex-specific alpha 2u-globulin, the protein accumulates in male rats, leading to increased nephrotoxicity, cell proliferation and renal tumours. Female rats and mice are not affected by an accumulation of alpha 2u-globulin. However, in a subchronic study, hexachloroethane caused nephrotoxicity in female F344 rats at 62 mg/kg body weight and day. Male and female B6C3F1 mice developed hepatocellular carcinomas after doses of 590 mg/kg body weight and day and above. Hexachloroethane was not found to have any relevant genotoxic potential, but it induced liver foci after initiation in rats and increased the replicative DNA synthesis in mouse hepatocytes. In developmental toxicity studies in rats, an increase in the number of resorptions, a smaller number of live foetuses per litter, reduced foetal weights and delayed ossification were observed at the dose level of 500 mg/kg body weight and day.

2 Mechanism of Action

In male F344 rats, hexachloroethane leads to the accumulation of hyaline droplets and increased cell proliferation and tumours in the kidneys, but not in female rats or in mice. These findings suggest a mechanism of tumour formation that is mediated by alpha 2u-globulin and occurs only in male rats. The protein itself was not detected in the studies of hexachloroethane, but in those carried out with the structurally similar pentachloroethane, which likewise causes renal tumours and increases cell proliferation only in male rats (Goldsworthy et al. 1988). In addition, linear mineralization of the renal papillae, which is typical of the alpha 2u-globulin mechanism (Hard et al. 1993), was found in the kidneys of male F344 rats following administration of hexachloroethane (NTP 1989). In male and female rats, hexachloroethane increased the incidence and severity of the nephropathy commonly found in rats and caused nephrotoxic effects in mice. This shows that other mechanisms of nephrotoxicity are involved besides the alpha 2u-globulin mechanism.

The development of phaeochromocytomas in male F344 rats is explained as follows: The analysis of several substances tested by the NTP, including hexachloroethane, revealed a significant association between the incidence of phaeochromocytomas in male F344 rats and a substance-induced increase in the severity of the chronic nephropathy commonly found in rats (Nyska et al. 1999). The increased severity of nephropathy led to the increased impairment of calcium homoeostasis and an increase over the spontaneous incidence (here 30%) of phaeochromocytomas. Hexachloroethane is one of the substances known to have this mechanism of action. However, this mechanism is not considered to be of relevance to humans (Greim et al. 2009).

Hepatocellular carcinomas were induced in male and female B6C3F1 mice (NCI 1978). Hexachloroethane is not genotoxic (Section 5.6). However, it increased the replicative DNA synthesis in mice (Miyagawa et al. 1995) and had promoting, but not initiating effects in a rat liver foci assay (Milman et al. 1988; Story et al. 1986). Therefore, the mechanisms involved in the development of tumours in B6C3F1 mice are probably cell proliferating effects with promoting effects on spontaneously initiated cells.

Another possible mechanism may be DNA damage or cytotoxic effects induced by the pentachloroethane radical that is presumed to form in the liver by reductive dechlorination. However, in studies that investigated the reductive dechlorination of several chloroalkanes in rat liver microsomes or hepatocytes, the extent of dechlorination or the formation of radicals was not correlated with the carcinogenic potency of the chloroalkanes in the liver of mice

(Nastainczyk et al. 1982; Salmon et al. 1981, 1985; Thompson et al. 1984; Tomasi et al. 1984). Therefore, it is unclear to which extent the formation of radicals contributes to the carcinogenic effects in the mouse liver.

In rats, after initiation hexachloroethane promoted gamma-glutamyltransferase-positive liver foci; these foci are different from those induced by phenobarbital promotion and their relevance as tumour precursors is uncertain (Milman et al. 1988; Story et al. 1986).

The central nervous effects (tremor and muscle twitches) after inhalation exposure of rats and dogs would be compatible with the effects of the structural class of chlorinated aliphatics. However, hexachloroethane stimulates rather than depresses the central nervous system (Weeks et al. 1979). Also after oral administration of hexachloroethane, neurotoxic symptoms were reported in sheep (US EPA 2011) and sporadically in rats (Weeks et al. 1979). However, this was not the case in most studies with rats.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

There are no quantitative data available for absorption after inhalation exposure.

The blood:air partition coefficient is 52.4 (Gargas et al. 1989).

When rats and mice were given oral hexachloroethane doses of 500 and 1000 mg/kg body weight, respectively, absorption was calculated to be about 95% from the amount of radioactivity excreted and the levels determined in the rest of the body (Mitoma et al. 1985).

A flux of 159 µg/cm^2 and hour was calculated for a saturated aqueous solution using the model of Fiserova-Bergerova et al. (1990). Assuming a surface area of 2000 cm^2 of skin and exposure for 1 hour (area of hands and forearms), this would correspond to an absorbed amount of 318 mg. A flux of 3.3 µg/cm^2 and hour and the corresponding absorption of 6.6 mg from a saturated aqueous solution were calculated using the algorithm of the IH SkinPerm model (Tibaldi et al. 2014).

Following absorption, hexachloroethane is distributed mainly in the adipose tissue. When male F344 rats were given a dose of 62 mg/kg body weight and day with the diet for 57 days, the hexachloroethane levels in the adipose tissue were 3 times as high as in the kidneys and more than 100 times as high as in the liver and blood. The half-lives were 2.7 days in the adipose tissue, 2.3 days in the liver and about 2.5 days in the blood. In another study with dietary administration for 110 days, the hexachloroethane levels in the kidneys of male F344 rats were up to 47 times as high as those in the kidneys of female rats. The levels in the blood, liver and adipose tissue, however, were similar. The authors assumed that higher exposure of the kidneys was the reason for the nephrotoxicity in male rats (Gorzinski et al. 1985; Table 1).

Tab. 1 Hexachloroethane concentrations (µg/g) in the tissues of F344 rats on the last day of exposure after administration with the diet for 110 days (Gorzinski et al. 1985)

Dose (mg/kg body weight and day)		Blood	Liver	Kidneys	Adipose tissue
1	4 ♂	0.079 ± 0.057	0.291 ± 0.213	1.356 ± 0.286	3.09 ± 0.33
	2–4 ♀	0.067 ± 0.039	0.260 ± 0.035	0.369 ± 0.505	2.59 ± 0.72
15	4 ♂	0.596 ± 0.653	1.736 ± 1.100	24.33 ± 5.73	37.90 ± 6.10
	3–4 ♀	0.162 ± 0.049	0.472 ± 0.204	0.69 ± 0.165	45.27 ± 11.33
62	4 ♂	0.742 ± 0.111	0.713 ± 0.343	95.12 ± 11.56	176.1 ± 14.5
	4 ♀	0.613 ± 0.231	0.631 ± 0.262	2.01 ± 0.66	162.1 ± 7.1

After ingestion of radioactively labelled hexachloroethane, rats and mice excreted about 70% of the radioactivity in the exhaled air, 2% as CO_2, and 6% to 16% in the urine and (mainly) in the faeces, with 6% to 20% remaining in the rest of the body. The radioactivity in the urine and faeces and in the rest of the body was attributed to metabolites. A total

amount of 24% to 30% was metabolized, but it was not investigated whether metabolites other than CO_2 were exhaled in the air. About 95% of the radioactivity was recovered (Mitoma et al. 1985).

3.2 Metabolism

There are no data available for humans.

The postulated metabolic pathway is shown in Figure 1 below and is based on data from rabbits in vivo and studies in vitro:

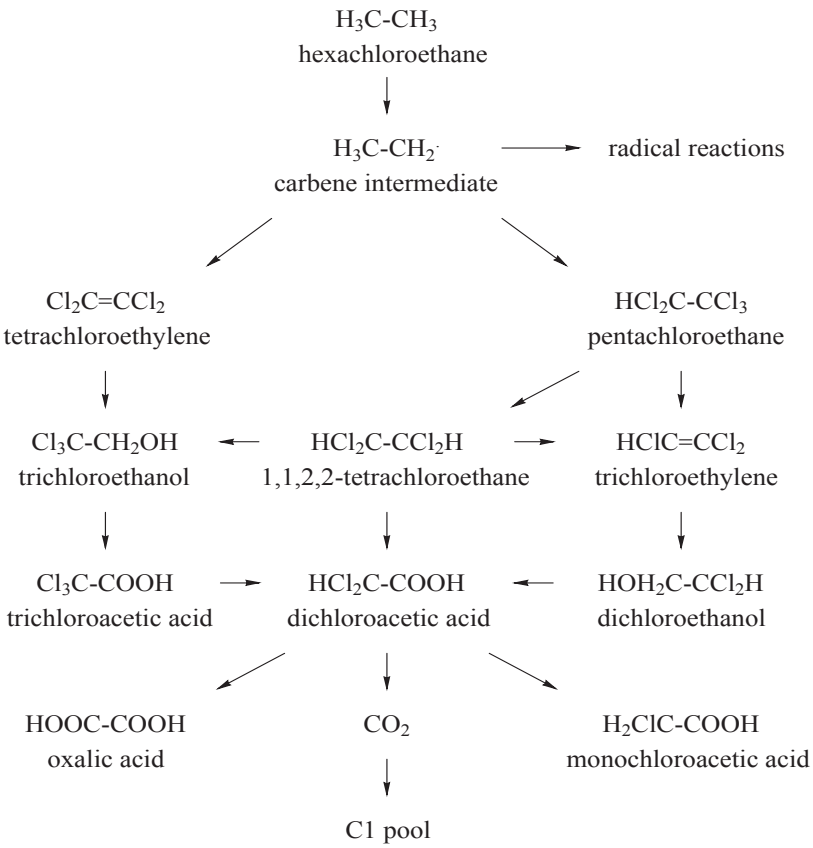

Fig. 1 Metabolism of hexachloroethane (US EPA 2011)

After oral administration of a hexachloroethane dose of 500 mg/kg body weight, the urine of rabbits contained 1.3% trichloroethanol, 0.4% dichloroethanol, 1.3% trichloroacetic acid, 0.8% dichloroacetic acid, 0.7% monochloroacetic acid and 0.1% oxalic acid. Hexachloroethane, carbon dioxide, tetrachloroethylene and 1,1,2,2-tetrachloroethane were determined in the exhaled air; however, the report did not include the percentage data (Jondorf et al. 1957).

After oral administration of hexachloroethane to sheep, pentachloroethane was identified as an elimination product (Henschler 1974; US EPA 2011).

Reductive dechlorination to pentachloroethane and tetrachloroethylene was detected also with rat hepatocytes in vitro. Mainly cytochrome P450 (CYP)3A enzymes, but also CYP2A and CYP2B enzymes, are responsible for metabolism. A study with recombinant rat CYP1A2 demonstrated metabolism to pentachloroethane, tetrachloroethylene and trichloroethylene; however, this was not confirmed by other studies (US EPA 2011).

4 Effects in Humans

Studies that investigated occupational exposure to hexachloroethane in the production of smoke bombs have not been included in the evaluation because there was simultaneous exposure to zinc oxide, titanium dioxide, aluminium powder, cryolite or zinc stearate (US EPA 2011).

The levels of hexachloroethane in the plasma were < 0.02 to 0.06 µg/l in 10 of 12 workers before beginning production of smoke munition, but increased to levels about 100 times as high during the 5-week production phase. When the workers were divided into groups according to low, medium and high levels of exposure, the corresponding plasma levels were 3.99, 7.14 and 10.75 µg/l, respectively. The workers used personal protective equipment (no other details). The workers were exposed to concentrations of up to 30 mg/m^3. Irritation of the skin was reported, but the symptoms were not attributed to exposure concentrations. The clinical examination did not reveal any unusual findings (US EPA 2011).

Irritation of the skin and mucous membranes was reported while handling heated hexachloroethane and after exposure to high dust concentrations (no other details; Weeks et al. 1979).

Apart from the data for irritation, no other data are available for humans.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

An LC_{50} cannot be derived from the available data. When groups of 6 Sprague Dawley rats were exposed for 8 hours, no effects were observed during exposure to a hexachloroethane concentration of 2500 mg/m^3. The body weight gains of the animals were slightly reduced during the subsequent observation period of 14 days. After 6-hour exposure to 17 000 mg/m^3, unsteady gait was observed in 2 rats and the body weight gains were slightly reduced. After 8-hour exposure to 57 000 mg/m^3, 2 of 6 animals died, and unsteady gait, reduced body weight gains, interstitial pneumonitis and vascular congestion were observed. Effects on the upper respiratory tract were attributed to a mycoplasma infection (Weeks et al. 1979). These were the nominal concentrations that were determined by the decreased weight of the hexachloroethane after it was heated to 25 °C or 50 °C to generate the test atmosphere.

5.1.2 Oral administration

The oral LD_{50} values in rats, rabbits and guinea pigs are above 1000 mg/kg body weight (US EPA 2011). In the rats that died (no other details), ataxia, tremor and convulsions were observed before death (Weeks et al. 1979).

5.1.3 Dermal application

The dermal LD_{50} for rabbits was above 3160 mg/kg body weight after the epicutaneous application of a paste consisting of hexachloroethane and methyl cellulose. Skin irritation was not observed (Esso Research and Engineering Company 1962).

The dermal LD_{50} for rabbits was > 32 000 mg/kg body weight, and skin irritation was not observed (Weeks et al. 1979; Weeks and Thomasino 1976).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

In a study with repeated inhalation exposure, rats, guinea pigs and dogs were exposed whole-body to hexachloroethane vapour at concentrations of up to 260 ml/m³. The concentrations in the exposure chamber were determined by passing the air in the chamber through 2 wash bottles filled with toluene that were connected in series; the sum of vapour and any aerosol present was determined using this method. Histopathological examinations were carried out in half of the animals after the last exposure and in the other half 12 weeks later; 22 tissues and organs including the lungs, trachea and nose were examined. Additional groups of rats were observed for behavioural toxicity. Tremor and reduced body weight gains, which presumably resulted in increased relative kidney, spleen and testis weights, were observed in rats at the high concentration. Dogs developed neurotoxic effects such as tremor, facial muscular fasciculations, hypersalivation and ataxia. In guinea pigs, the body weight gains were reduced and the relative liver weights were increased. Mycoplasma infection was found in rats. Therefore, it was difficult to interpret the local findings. They were increased at a hexachloroethane concentration of 260 ml/m³. The NOAEC (no observed adverse effect concentration) was 48 ml/m³ for all species (Table 2; Weeks et al. 1979). The study results were reported only in summary form.

Tab. 2 Effects of hexachloroethane after repeated inhalation exposure (Weeks et al. 1979)

Species, strain, number per group	Exposure	Findings
rat, Sprague Dawley, 25 ♂ and 25 ♀	**6 weeks**, 0, 15, 48, 260 ml/m³, 6 hours/day, 5 days/week, without and with a 12-week observation period	**48 ml/m³**: **NOAEC**; **260 ml/m³**: ♂: body weight gains ↓, relative kidney, spleen, testis weights ↑; ♀: relative liver weights ↑; ♂ and ♀: tremor, ruffled fur, red exudate around the eyes after 4 weeks; mycoplasma-dependent lesions in the nasal concha, trachea and lungs ↑, no other histopathological findings; observation period: no unusual findings
rat, Sprague Dawley, 15 ♂ 12–14 weeks old	**6 weeks**, 0, 15, 48, 260 ml/m³, 6 hours/day, 5 days/week	**48 ml/m³**: **NOAEC**; **260 ml/m³**: body weight gains ↓, avoidance latency and spontaneous motor activity unchanged, no clinical signs of toxicity
dog, beagle, 4 ♂	**6 weeks**, 0, 15, 48, 260 ml/m³, 6 hours/day, 5 days/week, without and with a 12-week observation period	**48 ml/m³**: **NOAEC**; **260 ml/m³**: mortality 1/4 after first exposure, in 3/4: tremor, ataxia, hypersalivation, head bobbing, facial muscular fasciculations, closed eyelids; no changes in the clinico-chemical parameters in the blood, no changes in pulmonary function; no histopathological findings; observation period: no unusual findings
guinea pig, Hartley, 10 ♂	**6 weeks**, 0, 15, 48, 260 ml/m³, 6 hours/day, 5 days/week, without and with a 12-week observation period	**48 ml/m³**: **NOAEC**; **260 ml/m³**: body weight gains ↓, relative liver weights ↑; no histopathological findings; observation period: no unusual findings

The neurotoxic lesions that were observed in this study were not reported among the findings of the studies with oral exposure of rats (Section 5.2.2). A first-pass effect in the liver may have led to deactivation.

5.2.2 Oral administration

After ingestion, the liver and kidneys are the target organs of the toxicity of hexachloroethane (Table 3). Hyaline droplets accumulated in the renal tubular epithelium of male rats even in subacute studies. In a subchronic study (NTP 1989), effects were determined in the liver in female rats, whereas in chronic studies, the kidneys were the main target organs in female F344 rats and in B6C3F1 mice (NCI 1978). Likewise, nephrosis was observed in rabbits.

Tab. 3 Effects of hexachloroethane after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rabbit, New Zealand White, 5 ♂	**12 days**, 0, 100, 320, 1000 mg/kg body weight and day, daily, gavage	**100 mg/kg body weight**: **NOAEL**; **320 mg/kg body weight and above**: body weight gains ↓, relative liver and kidney weights ↑, liver degeneration and necrosis, toxic tubular nephrosis of the convoluted tubules and corticomedullary region in the kidneys, minimal tubular nephrocalcinosis	Weeks et al. 1979
rat, F344/N, 5 ♂ and 5 ♀	**16 days**, 0, 187, 375, 750, 1500, 3000 mg/kg body weight and day, 5 days/week (12 doses over 16 days), gavage	**187 mg/kg body weight and above**: ♂: hyaline droplets in the tubular epithelium, regeneration, tubular casts; **375 mg/kg body weight**: ♀: **NOAEL**; **750 mg/kg body weight and above**: ♀: body weights ↓	NTP 1989
rat, F344/N, 5 ♂	**3 weeks**, 0, 146, 293 mg/kg body weight and day, 7 days/week, gavage	**146 mg/kg body weight and above**: hyaline droplets in the tubular epithelium, regeneration, tubular casts, absolute and relative kidney weights ↑, PCNA labelling index ↑, AST, NAG ↑; **293 mg/kg body weight**: relative liver weights ↑; urine volume ↑, creatinine, glucose, specific gravity ↓	NTP 1996
rat, Osborne Mendel, 5 ♂ and 5 ♀	**6 weeks**, 0, 178, 316, 562, 1000, 1780 mg/kg body weight and day, 5 days/week, 2-week observation period, gavage	**1000 mg/kg body weight and above**: mortality ↑, body weight gains ↓, no histopathology	NCI 1978
rat, F344/N, 10 ♂ and 10 ♀	**13 weeks**, 0, 47, 94, 188, 375, 750 mg/kg body weight and day, 5 days/week, gavage	**47 mg/kg body weight and above**: ♂: hyaline droplets in the tubular epithelium, regeneration, tubular casts; **94 mg/kg body weight and above**: ♂: relative kidney weights ↑; ♀: 6.5% increase in relative liver weights; **188 mg/kg body weight and above**: ♂: 9% increase in relative liver weights; ♀: hepatocellular centrilobular necrosis; **375 mg/kg body weight and above**: ♂: hepatocellular centrilobular necrosis; ♀: relative kidney weights ↑; **750 mg/kg body weight**: ♂: mortality 5/10; papillary necrosis; haemorrhagic necrosis in the urinary bladder; ♀: mortality 2/10, relative thymus weights ↓	NTP 1989

Tab. 3 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, F344, 10 ♂ and 10 ♀	**16 weeks**, 0, 1, 15, 62 mg/kg body weight and day, in the diet	**1 mg/kg body weight**: ♂: **NOAEL**; **15 mg/kg body weight and above**: ♂: hypertrophy, dilation of the proximal convoluted tubules of the kidneys (0, 1, 15, 62 mg/kg body weight: ♂: 0, 1, 7, 10; ♀: 0, 0, 0, 0); atrophy and degeneration of the renal tubules (♂: 1, 2, 7, 10; ♀: 1, 1, 2, 6); slight swelling of the hepatocytes (♂: 4, 3, 6, 8; ♀: 0, 0, 0, 0); ♀: **NOAEL**; **62 mg/kg body weight**: ♂: relative and absolute liver and kidney weights ↑; ♀: relative liver weights ↑, atrophy and degeneration of the renal tubules; no effects on urine and blood parameters or on an additional 35 organs and tissues	Gorzinski et al. 1985
rat, Osborne Mendel, 50 ♂ and 50 ♀, 20 control animals	**78 weeks**, 0, 250, 500 mg/kg body weight and day, from week 23 onwards: 1 week without exposure after 4 weeks of treatment (212, 423 mg/kg body weight and day on average), 5 days/week, observation period of 33–34 weeks, gavage	**212 mg/kg body weight and above**: ♂ and ♀: mortality earlier than in control animals (surviving animals after 70 weeks (♂/♀): vehicle control: 55%/70%, 212 mg/kg body weight: 48%/54%, 423 mg/kg body weight: 38%/48%), tubular nephropathy with degeneration, necrosis and large hyperchromatic regenerative epithelial cells (not observed in control animals); **423 mg/kg body weight**: ♂: mortality ↑; dose reduction because of early mortality	NCI 1978
rat, F344/N, 50 ♂ and 50 ♀	**103 weeks**, ♂: 0, 10, 20 mg/kg body weight and day, ♀: 0, 80, 160 mg/kg body weight and day, 5 days/week, gavage	**10 mg/kg body weight and above**: ♂: increase in the severity of the nephropathy commonly found in rats that was also observed in control animals (tubular cell degeneration and regeneration, tubular dilation and atrophy, glomerulosclerosis, interstitial fibrosis, chronic inflammation), linear mineralization of the renal papillae, hyperplasia of the transitional epithelium of the renal pelvis, renal tubule pigmentation; **80 mg/kg body weight and above**: ♀: increase in the severity and incidence of the nephropathy commonly found in rats	NTP 1989
mouse, B6C3F1, 5 ♂ and 5 ♀	**6 weeks**, 0, 316, 562, 1000, 1780, 3160 mg/kg body weight and day, 5 days/week, 2-week observation period, gavage	**1780 mg/kg body weight and above**: ♂: mortality ↑ (no other details), ♂ and ♀: body weight gains ↓, no histopathology	NCI 1978
mouse, B6C3F1, 50 ♂ and 50 ♀ 20 control animals, because of high mortality, vehicle controls pooled from other, concurrently performed studies	**78 weeks**, 0, 500, 1000 mg/kg body weight and day, from week 9 onwards: doses increased to 600 and 1200 mg/kg body weight and day (590 and 1180 mg/kg body weight and day on average), 5 days/week, observation period of 12–13 weeks, gavage	**590 mg/kg body weight and above**: ♂: unexpectedly small number of surviving animals (14%), ♂ and ♀: tubular nephropathy in almost all animals: degeneration of the convoluted tubule epithelium, regenerative tubular epithelium, infiltration of inflammatory cells, fibrosis, calcium deposits (not in control animals); **1180 mg/kg body weight**: ♂: mortality ↑	NCI 1978

AST: aspartate aminotransferase; NAG: N-acetylglucosaminidase; NOAEL: no observed adverse effect level; PCNA: proliferating cell nuclear antigen

Hexachloroethane increased the severity of the nephropathy that occurred already in the control animals. In male F344 rats, nephropathy was increased at the lowest dose tested of 10 mg/kg body weight and day and above and in the female F344 rats at 80 mg/kg body weight and day and above. In the females, the incidences of linear mineralization of the renal papillae, hyperplasia of the transitional epithelium of the renal pelvis or pigmentation of the renal tubules were not increased (NTP 1989).

In a toxicokinetics study (Gorzinski et al. 1985), the hexachloroethane levels in the kidneys of male rats were about 40 times as high as those in the kidneys of female rats after a dose of 15 mg/kg body weight. This may be due to the hexachloroethane binding to alpha 2u-globulin, which subsequently accumulates. This finding was observed only in male rats. As this mechanism is of no relevance to humans, the results obtained in male rats for nephrotoxicity are not applicable to humans. However, hexachloroethane caused nephrotoxicity also in mice at dose levels of 590 mg/kg body weight and day and above (NCI 1978) and in female F344 rats at 62 mg/kg body weight and day and above (Gorzinski et al. 1985). Possible differences in sensitivity between Osborne Mendel and F344 rats cannot be quantified. A NOAEL (no observed adverse effect level) for nephrotoxicity was not established for mice because lower doses were not examined. The NOAEL derived from the 16-week study (Gorzinski et al. 1985) for female F344 rats was 15 mg/kg body weight and day.

5.2.3 Dermal application

There are no data available.

5.2.4 Intraperitoneal injection

After daily intraperitoneal injection of 0.01 ml hexachloroethane on 17 days, scleroderma-like lesions of the skin were observed in 5 of 17 ddY mice (US EPA 2011). These kinds of effects were not described in other studies with rats and mice after repeated oral and inhalation exposure.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

The 24-hour application (no other details) of 500 mg hexachloroethane powder to the intact and abraded skin of New Zealand White rabbits did not cause irritation. When hexachloroethane was applied to the intact skin as an aqueous paste, it caused barely perceptible erythemas, but no oedemas (Weeks et al. 1979; Weeks and Thomasino 1976).

5.3.2 Eyes

When 100 mg hexachloroethane powder was instilled into the conjunctival sac of New Zealand White rabbits, moderate damage to the cornea, iritis and conjunctivitis was observed 24, 48 and 72 hours later. These effects had completely subsided after 7 days (Weeks et al. 1979; Weeks and Thomasino 1976).

After repeated exposure to a concentration of 260 ml/m^3, dogs kept their eyelids closed, presumably as a sign of irritation. This effect was not observed at the concentration of 48 ml/m^3 (Weeks et al. 1979).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

In a modified Draize test, 10 male guinea pigs were injected intradermally with a solution of 0.1% hexachloroethane in propylene glycol and physiological saline (1:29). None of the animals reacted to an injection carried out in the same way for the challenge (Weeks and Thomasino 1976).

5.4.2 Sensitizing effects on the airways

Groups of 10 male guinea pigs were exposed to concentrations of 0, 15, 48 or 260 ml/m^3 for 6 hours a day, on 5 days a week, for 12 weeks. None of the animals reacted to the subsequent intradermal injection of 0.1 ml of a 0.1% hexachloroethane solution in physiological saline (Weeks et al. 1979).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

There are no data available. The reproductive organs of rats and mice were not target organs in the subchronic and chronic studies.

5.5.2 Developmental toxicity

A research group carried out two developmental toxicity studies in Sprague Dawley rats with hexachloroethane. In the first study, groups of 22 rats were exposed to concentrations of 0, 15, 48 or 260 ml/m^3 for 6 hours a day from days 6 to 16 of gestation. The high concentration caused tremor and reduced body weight gains in the dams, but developmental toxicity or teratogenicity were not observed in the foetuses. The authors concluded that the increased incidences of mucopurulent nasal exudate found in the dams at concentrations of 48 ml/m^3 and above were caused by a mycoplasma infection. The maternal NOAEC was 48 ml/m^3 and the NOAEC for developmental toxicity was 260 ml/m^3 (Weeks et al. 1979). Foetuses with unusual gross-pathological findings and 2 male and 2 female foetuses per litter were examined for skeletal changes and another 2 male and 2 female foetuses were examined for soft tissue changes (OECD Test Guideline 414: examination of 50% of the foetuses per litter for skeletal and soft tissue alterations, respectively). The results were not published in the form of a table.

In the second study, groups of 22 rats were given gavage doses of hexachloroethane of 0, 50, 100 or 500 mg/kg body weight and day from days 6 to 16 of gestation. Tremor, reduced body weight gains, mucopurulent nasal exudate (70% of the animals) and subclinical pneumonitis (20%) were observed in the dams at the high dose level. In addition, the number of resorptions was increased and the number of live foetuses per dam was reduced. Anomalies of the skeleton and soft tissues or malformations were not found. Nasal exudate and subclinical pneumonitis were reported in about 10% of the control animals. The NOAEL for dams and developmental toxicity was 100 mg/kg body weight and day (Weeks et al. 1979).

Groups of 20 to 21 Wistar rats were given gavage doses of hexachloroethane of 0, 56, 167 or 500 mg/kg body weight and day from days 7 to 17 of gestation. Maternal body weight gains, feed consumption and motor activity were reduced after doses of 167 mg/kg body weight and day and above. Piloerection, subcutaneous haemorrhages and whitening of the liver were observed at 500 mg/kg body weight and day. In this dose group, the weights of the foetuses were reduced and the increased number of skeletal variations of the lumbar ribs was statistically significant (60.3%; control group: 1.3%). The ossification of sternebrae, phalanges and the spine was decreased at this dose level. Hexachloroethane did not induce visceral anomalies or malformations; one foetus of the group that received 500 mg/kg body weight had no tail. The NOAEL for dams was 56 mg/kg body weight and day, and the NOAEL for developmental toxicity was 167 mg/kg body weight and day (Shimizu et al. 1992; US EPA 2011). The study report of Shimizu et al. (1992) is in Japanese and includes an abstract, tables and figures in English.

5.6 Genotoxicity

5.6.1 In vitro

Data for in vitro genotoxicity are shown in Table 4.

Hexachloroethane did not cause gene mutations in Salmonella typhimurium or Salmonella cerevisiae, mitotic recombinations in Salmonella cerevisiae or gene conversions or aneuploidy in Aspergillus nidulans (see Table 4).

In the gamma-H2AX assay for DNA double-strand breaks in mouse lymphoma cells, hexachloroethane was tested as 1 of 3 examples of non-genotoxic cytotoxic substances to validate the test system together with substances that were classified as genotoxic. The histone H2AX, which is phosphorylated at serine139, is involved in the repair of DNA double-strand breaks. The amount of the protein in the nucleus is used as an indirect biomarker of double-strand breaks. At similar levels of cytotoxicity, the genotoxic substances markedly increased the incidences of DNA double-strand breaks. Hexachloroethane was classified as negative in this assay (Smart et al. 2011).

Binding to calf thymus DNA was detected with ^{14}C-labelled hexachloroethane after activation with microsomes from the liver and kidneys, but not from the lungs and stomach of Wistar rats or BALB/c mice. The highest levels of DNA binding were observed after activation with the cytosolic fractions from the 4 organs of both species. However, the type of DNA binding (DNA adducts or metabolic incorporation of ^{14}C) was not investigated (Lattanzi et al. 1988).

The SCE test with CHO cells (a cell line derived from Chinese hamster ovary) yielded positive results for hexachloroethane only if the cell cycle was delayed (Galloway et al. 1987).

Up to the highest concentration tested of 16 mM, the incidences of micronuclei in isolated human lymphocytes were increased sporadically with and without the addition of metabolic activation, but the increase was not dependent on the concentration (Tafazoli et al. 1998).

Tab. 4 Genotoxicity of hexachloroethane in vitro

End point	Test system	Concentration range [µg/ml]	Cytotoxicity	Result		References
				–m.a.	+m.a.	
SOS test	Salmonella typhimurium TA1535/pSK1002	up to 42	no data	–	–	Nakamura et al. 1987
gene mutation	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	no data	no data	–	–	US EPA 2011
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.1–500 µg/plate	[a]	–	–	Weeks et al. 1979
	Salmonella typhimurium TA98, TA100, TA1535, TA1537	100–10 000 µg/plate	[b]	–	–	Haworth et al. 1983
	Salmonella typhimurium TA98, TA100, TA1535, TA1537	no data	no data	–	–	Milman et al. 1988
	Salmonella typhimurium BA13	354–7080 µg/plate	[b]	–	–	Roldán-Arjona et al. 1991
mitotic recombination	Salmonella cerevisiae D3	no data	no data	–	–	US EPA 2011
gene mutation	Salmonella cerevisiae D4	0.1–500 µg/plate	[a]	–	–	Weeks et al. 1979
gene mutation	Salmonella cerevisiae D7	1185–2963	[b]	–	–	Bronzetti et al. 1989
mitotic gene conversion	Salmonella cerevisiae D7	1185–2963	[b]	–	–	Bronzetti et al. 1989
mitotic crossing-over	Aspergillus nidulans P1	25–400	≥ 200	–	n. t.	Crebelli et al. 1988, 1992, 1995
aneuploidy	Aspergillus nidulans P1	25–400	≥ 200	–	n. t.	Crebelli et al. 1988, 1992, 1995

Tab. 4 *(continued)*

End point	Test system	Concentration range [µg/ml]	Cytotoxicity	Result		References
				–m.a.	+m.a.	
SCE	CHO cells	10–1000	[b]	–	+[d]	Galloway et al. 1987
comet assay	human lymphocytes	237–3792	[c]	–	–	Tafazoli et al. 1998
gamma-H2AX assay DNA double-strand breaks	mouse lymphoma cells L5178Y	23.7–474	[a]	–	–	Smart et al. 2011
covalent DNA binding	calf thymus DNA	about 13.5			+	Lattanzi et al. 1988
CA	CHO cells	150–1000	[b]	–	–	Galloway et al. 1987
MN	human lymphocytes from 2 donors	11.9–3792	[c]	±[e]	±[e]	Tafazoli et al. 1998
MN	human lymphoblastoid cell lines AHH-1, MCL-5, h2E1	2.37–23.7	≥ 11.9	–	n. t.	Doherty et al. 1996

[a] tested up to toxicity
[b] tested up to the solubility limit
[c] tested up to the recommended maximum concentration
[d] only with delays in the cell cycle
[e] not dose-dependent
CA: chromosomal aberrations; MN: micronuclei; n. t.: not tested; SCE: sister chromatid exchange

5.6.2 In vivo

Hexachloroethane increased the replicative DNA synthesis as a measure of cell proliferation in the hepatocytes of B6C3F1 mice treated with a gavage dose of 1000 mg/kg body weight, but not in mice treated with 2000 mg/kg body weight (Miyagawa et al. 1995).

The positive result obtained with 10 mM hexachloroethane in Drosophila in the test for mitotic recombination (eye mosaic assay) was interpreted by the authors as more likely to be a non-specific, probably non-genotoxic effect (Vogel and Nivard 1993).

Male BALB/c mice were given single intraperitoneal injections of a hexachloroethane dose of 900 mg/kg body weight. An increase in DNA strand breaks in the liver was not determined by alkaline unwinding (Taningher et al. 1991).

Radioactively labelled hexachloroethane injected intraperitoneally at a dose of about 2 mg/kg body weight induced DNA binding. The level of binding was determined 22 hours after treatment based on the covalent binding index in the liver and was assessed as "weak" in Wistar rats and "moderate" in BALB/c mice (Lattanzi et al. 1988). DNA adducts were not examined. Therefore, metabolic incorporation of radioactive carbon into the DNA may have occurred.

A micronucleus test with bone marrow cells from groups of 5 male and 5 female CD-1 mice that were given single intraperitoneal injections of 2000 or 4000 mg/kg body weight yielded negative results. Marked clinical signs of toxicity were observed in the animals; they were examined 24 and 48 hours following treatment. The ratio of polychromatic to normochromatic erythrocytes was unchanged (Crebelli et al. 1999).

Overall, the results did not provide evidence of a relevant genotoxic potential.

5.7 Carcinogenicity

5.7.1 Short-term studies

Hexachloroethane was tested for the initiation and promotion of gamma-glutamyl-transferase-positive foci in the liver of Osborne Mendel rats. After administration of diethylnitrosamine, hexachloroethane was found to be a promoter, but not an initiator, at a dose of 500 mg/kg body weight and day. The number of liver foci did not increase after treatment with hexachloroethane alone (Milman et al. 1988; Story et al. 1986). In a cell transformation assay with BALB/c-3T3 cells, hexachloroethane yielded negative results at the concentrations tested of 0.16 to 100 µg/ml (Milman et al. 1988; Tu et al. 1985).

5.7.2 Long-term studies

The National Cancer Institute carried out long-term studies with oral administration to B6C3F1 mice and Osborne Mendel rats. The study with rats did not find a statistically significant increase in the incidence of tumours up to the high dose of 423 mg/kg body weight and day. The incidences of hepatocellular carcinomas were increased in male and female B6C3F1 mice at the low dose of 590 mg/kg body weight and day and above. Adenomas were not reported (Table 5; NCI 1978).

Oral administration of hexachloroethane to male F344 rats for 2 years induced renal adenomas and carcinomas at the high dose of 20 mg/kg body weight and day; when the incidences were combined, the increases were statistically significant. At this dose level, also the increased incidence of tubular hyperplasia, which is a tumour precursor, was statistically significant. Increases in the incidences of phaeochromocytomas were statistically significant at 10 mg/kg body weight and day. No tumours were found in the females after treatment with the 8-fold dose (Table 5; NTP 1989). The analysis of several substances tested by the NTP, including hexachloroethane, revealed a significant association between the incidence of phaeochromocytomas in male F344 rats and the substance-induced increase in the severity of the chronic nephropathy commonly found in rats (Nyska et al. 1999). The increased severity of nephropathy led to a more severe impairment of calcium homoeostasis and an increase over the spontaneous incidence (here 30%) of phaeochromocytomas. Hexachloroethane is one of the substances known to have this mechanism of action. However, this mechanism is not considered to be of human relevance (Greim et al. 2009). In mice, the spontaneous incidence of phaeochromocytomas is low. Therefore, these tumours do not develop in spite of nephrotoxicity.

Tab. 5 Studies of the carcinogenicity of hexachloroethane

Author:		NCI 1978		
Substance:		hexachloroethane (> 98% pure)		
Species:		**mouse**, B6C3F1, 50 ♂ and 50 ♀ exposed animals, 20 ♂ and 20 ♀ control animals		
Administration route:		gavage		
Dose:		0, 500, 1000 mg/kg body weight and day, increased to 600 and 1200 mg/kg body weight and day, respectively, from week 9 onwards (590, 1180 mg/kg body weight and day on average; see Table 1)		
Duration:		78 weeks, 5 days/week, observation period of 12–13 weeks		
Toxicity:		590 mg/kg body weight and day and above: nephrotoxicity (see Section 5.2.1)		
		dose (mg/kg body weight and day)		
		vehicle control	**590**	**1180**
surviving animals	♂	5/20 (25%)	7/50 (14%)	29/50 (58%)
	♀	16/20 (80%)	40/50 (80%)	34/50 (68%)
tumours				
liver:				
hepatocellular carcinomas	♂	6/60 (10%)[a)]	15/50 (30%)*	31/49 (63%)**
	♀	2/60 (3%)[a)]	20/50 (40%)**	15/49 (31%)**

Tab. 5 *(continued)*

Author:	NCI 1978			
Substance:	hexachloroethane (>98% pure)			
Species:	**rat**, Osborne Mendel, 50 ♂, 50 ♀			
Administration route:	gavage			
Dose:	0, 250, 500 mg/kg body weight and day (212, 423 mg/kg body weight and day on average, see Table 1)			
Duration:	78 weeks, 5 days/week, from week 23 onwards: 1 week without exposure after 4 weeks, observation period of 33–34 weeks			
Toxicity:	212 mg/kg body weight and day and above: mortality ↑, nephrotoxicity (see Section 5.2.1)			
		dose (mg/kg body weight and day)		
		vehicle control	**212**	**423**
surviving animals	♂	11/20 (55%)	24/50 (48%)	19/50 (38%)
	♀	14/20 (70%)	27/50 (54%)	24/50 (48%)
tumours				
kidneys:				
tubular adenomas	♂	0/18	4/37 (11%)	0/29
	♀	0/20	0/50	0/49
hamartomas	♂	0/18	0/37	0/29
	♀	0/20	0/50	3/49 (6%)
thyroid gland:				
follicle cell adenomas and carcinomas	♂	2/18 (11%)	3/36 (8%)	5/28 (18%)
	♀	2/20 (10%)	3/47 (6%)	3/47 (6%)
testes:				
interstitial cell tumours	♂	0/18	0/36	3/29 (10%)
Author:	NTP 1989			
Substance:	hexachloroethane (> 99% pure)			
Species:	**rat**, F344/N, 50 ♂, 50 ♀			
Administration route:	gavage			
Dose:	♂: 0, 10, 20 mg/kg body weight and day, ♀: 0, 80, 160 mg/kg body weight and day			
Duration:	2 years, 5 days/week			
Toxicity:	10/80 mg/kg body weight and above: nephrotoxicity (see Section 5.2.1)			
		dose (mg/kg body weight and day)		
		0	**10/80**	**20/160**
surviving animals	♂	31/50 (62%)	29/50 (58%)	26/50 (52%)
	♀	32/50 (64%)	27/50 (54%)	32/50 (64%)
tumours and pre-neoplasias				
kidneys:				
tubular hyperplasia	♂	2/50 (4%)	4/50 (8%)	11/50 (22%)*
	♀	0/50	0/50	0/50
tubular adenomas	♂	1/50 (2%)	2/50 (4%)	4/50 (8%)
	♀	0/50	0/50	0/50
tubular carcinomas	♂	0/50	0/50	3/50 (6%)
	♀	0/50	0/50	0/50
tubular adenomas and carcinomas	♂	1/50 (2%)	2/50 (4%)	7/50 (14%)***
	♀	0/50	0/50	0/50

Tab. 5 *(continued)*

		dose (mg/kg body weight and day)		
		0	**10/80**	**20/160**
adrenal gland:				
focal hyperplasia	♂	6/50 (12%)	4/45 (9%)	10/49 (20%)
	♀	0/50	0/50	0/50
phaeochromocytomas	♂	14/50 (28%)	26/45 (58%)***	19/49 (39%)
	♀	0/50	0/50	0/50
complex phaeochromocytomas	♂	0/50	0/45	2/49 (4%)
	♀	0/50	0/50	0/50
malignant phaeochromocytomas	♂	1/50 (2%)	2/45 (4%)	1/49 (2%)
	♀	0/50	0/50	0/50
all phaeochromocytomas	♂	15/50 (30%)	28/45 (62%)***	21/49 (43%)
	♀	0/50	0/50	0/50

*p < 0.01; **p < 0.001; ***statistically significant in the life table test and logistic regression test
a) pooled vehicle controls from other studies that were carried out simultaneously because of the high mortality of the test control animals

6 Manifesto (MAK value/classification)

Critical effects are irritation, neurotoxicity, hepatotoxicity and nephrotoxicity.

Carcinogenicity. The renal tumours that were observed in male F344 rats at the dose of 20 mg/kg body weight and day can plausibly be explained by the accumulation of sex and species-specific alpha 2u-globulin bound to hexachloroethane, resulting first in cytotoxicity and regenerative hyperplasia and then in the development of adenomas and carcinomas (NTP 1989). This accumulation may also explain the tubular adenomas that were reported in male Osborne Mendel rats after a dose of 212 mg/kg body weight and day. Increased cell proliferation was detected in the kidneys of male F344 rats (NTP 1996). In agreement with these findings, female rats and B6C3F1 mice were not affected by renal tumours. Evidence of the accumulation of alpha 2u-globulin in the kidneys of male F344 rats was not provided for hexachloroethane, but for the structurally related pentachloroethane, which induced a low incidence of renal tumours also only in male F344 rats. The tumours induced by these mechanisms are not relevant to humans. The same applies to the occurrence of phaeochromocytomas in male F344 rats; these tumours very probably develop as a result of the impairment of calcium homoeostasis arising from the increase in the severity of the chronic nephropathy commonly found in rats (Greim et al. 2009; Nyska et al. 1999). Phaeochromocytomas were not observed in female F344 and Osborne Mendel rats or in B6C3F1 mice in spite of markedly higher doses and nephrotoxicity.

Hexachloroethane caused hepatocellular carcinomas in male and female B6C3F1 mice at dose levels of 590 mg/kg body weight and day and above (NCI 1978). Hexachloroethane has no relevant genotoxic potential. However, as hexachloroethane was found to be a promoter, but not an initiator in a rat liver foci assay, the liver tumours in mice were very probably caused by the promotion of spontaneously initiated cells in the liver. A large number of these cells are found in B6C3F1 mice; they cause the relatively high spontaneous incidences of liver tumours in this mouse strain that is sensitive to hepatocarcinogenicity. Although evidence was provided that radioactively labelled hexachloroethane binds to liver DNA in mice (Lattanzi et al. 1988), experimental findings were not able to rule out the possibility that this may be due to the metabolic incorporation of ^{14}C into the DNA. Hexachloroethane did not cause liver tumours in Osborne Mendel rats at dose levels of up to 500 mg/kg body weight and day. Therefore, the most relevant mechanism is non-genotoxic. The tumour spectrum and species sensitivity after exposure to hexachloroethane are similar to that of the pentachloroethane metabolite (rat: kidneys; mouse: liver), but hexachloroethane has a lower carcinogenic potential in the mouse liver. The radicals that form during metabolism or oxidative metabolites may have cytotoxic effects on the liver, thereby leading to the development of liver tumours. This mechanism may be relevant to humans. In rats, hexachloroethane promoted gamma-glutamyltransferase-positive liver foci; these foci are different from those induced

by phenobarbital and their relevance as tumour precursors is uncertain. Overall, hexachloroethane is suspected of causing carcinogenicity in the liver, but genotoxicity is not considered to play an important role. However, this has not been confirmed in a second species. Therefore, hexachloroethane has been classified in Carcinogen Category 3B.

MAK value. There are no data available for human exposure that are suitable for the derivation of a MAK value. Hexachloroethane is nephrotoxic in both rats and mice. In a 16-week study with administration of hexachloroethane in the diet, the NOAEL was 1 mg/kg body weight and day for male rats and 15 mg/kg body weight and day for female rats. The LOAEL (lowest observed adverse effect level) was 15 and 62 mg/kg body weight and day, respectively. At these doses, the hexachloroethane levels in the kidneys of male rats were up to 47 times as high as those in the kidneys of female rats (Gorzinski et al. 1985). This difference may arise from the binding of hexachloroethane to the alpha 2u-protein (see above); this protein is found in only very low concentrations in female rats and does not occur in humans. Therefore, the NOAEL for male rats was not used to derive a MAK value. The NOAEL for female rats was determined on the basis of the increased severity and incidence of the nephropathy commonly found in rats and is only of limited relevance to humans because this syndrome is not observed in humans (Hard et al. 2009). Therefore, the MAK value derived on the basis of this end point represents the worst case. In mice, the LOAEL for renal toxicity was 590 mg/kg body weight and day, which was the lowest dose tested in a 2-year study. Therefore, the MAK value was derived from the NOAEL obtained in the subchronic study of 15 mg/kg body weight and day for female rats.

The following toxicokinetic data are used to extrapolate this NOAEL 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 corresponding toxicokinetic species-specific correction value for the rat (1:4), the oral absorption of 95%, the body weight (70 kg) and respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The NOAEL may be lower after chronic exposure (1:2). The concentration calculated from this is 17.5 mg/m^3, or 1.7 ml/m^3, as hexachloroethane may occur as vapour at this concentration. As this value was based on a NOAEL from animal studies and is regarded as the worst case for humans, the current MAK value of 1 ml/m^3 has been retained. The NOAEL also applies to effects on the liver. Therefore, the MAK value provides protection from toxicity in the liver and thus from possible hepatocarcinogenicity.

If the derivation were carried out on the basis of a 6-week inhalation study with exposure on 5 days per week (Weeks et al. 1979), the results would be consistent with this value. The findings of the study are of limited validity; the critical effects were the clinical signs of central neurotoxicity observed in rats and dogs and the reduced body weight gains observed in guinea pigs at a concentration of 260 ml/m^3. The NOAEC of this study was 48 ml/m^3. The NOAEC may be lower after chronic exposure (1:6). The concentration calculated from this is 8 mg/m^3. Taking into consideration that this value was derived from a NOAEC from animal studies (1:2) and that the respiratory volume is increased (1:2), this would result in a concentration of 2 ml/m^3 (19.6 mg/m^3).

A concentration of 260 ml/m^3 caused eye irritation in dogs. The NOAEC was 48 ml/m^3. In a subacute study, no substance-induced findings were obtained in the histopathological examination of the nasal conchae of rats after treatment at a concentration of 48 ml/m^3. At 260 ml/m^3, lesions were observed in the nasal conchae, but these may have been caused by a mycoplasma infection. A concentration of 2.7 ml/m^3 was derived from the clear NOAEC of 48 ml/m^3 according to the method of Brüning et al. (2014) taking into consideration the extrapolation of subacute to chronic exposure (1:6) and the extrapolation of data for local effects from animal studies to humans (1:3). Therefore, no sensory irritation is expected at a MAK value of 1 ml/m^3.

Peak limitation. In view of the critical systemic effects, hexachloroethane remains classified in Peak Limitation Category II. An excursion factor of 8 would be possible because of the long half-life. However, to avoid irritation, the excursion factor of 2 has been retained because no sensory irritation is expected at the short-term concentration of 2 ml/m^3 permitted in this case (see above).

Prenatal toxicity. In a prenatal developmental toxicity study in Sprague Dawley rats, no toxic effects on development were reported after inhalation of hexachloroethane concentrations of up to 260 ml/m^3, which was a maternally toxic concentration (tremor and reduced body weight gains). The NOAEC for maternal toxicity was 48 ml/m^3 and the

NOAEC for developmental toxicity was 260 ml/m³ (Weeks et al. 1979). In a prenatal developmental toxicity study with administration by gavage, an increased number of resorptions and a reduced number of foetuses per dam were observed in the same rat strain at the maternally toxic dose of 500 mg/kg body weight and day (tremor and reduced body weights). The NOAEL for maternal toxicity and developmental toxicity was 100 mg/kg body weight and day (Weeks et al. 1979). Another prenatal developmental toxicity study in Wistar rats with administration by gavage reported maternally toxic effects, such as reduced body weight gains and motor activity, after doses of 167 mg/kg body weight and day and above. At the next-higher dose of 500 mg/kg body weight and day, reduced body weights, increased incidences of skeletal variations and delayed ossification were observed in the foetuses. The NOAEL for maternal toxicity was 56 mg/kg body weight and day, and the NOAEL for developmental toxicity was 167 mg/kg body weight and day (Shimizu et al. 1992; US EPA 2011). On the basis of the above assumptions (see Section "MAK value" without extrapolation of the daily exposure of the animals to 5 days per week exposure at the workplace (7:5)) and the oral NOAELs for developmental toxicity of 100 and 167 mg/kg body weight and day, the concentrations in air are determined to be 166 and 278 mg/m³, respectively. Taking into consideration the increased respiratory volume at the workplace (1:2; blood:air partition coefficient > 5; Section 3.1), the NOAEC for developmental toxicity is 130 times as high and the two converted NOAELs are 17 and 28 times as high as the MAK value of 1 ml/m³ (9.8 mg/m³). As no teratogenicity was observed, these margins are regarded as sufficiently large. Therefore, hexachloroethane has been classified in Pregnancy Risk Group C.

Germ cell mutagenicity. There are no studies available in germ cells. As hexachloroethane was not mutagenic in bacteria in the in vitro tests and was not clastogenic either in mammalian cells or in vivo, classification in one of the germ cell mutagen categories is not required.

Absorption through the skin. On the basis of a model calculation (Section 3.1), the maximum amount dermally absorbed was estimated to be 318 mg for humans after exposure to a saturated aqueous solution under standard conditions (2000 cm² surface area of skin and 1-hour exposure). Assuming 100% absorption by inhalation and a respiratory volume of 10 m³, the systemic NOAEC in humans is estimated to be 17.5 mg/m³ and the systemically tolerable amount 175 mg. Absorption through the skin thus accounts for more than 25% of the systemically tolerable amount, and hexachloroethane has been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There are no findings available from humans for sensitizing effects and no positive results from animal studies or from in vitro tests. Hexachloroethane has therefore not been designated with either "Sh" or "Sa" (for substances which cause sensitization of the skin or 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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