1,3-Dichlorobenzene

Supplement 2008	
MAK value (2007)	**2ml/m³ ≙ 12mg/m³**
Peak limitation (2007)	**Category II, excursion factor 2**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2007)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Chemical name (CAS)	**1,3-dichlorobenzene**
CAS number	541-73-1
Molecular weight	147
log K_{OW}[1)]	3.53 (SRC 2006)
Solubility in water at 20 °C	**125 mg/l (**SRC 2006**)**

New information has appeared since the 1988 documentation (documentation, "1,3-Dichlorobenzene", 1990), making publication of this Supplement necessary.

Toxic Effects and Mode of Action

1,3-Dichlorobenzene is hydroxylated by cytochrome P450-dependent monooxygenases via a reactive epoxide to a phenolic compound in the liver and is eliminated in the form of a glutathione conjugate, glucuronide or sulfate.

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1) *n*-octanol/partition coefficient

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New data are now available, especially from studies with repeated oral administration of 1,3-dichlorobenzene to rats. The effects in the liver and kidneys already described have been confirmed. A 28-day study in rats shows that 1,3-dichlorobenzene induces glucuronosyl transferase I and other xenobiotic metabolizing enzymes from the lowest dose used of 4 mg/kg body weight and day.

1,3-Dichlorobenzene affects the endocrine system. In a 90-day study, an increased number of follicles having a reduced colloidal density were observed in the thyroid gland at doses of 35 mg/kg body weight and day and above. At 147 mg/kg body weight and above the relative liver weights in male and female rats as well as the relative kidney weights in male rats were increased.

1,3-Dichlorobenzene caused moderate irritation on the skin and slight irritation to the conjunctiva.

Although 1,3-dichlorobenzene is not mutagenic in *Salmonella typhimurium* and *Escherichia coli* WP2 uvrA in the gene mutation test, it is mutagenic in *Aspergillus nidulans*. 1,3-Dichlorobenzene produced mitotic recombinations in *Saccharomyces cerevisiae* D3. In the studies performed since the 1988 documentation (documentation "1,3-dichlorobenzene" 1990), 1,3-dichlorobenzene induced no DNA repair synthesis in A549 cells or in primary rat hepatocytes. In hamsters, 1,3-dichlorobenzene did not significantly increase the frequency of chromosome aberrations in bone marrow cells. Owing to its methodological shortcomings, a micronucleus test in mice cannot be used in this evaluation. To summarize, no genotoxic activity can be derived from the data available for 1,3-dichlorobenzene. A conclusive assessment is, however, not possible.

Mechanism of Action

The liver damage caused in rats by 1,3-dichlorobenzene (Hoechst AG 1989; McCauley et al. 1995) is accompanied by induction of xenobiotic metabolizing enzymes of the phenobarbital type.

In rats, a disturbance in thyroid homoeostasis can develop by hepatic enzyme induction (Capen 1994): 1,3-dichlorobenzene induces glucuronosyl transferases (Hoechst AG 1989). The conjugation of the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) is increased by the induction of glucuronosyl transferases. This leads to an increased release of T_4 and T_3 in the thyroid follicles.

In humans and rats, the biosynthesis of thyroid hormones takes place in the epithelial cells of thyroid gland follicles at the tyrosine residues of a high molecular weight protein, thyreoglobulin. Iodized thyreoglobulin is stored in the interior of the follicles, the colloid. To release hormones, the iodized thyreoglobulin is reabsorbed into the epithelial cells by means of pinocytosis. The thyronine precursors of the hormones are then formed through the corresponding coupling, and T_4 and T_3 are released during thyreoglobulin degradation. The thyroid hormones diffuse into the blood through the adjacent capillaries. As hormone release increases, the follicles become smaller and the colloid density decreases (Thews et al. 1989).

A decrease in serum thyroid hormone concentration caused by the induction of glucuronosyl transferases results in an increase in thyroid-stimulating hormone (TSH or thyreotropin) in the serum of rats.

TSH is seen to be the principal growth factor of the thyroid gland in rats. However, TSH is not the only stimulation factor in humans. In the human thyroid gland, IGF (insulin-like growth factor), EGF (epidermal growth factor) and TGF-α (transforming growth factor) are also stimulated by TSH to a lesser extent. Each of these factors has signal transduction pathways of its own, which end with an increase in cell proliferation. The IGF-I-dependent signal pathway plays an important role in growth regulation of the thyroid gland. Excess IGF-I synthesis can result in abnormal thyroid gland growth and finally produce adenomas (Derwahl et al. 1999).

In humans and rats, TSH increases the synthesis and release of T_4 and T_3 in the thyroid gland. The number of follicular cells and the organ weight remain unchanged at first. After continued TSH stimulation, a proliferation of follicular cells follows this regulatory phase, which terminates in a marked increase in thyroid gland size (Andrae and Greim 1992).

In rats, however, continued stimulation of the thyroid function by TSH frequently results in progressive proliferative changes and, subsequently, in the formation of neoplasms in follicular cells (Hill et al. 1998).

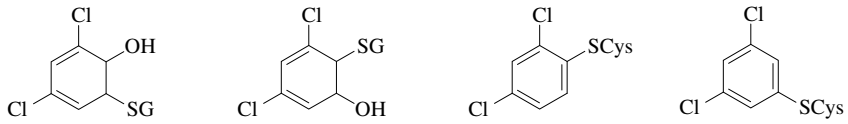
The enzymatic and morphological changes in rats occurring in the 28-day and 90-day studies after administration of 1,3-dichlorobenzene (Hoechst AG 1989; McCauley et al. 1995) indicate the beginning of this pathological process in the thyroid gland, which could also be relevant for humans (Hill et al. 1998). There are, however, qualitative and quantitative differences. Humans are able to store thyroid hormones with the thyroxine-binding globulin, which is not found in rodents (Capen 1994). In rats, the half-life of T_4 in the serum is less than a day, but it is six to nine days in humans. This means that the demand for endogenous T_4 is approximately ten times higher in rats than in humans. After about six hours, the half-lives of T_3 are much shorter in rats than after 24 hours in humans. In rats, TSH concentrations are 6-fold to 60-fold higher than in humans (Hill et al. 1998). Humans are therefore better able to compensate for degradation of thyroid hormones than rats, which react very quickly with feedback mechanisms.

Toxicokinetics and Metabolism

1,3-Dichlorobenzene is readily absorbed after ingestion.

No experimental data on the dermal penetration of 1,3-dichlorobenzene are available. On the basis of the physico-chemical properties of 1,3 dichlorobenzene, the models of Guy and Potts (1993) and Wilschut et al. (1995) yield a calculated dermal flux of 9.26 µg/cm² and hour and 4 µg/cm² and hour, respectively. Assuming a skin area of 2000 cm, the amount of 1,3 dichlorobenzene absorbed in one hour would be 18.5 mg and 9 mg, respectively.

Like other halogenated benzenes (den Besten et al. 1994), 1,3-dichlorobenzene is also hydroxylated in the liver by cytochrome P450-dependent monooxygenases of the phenobarbital type via a reactive epoxide to form a phenolic compound which is eliminated as a glutathione conjugate, glucuronide or sulfate. At least twelve metabolites, for the most part glutathione conjugates and their degradation products, were isolated in the gallbladder of rats after intraperitoneal injection of 1,3-dichlorobenzene. The main metabolites were *trans*-2,4-dichloro-6-(glutathion-S-yl) cyclohexa-2,4-dien-1-ol and *trans*-3,5-dichloro-6-(glutathion-S-yl)cyclohexa-2,4-dien-1-ol as well as their resultant cysteine conjugates. Eliminated as further metabolites were 3,5-dichlorophenyl conjugates with glutathione or cysteine and 3,5-dichlorophenyl mercapturic acids and their 2,4-dichlorophenyl isomers, including S-(2,4-dichlorophenyl)cysteine and S-(3,5-dichlorophenyl)cysteine (Kimura et al. 1992). The structural formulae of the main metabolites are shown in Figure 1.



trans-3,5-dichloro-6-(glutathion-S-yl)cyclohexa-2,4-dien-1-ol

trans-2,4-dichloro-6-(glutathion-S-yl)cyclohexa-2,4-dien-1-ol

S-2,4-dichlorophenylcysteine

S-3,5-dichlorophenylcysteine

SG: glutathionyl, Cys: cysteine

Figure 1 Main metabolites of 1,3-dichlorobenzene (according to Kimura et al. 1992)

Cultivation of liver slices from Sprague-Dawley rats showed that about 70% of the 1,3-dichlorobenzene is metabolized to glutathione and cysteine conjugates and only small quantities, about 3% to 5%, to glucuronides or sulfates. In cultivated human liver slices, on the other hand, about 40% of the substance is found as glucuronide or glutathione conjugates and about 20% as sulfates (Fisher et al. 1990). A further study with human liver slices and liver slices from Fischer-344 and Sprague-Dawley rats confirmed that mainly glutathione and cysteine conjugates and in human liver slices noticeably more glucuronides occur than in liver slices from rats. The extent of metabolism was greater in Fischer-344 rats than in Sprague-Dawley rats. Covalent binding of 1,3-dichlorobenzene to liver proteins was found to be higher in human liver preparations than in the liver tissue of Fischer-344 or Sprague-Dawley rats. The extent of this covalent binding does not correlate with the toxicity (Fisher et al. 1995).

1,3-Dichlorobenzene and its metabolites are inducers of xenobiotic metabolizing enzymes of the phenobarbital type in the liver. Increased activities of aniline hydroxylase, aminopyrine *N*-demethylase, ethoxyresorufin-O-deethylase, δ-aminolevulinic acid synthetase and NADPH cytochrome c-reductase as well as of glucuronosyl transferases I and II were found (Hoechst AG 1989; Kato et al. 1986, 1988 a, b; Kimura et al. 1983, 1985).

Effects in Humans

There are no new data available for the effects of 1,3-dichlorobenzene in humans.

Animal Experiments and *in vitro* Studies

Acute Toxicity

Inhalation

In a study carried out according to OECD Test Guideline 403, a 4-hour LC_{50} of > 17600 mg/m³ (2933 ml/m³) was found for the rat (ECB 2000).

Ingestion

Groups of 5 male B6C3F1 mice received 1,3-dichlorobenzene doses of 0, 120, 200 or 300 mg/kg body weight by single oral administration (no other details). No effects were observed at 120 mg/kg body weight. There was a significant increase in alanine aminotransferase (ALT) activity in the plasma, extensive centrilobular liver cell necrosis and greatly increased liver cell proliferation at 200 and 300 mg/kg body weight. At 300 mg/kg body weight, 1,3-dichlorobenzene produced a rapid increase in plasma ALT activity, which reached its highest value after one day, and was still above control values on the second day. Liver necrosis was also most severe after one day, and still recognizable three days after administration. A significant increase in the labelling index as a measure for increased frequency of liver cell division occurred from the second day and was still demonstrable seven days after administration. The increase in cell proliferation occurred only at dose levels also causing liver cell necrosis (Umemura et al. 1996).

In a study with one animal per dose, 1,3-dichlorobenzene was administered once by gavage at doses between 0 and 2800 mg/kg body weight to a total of 25 male Fischer-344 rats. In the liver, 1,3-dichlorobenzene caused an induction of total cytochrome P450 at 77 mg/kg body weight and above, increased relative liver weights and degeneration at 129 mg/kg body weight and above, and liver cell necroses with increased ALT and aspartate aminotransferase (AST) activities at 450 mg/kg body weight and above 24 hours after administration (Allis et al. 1992).

Intraperitoneal injection

Male Fischer-344 rats (no data on number of animals per group) received single intraperitoneal injections of 1,3-dichlorobenzene in corn oil at doses of 0 to 794 mg/kg body weight (0.9 to 5.4 mmol/kg). A dose-dependent increase in plas-

ma ALT activity at 397 mg/kg body weight and above occurred 24 hours after injection. Histological examination revealed centrilobular hepatocellular necrosis in the liver. The hepatotoxicity was potentiated by pretreatment with phenobarbital. Administration of 1,3-dichlorobenzene caused significant glutathione depletion in the liver. Depletion of hepatic glutathione by pretreatment with phorone prior to 1,3-dichlorobenzene administration markedly increased the ALT activity in plasma (Stine et al. 1991).

In comparative studies, Fischer-344 rats were found to be markedly more sensitive to the hepatotoxicity of 1,3-dichlorobenzene than Sprague-Dawley rats (Gunawardhana and Sipes 1991).

After single intraperitoneal injection of 1,3-dichlorobenzene at doses of 0, 294, 441 or 588 mg/kg body weight in corn oil to 4 male Fischer 344 rats per group, no increase in plasma ALT activity and no histological liver changes were found. The relative kidney weights were increased at 441 mg/kg body weight; there was a decrease in renal accumulation of p-aminohippurate at 441 and 588 mg/kg body weight, which was not dose-dependent (Valentovic et al. 1993). These results are not in line with those obtained in the other studies.

Subacute, subchronic and chronic toxicity

Ingestion

Table 1 gives the studies following in detail.

Table 1 Effects of 1,3-dichlorobenzene after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 10 ♂, 10 ♀	10 days 0, 37, 147, 368, 735 mg/kg body weight and day; gavage	37 mg/kg body weight: NOAEL	McCauley et al. 1995
		147 mg/kg body weight and above: liver: ♂: relative weights increased, ♂, ♀: sporadic cell necrosis	
		368 mg/kg body weight: ♂: relative kidney weights increased;	
		368 and 735 mg/kg body weight: ♀: serum cholesterol increased; liver: ♀: relative weights increased	
		735 mg/kg body weight: ♂, ♀: body weights decreased (♂ 20%, ♀ 13%); liver: hepatocellular degeneration (♂ 9/10, ♀ 9/9); thymus atrophy (♂ 2/10, ♀ 2/9)	

Table 1 (Continued)

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 5 ♂, 5 ♀	28 days 0, 4, 20, 100, 500 mg/kg body weight and day; gavage	4 mg/kg body weight and above: ♂: LOEL: xenobiotic metabolizing enzyme activity increased (aminopyrine-N-demethylase, ethoxyresorufin-O-deethylase, glucuronosyl transferase-I activities) 20 mg/kg body weight and above: ♂: serum glutathione increased, glucuronosyl transferase-II activity increased 100 mg/kg body weight: ♂: vacuolization of epithelia and degeneration of the brush-border membrane of the proximal convoluted tubules of the kidneys 100 mg/kg body weight and above: ♂, ♀: relative liver weights increased; ♀: activity of xenobiotic metabolizing enzymes increased, ♀: serum glutathione increased 500 mg/kg body weight: ♂, ♀: body weights decreased, food and water consumption increased; hepatocellular hypertrophy; activity of ALT and γ-GT increased; relative kidney weights increased; urine volume increased	Hoechst AG 1989
rat, Sprague Dawley, 10 ♂, 10 ♀	90 days 0, 9, 37, 147, 588 mg/kg body weight and day; gavage	9 mg/kg body weight: ♂, ♀: NOAEL: incidence of thyroid follicles with reduced colloid density minimally increased 9 mg/kg body weight and above: ♂: activity of AST increased and LDH decreased, cholesterol increased 37 mg/kg body weight and above : ♂, ♀: incidence and severity of thyroid follicles with reduced colloid density increased; ♂, ♀: calcium concentration increased 147 and 588 mg/kg body weight: ♂, ♀: relative liver weight increased, ♂: relative kidney weights increased, pituitary: vacuolization in cells of the *pars distalis*; vacuoles of different sizes, irregularly formed; frequency and severity dose-dependently increased 588 mg/kg body weight: ♂, ♀: body weights decreased (♂ 24%, ♀ 10%), ♀: relative kidney weights increased; leukocyte count increased, ♂: erythrocyte count increased	McCauley et al. 1995

ALT: alanine aminotransferase, AST: aspartate aminotransferase,
γ-GT: γ-glutamyl transpeptidase, LDH: lactate dehydrogenase,
LOEL: lowest observed effect level, NOAEL: no observed adverse effect level

In Sprague-Dawley rats treated by gavage for 10 days with doses of 1,3-dichlorobenzene of up to 735 mg/kg body weight and day in corn oil, the deaths which occurred were, with one exception, a result of improper gavage administration. Determination of blood parameters did not yield any unusual findings. Values for alkaline phosphatase, calcium, urea nitrogen, glucose and ALT activity were within the reference range. The target organ for 1,3-dichlorobenzene doses of 147 mg/kg body weight and day and above was the liver, for doses of 368 mg/kg body weight and day and above the kidney. The number and severity of damaged sites in the liver tended to increase in a dose-dependent manner in the male animals (McCauley et al. 1995). With regard to the increase in relative liver weights in the male animals, a no observed adverse effect level (NOAEL) of 37 mg/kg body weight and day can be derived from this study.

In a study carried out according to OECD Test Guideline 407, Wistar rats were treated with 1,3-dichlorobenzene in sesame oil by gavage on 28 consecutive days at dose levels of up to 500 mg/kg body weight and day. As primary effect, enzymes were induced in the livers of the male animals. Phase I and phase II xenobiotic metabolizing enzymes were dose-dependently induced. The authors state that "in general" the enzyme activities were significantly increased at the two highest doses (100 and 500 mg/kg body weight and day), some even at the second lowest dose of 20 mg/kg body weight and day. However, the original data reveal a significant increase in the activities of glucuronosyl transferase I, aminopyrine-*N*-demethylase and ethoxyresorufin-O-deethylase even from 4 mg/kg body weight and day. Liver weights were increased, and, in male animals, pathological changes in the kidneys occurred at 100 mg/kg body weight and day. No changes were found in other organs (no other details). The authors give 20 mg/kg body weight and day as the NOAEL (Hoechst AG 1989). Owing to the significantly increased enzyme activities at 4 mg/kg body weight and day, however, this dose is assessed to be a LOEL (lowest observed effect level).

Ten male and ten female Sprague-Dawley rats were treated by gavage with 1,3-dichlorobenzene in corn oil at doses of up to 588 mg/kg body weight and day for 90 days. No deaths occurred. At 9 mg/kg body weight and day and above in male and female animals, a slightly increased incidence of thyroid changes occurred in the form of a decreased colloid density in the thyroid follicles, which was considered to be relevant from 37 mg/kg body weight and day, as there was no increase in severity below this dose. In the pituitary gland of the male animals, vacuolization in the *pars distalis* occurred from 147 mg/kg body weight and day. Comparable pituitary changes are observed in castrated or elderly rats with testicular atrophy. The increase in the cholesterol level and the serum calcium concentration at 37 mg/kg body weight and above was attributed by the authors to a possible disturbance in hormonal feedback mechanisms or to the effects on other endocrine organs. The significantly increased AST activity and the significant decrease in lactate dehydrogenase activity in the male animals at 9 mg/kg body weight and day and above can possibly be attributed to the unusually high or low control values with conspicuously high standard deviations. Glucuronosyl transferase I activity

was not determined. Histopathological liver changes were found to different extents in animals of all dose groups and consisted of infiltrations of lymphocytes and macrophages, often in degenerated or necrotic liver cell regions, homogeneous eosinophilic cytoplasmatic inclusions and sporadic or focal liver cell necroses. However, the authors do not consider the liver changes to be treatment-related below doses of 588 mg/kg body weight and day. They give no NOAEL due to significantly changed parameters in all dose groups (McCauley et al. 1995). This study revealed a decrease in colloid density in the thyroid follicles at the lowest dose of 9 mg/kg body weight and day in male and female animals. This was not, however, assessed as being adverse due to the minimal severity. As a result, a NOAEL of 9 mg/kg body weight and day can be derived for the thyroid changes.

The decrease in colloid density in thyroid gland follicles observed in this 90-day study must be brought into context with the increased glucuronosyl transferase I activity in the 28-day study (see Table 1) (see also Mechanism of Action).

Local effects on skin and mucous membranes

Skin

Moderate skin irritation (no other details) occurred in rabbits after 24-hour occlusive application of 0.5 ml 1,3-dichlorobenzene (Hoechst AG 1979).

A primary irritation score of 3.9 out of 8 was obtained after application of 1,3-dichlorobenzene on the intact or scarified skin. The substance was classified as moderately irritating (Bayer AG 1980 a).

Eye

After single instillation of 0.1 ml 1,3-dichlorobenzene into the conjunctival sac, the substance was assessed as not irritating to the rabbit eye following examinations after 24, 48 and 72 hours as well as after 8 days (Bayer AG 1980 b). Exposure of the animals' eyes to 0.1 ml 1,3-dichlorobenzene for 24 hours caused a slight to marked conjunctival swelling and a slight corneal opacity (no other details). The substance was classified as slightly irritating (Hoechst AG 1979).

Developmental toxicity

The results of studies on the developmental toxicity of 1,3-dichlorobenzene and its 1,2-dichlorobenzene and 1,4-dichlorobenzene isomers are shown in Table 2.

In the 1988 documentation, (documentation "1,3-Dichlorobenzene", 1990) an oral developmental toxicity study in rats was described, in which 1,3-dichloroben-

Table 2 Studies of the developmental toxicity of 1,2-, 1,3- and 1,4-dichlorobenzene

Species, strain, number per group	Exposure	Findings	References
1,2-Dichlorobenzene			
rat, F344, 31–32 ♀	GD 6–15, 0, 100, 200, 400 ml/m³, 6 hours/day, inhalation	100 ml/m³ and above: dams: body weight gain decreased 400 ml/m³: dams: liver weight increased; foetuses: no embryotoxic, foetotoxic or teratogenic effects	Hayes et al. 1985; see documentation 1988 documentation "1,2-Dichlorobenzene" 1990
rat, Sprague Dawley, no other details	GD 6–15, 0, 50, 100, 200 mg/kg body weight and day, oral	up to 200 mg/kg body weight: foetuses: no teratogenic effects (no other details); study only available as summary	Ruddick et al. 1983; see documentation 1988 Documentation "1,2-Dichlorobenzene" 1990
rabbit, New Zealand White, 28–30 ♀	GD 6–18, 0, 100, 200, 400 ml/m³, 6 hours/day, inhalation	100 ml/m³ and above: dams: body weight gain decreased 400 ml/m³: foetuses: no embryotoxic, foetotoxic or teratogenic effects; study only available as summary	Hayes et al. 1985; see documentation 1988 Documentation "1,2-Dichlorobenzene"1990
1,3-Dichlorobenzene			
rat, Sprague Dawley, no other details	GD 6–15, 0, 50, 100, 200 mg/kg body weight and day, oral	up to 200 mg/kg body weight: foetuses: no teratogenic effects (no other details); study only available as summary	Ruddick et al. 1983; see documentation *1988* documentation, "1,3-Dichlorobenzene", 1990
1,4-Dichlorobenzene			
rat, no other details	GD 6–15, 0, 74, 197, 492 ml/m³, 6 hours/day, inhalation	up to 492 ml/m³: dams: body weight gain normal; foetuses: no effects	see documentation 1991 documentation "1,4-Dichlorobenzene" 1992; see documentation 2001 documentation "1,4-Dichlorobenzene" 2003

Table 2 (Continued)

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, no other details	GD 6–15, 0, 50, 100, 200 mg/kg body weight and day, oral	up to 200 mg/kg body weight and above: foetuses: no teratogenic effects (no other details); study only available as summary	Ruddick et al. 1983; see documentation 1991 documentation "1,4-Dichlorobenzene" 1992; see documentation 2001 documentation "1,4-Dichlorobenzene" 2003
rat, CD, 13–17 ♀	GD 6–15, 0, 250, 500, 750, 1000 mg/kg body weight and day, oral	500 mg/kg body weight: dams: food intake and body weight gain decreased 750 mg/kg body weight and above: foetuses: foetal weight decreased; variations (additional ribs) increased	Giavini et al. 1986; see documentation 1991 documentation "1,4-Dichlorobenzene" 1992; see documentation 2001 documentation "1,4-Dichlorobenzene" 2003
rabbit, New Zealand White, 29–30 ♀	GD 6–18, 0, 100, 300, 800 ml/m³ (no other details)	800 ml/m³: dams: body weight gain decreased; foetuses: no effects	Hayes et al. 1985; see documentation 1991 Documentation "1,4-Dichlorobenzene" 1992; see documentation 2001 documentation "1,4-Dichlorobenzene" 2003

GD: gestation day

zene produced no teratogenic effects up to the highest dose tested of 200 mg/kg body weight and day. Nor were teratogenic effects reported with the 1,2-dichlorobenzene and 1,4-dichlorobenzene isomers up to doses of 200 mg/kg body weight and day (Ruddick et al. 1983). However, this study is only available as a summary.

Developmental toxicity studies after inhalation exposure of rats and rabbits to the 1,2-dichlorobenzene isomer revealed no embryotoxic effects up to the highest concentration investigated of 400 ml/m³, although body weight gains of the dams were decreased.

No embryotoxic effects were found with the isomer 1,4-dichlorbenzene at concentrations of 210 ml/m³ in rats and 800 ml/m³ in rabbits.

Genotoxicity

In vitro

It was reported in the 1988 documentation that 1,3-dichlorobenzene was not mutagenic in the gene mutation test with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* WP2 uvrA, but increased the incidence of back mutations in *Aspergillus nidulans*. In *Saccharamyces cerevisiae* D3, 1,3-dichlorobenzene produced mitotic recombinations.

In a valid study with mammalian cells, it was shown using bromodeoxyuridine density gradient centrifugation that 1,3-dichlorobenzene induced no DNA repair synthesis in A549 cells and in primary rat hepatocytes. A concentration range of 3 to 300 mg/ml in the absence of a metabolic activation system was investigated (Hoechst AG 1992).

In vivo

In a micronucleus test already described in the documentation of 1988, 5 NMRI mice (no other details) received two intraperitoneal injections of 1,3-dichlorbenzene totalling 175, 350, 525 or 700 mg/kg body weight 24 hours apart. Ten control animals received corn oil only. The animals were killed 30 hours after the first injection. A significant and dose-dependent increase in the number of micronuclei was found in the animals' bone marrow. Cytotoxicity was not investigated (Mohtashamipur et al. 1987). One point of criticism of this study is that only one vehicle control group consisting of 10 animals was used for the large number of substances tested (nine different substances at four dose levels each). There was no positive control group. Half of each dose was administered at 24-hour intervals, and the bone marrow was examined six hours after the last dose. As erythroblasts need ten hours after final mitosis to appear as polychromatic erythrocytes in the bone marrow, the second dose administered six hours previously was ineffective. The authors attributed the induction of micronuclei to clastogenicity. An aneugenic effect due to chromosome loss was not considered. Owing to these shortcomings, the results of this study cannot be used for this evaluation.

A single dose (1000 mg/kg body weight) of 1,3-dichlorobenzene was administered by gavage to 15 male and 15 female hamsters. Animals in a control group of equal size received sesame oil only, and 5 male and 5 female animals cyclophosphamide as a positive control. Five animals of each sex in the treated and control groups were killed 12, 24 and 48 hours after administration, and 50 metaphases in bone marrow cells evaluated. 1,3-Dichlorobenzene caused no significant increase in the frequency of structural chromosome aberrations in the bone marrow cells of the treated animals compared with the animals of the control group. On the other hand, the animals treated with cyclophosphamide showed a clear increase in frequency of aberrations. Data on cytotoxicity are not available (BUA 1994).

Genotoxic effects of 1,3-dichlorobenzene can not be derived from the available data. These data, however, do not allow a conclusive assessment of its genotoxicity.

Carcinogenicity

Short-term studies

A study on the tumour-promoting effect of 1,3-dichlorobenzene in the rat liver foci test was described in the 1988 documentation. Male Sprague-Dawley rats received single doses of 51 mg diethylnitrosamine by gavage. One week and five weeks following this, they were given 1,3-dichlorobenzene doses of 147 mg/kg body weight by intraperitoneal injection. No tumour-promoting effect was found two weeks later (Herren-Freund and Pereira 1986).

Long-term studies

There are no data available.

Manifesto

There are no suitable data available from studies of exposed persons that would permit the establishment of a MAK value. In a 28-day study, a significant increase in the activity of glucuronosyl transferase I and other enzymes such as aminopyrine-*N*-demethylase and ethoxyresorufin-O-deethylase was found at the lowest dose tested of 4 mg/kg body weight and day (LOEL). The induction of liver enzymes has to be considered as causal factor for the changes in thyroid follicles found in rats, which were significantly increased by 1,3-dichlorobenzene from 37 mg/kg body weight in a 90-day study. The changes observed in the thyroid are also relevant for humans although they do occur at higher doses than in the rat (Lewandowski et al. 2004). This is because humans are able to store thyroid hormones, thus providing themselves with improved protective mechanisms (see Mechanism of Action). Taking into account the LOEL for enzyme changes, a MAK value of 2 ml/m^3 is established. This is based on the following: the NOAEL of 9 mg/kg body weight and day for histopathological thyroid gland changes from the 90-day study (corresponding to a concentration of 63 mg/m^3 or 10.5 ml/m^3 for a person of 70 kg body weight with a ventilation of 10 m^3 during an 8-hour working day); the LOEL of 4 mg/kg body weight and day for the increase in the activity of glucuronosyl transferase I from the 28-day study (corresponding to a concentration of 28 mg/m^3 or 4.60 ml/m^3); the fact that rats react with particular sensitivity to substances acting

on thyroid hormones. As no data are available on toxicokinetics in humans, 1,3-dichlorobenzene is classified in Peak Limitation Category II with an excursion factor of 2.

No embryotoxic effects were described in the developmental toxicity study with 1,3-dichlorobenzene in rats up to the highest dose tested of 200 mg/kg body weight and day. This corresponds to a concentration in the air of 1400 mg/m³ for a person with 70 kg body weight and an inhaled air volume of 10 m³ in eight hours. As this study exists only in summary form, it can provide merely an indication that no embryotoxic effects will occur when the MAK value for 1,3-dichlorobenzene of 2 ml/m³ (12 mg/m³) is observed. As no embryotoxic effects have been found with the isomers 1,2-dichlorobenzene and 1,4-dichlorobenzene—for which sufficient data are available—and as 1,2-dichlorobenzene has been classified in Pregnancy Risk Group C (as was also 1,4-dichlorobenzene until the MAK value was withdrawn), the available database permits classification of 1,3-dichlorobenzene in Pregnancy Risk Group C.

In vitro, 1,3-dichlorobenzene was not mutagenic in the gene mutation test with *Salmonella typhimurium* and *Escherichia coli* WP2 uvrA, but was mutagenic with *Aspergillus nidulans*. 1,3-Dichlorobenzene produces mitotic recombinations in *Saccharomyces cerevisiae* D3. In the studies performed since the 1988 documentation, 1,3-dichlorobenzene did not induce DNA repair synthesis in A549 cells and in primary rat hepatocytes. *In vivo*, 1,3-dichlorobenzene induced no significant increase in the frequency of structural chromosome aberrations in the bone marrow cells of hamsters. Owing to its methodological shortcomings, a micronucleus test in mice cannot be used in this evaluation. No genotoxic effects can be derived from the valid studies. Altogether, the available data are not sufficient to assess the genotoxicity of 1,3-dichlorobenzene conclusively.

That 1,3-dichlorobenzene has no tumour-promoting effect in the rat liver was described in the documentation of 1988. However, due to the induction of liver enzymes of the phenobarbital type, a tumour-promoting effect of 1,3-dichlorobenzene in the rat liver is conceivable. No long-term carcinogenicity studies are available. Classification in one of the categories of carcinogens or germ cell mutagens is therefore not necessary.

From the physico-chemical data, it can be calculated that 9.0 to 18.5 mg of 1,3-dichlorobenzene are absorbed from a skin surface of 2000 cm² in one hour. Compared with the LOAEL (of 4 mg/kg body weight (280 mg 70 kg body weight), the amount absorbed through the skin is small. 1,3-dichlorobenzene is therefore not designated with an “H”.

As a result of the lack of data, 1,3-dichlorobenzene is not designated with “Sa” or “Sh”.

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