The MAK Collection for Occupational Health and Safety

Chlorobenzene

MAK Value Documentation, addendum – Translation of the German version from 2018

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Keywords: chlorobenzene; MAK value; maximum workplace concentration; peak limitation; developmental toxicity; central nervous system

Citation Note: Hartwig A, MAK Commission. Chlorobenzene. MAK Value Documentation, addendum – Translation of the German version from 2018. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2019 Nov;4(4):1893-1909]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/mb10890e6519_w

Republished (online): 08 Aug 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.mb10890e6519

Addendum completed: 22 Mar 2017

Published (online): 13 Nov 2019

The commission established rules and measures to avoid conflicts of interest.

Chlorobenzene

MAK value documentation

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DOI: 10.1002/3527600418.mb10890e6519

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated chlorobenzene [108-90-7] to derive a maximum concentration at the workplace (MAK value), considering all toxicity endpoints. Adverse effects are a depression of the central nervous system in humans and histological alterations in liver and kidney in rats. A 2 generation reproduction study with chlorobenzene vapour in Sprague-Dawley rats resulted in a NOAEC of 50 ml/m³. Based on this NOAEC, a MAK value of 5 ml/m³ is derived. This value is supported by a study with healthy volunteers at rest, who showed no neurotoxic or chemosensory effects at a NAEC of 5.9 ml/m³, which takes the increased respiratory volume at the work place into account. As systemic effects are critical, the substance remains assigned to Peak Limitation Category II and the excursion factor of 2 is confirmed. Chlorobenzene caused a statistically significantly increased incidence of neoplastic nodules in the liver of male F344 rats in a carcinogenicity study at the highest dose of 120 mg/kg body weight and day. As chlorobenzene is genotoxic only at high doses given intraperitoneally, and female rats and mice did not show any tumours in this study, it remains regarded neither as a carcinogen nor as a germ cell mutagen. From a synopsis of all data, the classification of chlorobenzene in Pregnancy Risk Group C is maintained. Chlorobenzene did not lead to contact sensitization in mice and guinea pigs.

Keywords

chlorobenzene; benzene chloride; chlorobenzol; monochlorobenzene; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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Chlorobenzene

[108-90-7]

Supplement 2018

MAK value (2017)	**5 ml/m³ (ppm) ≙ 23 mg/m³**
Peak limitation (2001)	**Category II, excursion factor 2**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (1985)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value (2009)	**150 mg 4-chlorocatechol (after hydrolysis)/g creatinine** sampling time: end of exposure or end of shift **25 mg 4-chlorocatechol (after hydrolysis)/g creatinine** sampling time: at the beginning of next shift
1 ml/m³ (ppm) ≙ 4.671 mg/m³	**1 mg/m³ ≙ 0.214 ml/m³ (ppm)**

For chlorobenzene, documentation from 1972 and supplements from 1987, 1995 (combined into one translation; documentation "Chlorobenzene" 1999), and 2001 (supplement "Chlorbenzol" 2001, in German only) are available.

In 2016, the Commission began using a revised approach for assessing substances with a MAK value based on systemic effects and derived from inhalation studies in animals or studies with volunteers at rest; this new approach takes into account that the respiratory volume at the workplace is higher than under experimental conditions. However, this does not apply to gases and vapours with a blood:air-partition coefficient < 5 (see List of MAK and BAT Values, Section I b and I c). The blood:air partition coefficient of chlorobenzene is about 30 (Béliveau and Krishnan 2000). This supplement evaluates whether the MAK value and the Pregnancy Risk Group of chlorobenzene need to be re-assessed as a result of the higher respiratory volume at the workplace.

1 Toxic Effects and Mode of Action

At 60 ml/m^3, chlorobenzene produces effects on the central nervous system in humans, such as sleepiness, headaches or throbbing pain in the eyes. In individual cases, also fatigue, nausea or torpidity have been reported.

In rats, liver and kidney changes have been observed at concentrations of 150 ml/m^3 and above in studies with repeated inhalation exposure to chlorobenzene. After long-term oral administration of chlorobenzene to male rats, the frequency of neoplastic nodules in the liver was significantly increased at the highest dose tested of 120 mg/kg body weight and day. Chlorobenzene is evaluated as not carcinogenic in rats and mice.

In mice, the RD_{50} is 1054 ml chlorobenzene/m^3. Chlorobenzene is irritating to the skin of rabbits, but not the eyes.

Chlorobenzene is not mutagenic in bacteria. DNA damage in mammalian cells was observed only in the higher concentration range (0.2 to 1 mg/ml).

In mice, after three daily intraperitoneal injections of a chlorobenzene dose of 750 mg/kg body weight and day, DNA strand breaks occurred to an increased extent in the peripheral lymphocytes. In rats, increased incidences of micronuclei in bone marrow cells were observed after a single intraperitoneal dose of 1250 mg/kg body weight.

In a 2-generation study with Sprague Dawley rats, the LOAEC (lowest observed adverse effect concentration) for the degeneration of the testicular germinal epithelium of the F1 animals was 150 ml/m^3. In studies of developmental toxicity, a LOAEC of 590 ml chlorobenzene/m^3 was obtained in rats, while the NOAEC (no observed adverse effect concentration) in rabbits was the highest concentration tested of 590 ml chlorobenzene/m^3. These concentrations were toxic to the dams.

Chlorobenzene did not induce skin sensitization in guinea pigs and mice.

2 Mechanism of Action

Chlorobenzene induces the release of inflammatory mediators from lung cells in vitro. However, no lung damage occurred in the animal studies after inhalation exposure (documentation "Chlorobenzene" 1999).

Using an in vitro system, lung carcinoma cells (A549) stimulated with the tumour necrosis factor α (TNF-α) were exposed to chlorobenzene at concentrations between 1 ng/m^3 and 100 g/m^3 via the gaseous phase without a surrounding medium. After 20-hour exposure to non-cytotoxic chlorobenzene concentrations between 10 and 100 µg/m^3, the release of the proinflammatory cytokine MCP-1 (monocyte chemoattractant protein-1) was increased by 20% to 40%. MCP-1 stimulates the migration of monocytes into ischaemic tissue and controls their attachment to endothelial cells. The chlorobenzene concentrations used in this study were in part as high as those of the volatile organic compounds (up to 20 µg/m^3) measured in normal houses. At the highest concentration of 100 g chlorobenzene/m^3, the release of MCP-1 was decreased. The release of interleukin-8 (IL-8) was only increased at toxic concentrations between 1 and 10 g chlorobenzene/m^3. Likewise, other aromatic volatile

organic compounds, such as styrene and 1,3-xylene, stimulated the increased release of MCP-1 at non-toxic concentrations in this system (Fischäder et al. 2008).

Under the same in vitro study conditions with directly exposed human peripheral blood mononuclear cells or with A549 cells, there were no changes in viability or proliferation ability at the highest concentration tested of 100 g chlorobenzene/m³. After pretreatment of the A549 cells with human rh-TNF-α (recombinant TNF-α) chlorobenzene induced the increased release of MCP-1 at concentrations of 10 µg/m³ to 1 g/m³ and an increase in IL-8 formation at 100 g chlorobenzene/m³. Chlorobenzene induced no cytokine release in -human peripheral blood mononuclear cells stimulated with anti-CD3/anti-CD28 antibodies (Lehmann et al. 2008).

TNF-α is not only an important factor in the cytokine cascade, but also activates the signal transduction pathways of p38 MAPK (mitogen-activated protein kinase) and NF-κB (nuclear transcription factor in B lymphocytes binding to the promotor of light κ chains) which also participate in the inflammatory response. To investigate the effect of chlorobenzene on these two intracellular signal transduction pathways, A549 cells stimulated with 1 ng TNF-α/ml in the presence and absence of specific inhibitors of p38 MAPK or NF-κB signal transmission were exposed to chlorobenzene concentrations in the range between 0.1 µg/m³ and 100 g/m³. Without inhibitor, all non-toxic chlorobenzene concentrations caused the increased release of MCP-1 by a factor of 1.4, and increased the relative activity of p38 MAPK as well as the expression of NF-κB. In the presence of a specific inhibitor of p38 MAPK or NF-κB signal transduction, however, the chlorobenzene-induced release of MPC-1 was inhibited, thus indicating that an increased release of MCP-1 is mediated via the activation of both signal pathways (Roeder-Stolinski et al. 2008).

Oxidative stress was assumed to be the trigger for the induction of p38 MAPK and NF-κB signal transduction pathways in response to chlorobenzene. Therefore, in a similar in vitro system, the cellular markers for oxidative stress in TNF-α-stimulated A549 cells were investigated. After 24-hour exposure to chlorobenzene concentrations of 100, 1000 or 10 000 µg/m³, the activities of haemoxidase-1 (HO-1), glutathione *S*-transferase π1 (GSTP1), superoxide dismutase (SOD1), prostaglandin-endoperoxide synthase 2 (PTGS2), and dual specificity phosphatase 1 (DUSP1; causes the inactivation of MAPK due to dephosphorylation) were increased, and reactive oxygen species detectable. The GSH level decreased in a concentration-dependent manner by about 29% to 63%. The results indicate that aromatic compounds such as chlorobenzene are potentially able to produce an inflammatory response in cultured lung cells by the induction of oxidative stress (Feltens et al. 2010).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Chlorobenzene can be absorbed by humans and animals both by inhalation or ingestion (documentation "Chlorobenzene" 1999). The excretion of chlorobenzene with the urine takes place in the form of metabolites, such as sulfate and glucuronic acid conjugates of chlorophenols and chlorocatechols. The 4-chlorocatechol excreted

with the urine is used as a marker for exposures occurring at the workplace or for observance of the BAT value (Göen 2016).

Using a PBPK model for F344 rats, the amount absorbed after oral administration was calculated to be 96%. Of this amount, 36% is exhaled (Thrall et al. 2004).

3.2 Metabolism

The most important metabolic pathways are described in the supplement of 1995 (documentation "Chlorobenzene" 1999).

The first step in the metabolism of chlorobenzene is its oxidation to chlorobenzene-3,4-epoxide and, to a lesser extent, to chlorobenzene-2,3-epoxide. In humans, it seems that the inactivation of epoxides via epoxide hydrolase generally takes place more rapidly than in rodents (Guenthner and Luo 2001); under the same exposure conditions, the epoxide burden in humans is smaller. Rodents could therefore be assumed to be more sensitive to the toxic effects produced by the epoxides than humans. However, the metabolite dihydrodihydroxychlorobenzene is rapidly metabolized to the toxic 4-chlorocatechol or also to the *o*, *m* or *p*-isomers of chlorophenol (Knecht and Woitowitz 2000).

4 Effects in Humans

There are no new studies or data available for repeated exposure, skin irritation, allergenic effects, reproductive toxicity, genotoxicity and carcinogenicity.

Inhalation

During a 7-hour exposure (three hours exposure, one hour pause, four hours exposure; unclear whether the study was blind) to a chlorobenzene concentration of 60.2 ml/m^3 in an exposure chamber, all four test persons complained of an unpleasant smell and sleepiness, three complained of headaches, two of throbbing pain in the eyes and one of a dry throat. After the first three hours the values determined in the so-called flicker fusion frequency test were significantly reduced relative to the control values, but they were no longer reduced after the second (4-hour) exposure. After exposure to 11.8 ml/m^3 no complaints were reported by the test persons (documentation "Chlorobenzene" 1999; Ogata et al. 1991). Limitations of this study are that the sample size is very low with only four male volunteers, the recording of the reported symptoms was not well documented, and that also no objective data were obtained from which sensory irritation could be physiologically confirmed. The data from the flicker fusion frequency test are difficult to interpret, as the initial values of the exposed and the control persons were markedly different.

In individual cases, workers reported transient irritation of the upper airways and intermittently occurring symptoms, such as exhaustion, nausea, vomiting and torpidity after exposure to chlorobenzene (no other details) (documentation "Chlorobenzene" 1999).

Ingestion

Ingestion of 140 ml of a 90% chlorobenzene solution with suicidal intent produced severe liver necrosis in a 40-year-old male alcoholic. It was assumed that chronic alcohol consumption increased the liver damage caused by chlorobenzene (Babany et al. 1991; Reygagne et al. 1992).

Local effects on skin and mucous membranes

According to earlier reports, irritation of the eyes and nasal mucosa occurred at concentrations of 200 ml/m^3 and above (documentation "Chlorobenzene" 1999).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

After inhalation, the RD_{50} was 1054 ml chlorobenzene/m^3 in mice (de Ceaurriz et al. 1981). The LC_{50} values were 66 000 mg/m^3 (ECHA 2017 a) after two hours exposure (OECD Test Guideline 403, calculated) and between 13 870 and 18 016 mg/m^3 after six hours exposure in rats and 8822 mg/m^3 in mice (documentation "Chlorobenzene" 1999).

Ten male and ten female rats and guinea pigs were exposed to chlorobenzene concentrations of 2990, 5850 or 7970 ml/m^3 (13 966, 27 325 or 37 227 mg/m^3) for 30 minutes, with a 14-day recovery period. Reversible irritation of the eyes and nose was observed in half of the exposed rats and guinea pigs at 2990 ml/m^3 (13 966 mg/m^3). Ataxia and narcosis were observed at 5850 ml/m^3 (27 325 mg/m^3) and above, progressing to twitching movements at 7970 ml/m^3 (37 227 mg/m^3). The guinea pigs were more sensitive to chlorobenzene than rats. At 5850 ml chlorobenzene/m^3, most rats were narcotic by 25 minutes but recovered rapidly after removal from the exposure chamber. All guinea pigs were narcotic at 30 minutes (ATSDR 2013).

5.1.2 Oral administration

The LD_{50} values were between 1427 and 3400 mg/kg body weight in rats, between 778 and 2390 mg/kg body weight in mice, between 2250 and 2830 mg/kg body weight in rabbits and 5060 mg/kg body weight in guinea pigs (supplement 1995; ECHA 2017 a).

5.1.3 Dermal application

All five rabbits survived 24-hour occlusive application of 2 ml chlorobenzene/kg body weight (2212 mg/kg body weight) to the shaved dorsal skin (documentation "Chlorobenzene" 1999).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

The well-documented studies of repeated toxicity in rats after inhalation were already evaluated in the supplement of 1995 documentation "Chlorobenzene" 1999), but are described once more below.

In a 2-generation study with groups of 30 male and 30 female Sprague Dawley rats, the animals inhaled chlorobenzene concentrations of 0, 50, 150 or 450 ml/m^3 (0, 234, 701 or 2102 mg/m^3) for 6 hours per day, on 7 days per week. Exposure in the F0 generation began ten weeks prior to mating and in the F1 generation one week after weaning. In both generations, it was continued during mating, gestation and lactation. At the final examination at the end of the exposure, the absolute and relative liver weights were increased in a dose-dependent manner. The relative liver weight was increased by 12% at 150 ml/m^3 and above in the male F0 animals, and by 5% in the females. In the males of the F1 generation, there was a 7% increase in relative liver weight at 50 ml/m^3 and a 20% increase at 150 ml/m^3. The increased liver weight at 150 ml/m^3 and above was accompanied by hepatocellular hypertrophy in the F0 generation. Kidney changes such as interstitial nephritis, foci of regenerative epithelium or tubular dilation with eosinophilic deposits were significantly increased in the male rats of the F0 and F1 generations at 150 ml/m^3 and above (see Table 1). The incidence of bilateral degeneration of the testicular germinal epithelium was increased in the F0 generation at 450 ml/m^3 as was that of unilateral degeneration in the F1 generation at 150 ml/m^3 and above. No evidence of adverse effects on reproduction was found (see Section 5.6) (Nair et al. 1987).

In view of the increase in relative liver weights by 20%, accompanied by hepatocellular hypertrophy at concentrations of 150 ml/m^3 and above in the F0 and F1 generations as well as the renal changes at 150 ml/m^3 and above in male Sprague Dawley rats of the F0 and F1 generations, the NOAEC in this study was 50 ml/m^3.

In an 11-week study with male Sprague Dawley rats, the effects produced at chlorobenzene concentrations of 75 ml/m^3 and above (no other details) included changes in the adrenal glands (formation of vacuoles) and kidneys (regenerating cortical tubules), and increased liver weights (Dilley 1977; Dilley and Lewis 1978).

After 120 exposures within 24 weeks, increased absolute (36%) and relative (31%) liver weights, and increased relative kidney weights (13%), were found in male Sprague Dawley rats at 250 ml chlorobenzene/m^3. The increases in organ weights were observed at chlorobenzene concentrations of 75 ml/m^3 and above and amounted to 13% for the liver and 11% for the kidneys (Dilley 1977).

Conclusions: From the studies with repeated inhalation exposure, a NOAEC of 50 ml chlorobenzene/m^3 and a LOAEC of 150 ml/m^3 for liver and kidney changes were obtained in rats.

Table 1 Effects of chlorobenzene on the liver and kidneys of male Sprague Dawley rats after inhalation (Nair et al. 1987)

Exposure	Findings	Concentration [ml/m³]			
		0	50	150	450
2-generation study, Sprague Dawley, groups of 30 ♂ and 30 ♀, start: 10 weeks prior to mating, termination: day 4 after birth of offspring	**liver**				
	relative weights (g/100 g body weight) [1] F0	3.61	3.60	4.06 (12%)*	4.12 (14%)*
	relative weights (g/100 g body weight) [1] F1	3.47	3.73 (7%)*	4.15 (20%)**	4.44 (28%)**
	hepatocellular hypertrophy [2, 3] F0	0	0	5 (17%)*	14 (47%)***
	hepatocellular hypertrophy [2, 3] F1	2 (7%)	0	3 (10%)	7 (23%)
	kidneys (occurring bilaterally)				
	tubular dilation with eosinophilic deposits [1] F0	0	1	4 (13%)	15 (50%)***
	tubular dilation with eosinophilic deposits [1] F1	4 (13%)	3 (10%)	8 (27%)	16 (53%)*
	interstitial nephritis [1] F0	1 (3%)	2 (7%)	7 (23%)*	9 (30%)**
	interstitial nephritis [1] F1	0	1 (3%)	6 (20%)*	11 (37%)**
	foci of regenerative epithelium [1] F0	0	1 (3%)	5 (17%)*	8 (27%)**
	foci of regenerative epithelium [1] F1	1 (3%)	0	4 (13%)	10 (33%)**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, [1] (Dunnett's test); [2] severity minimal to slight (no other details); [3] calculated later (Fisher's exact test)

5.2.2 Oral administration

All the studies of repeated oral administration are given in Table 3 of the 1995 supplement (documentation "Chlorobenzene" 1999). The following studies are only described to provide a complement to the changes in the liver and kidneys occurring after inhalation.

In a 13-week study, 10 male and 10 female F344 rats were given chlorobenzene doses of 0, 60, 125, 250, 500 or 750 mg/kg body weight and day by gavage on five days per week. Mortality was increased at 500 mg/kg body weight and day and above and hepatocellular necrosis and degeneration, nephropathy, depletion of myeloid cells of the bone marrow at 500 mg/kg body weight and day and above and of lymphoid cells of the spleen at 750 mg/kg body weight and day. In the subsequent two-year carcinogenicity study with gavage administration of chlorobenzene at 0, 60 or 120 mg/kg to groups of 50 male and 50 female F344 rats, the incidence of neoplastic nodules of the liver of male F344 rats was significantly increased at 120 mg/kg body weight and day and one renal adenocarcinoma was found in a female at this dose. The liver and kidney damage as well as the loss of erythropoietic precursor cells in bone marrow and the spleen observed in the 13-week study at 750 mg/kg body weight and day was not found at chlorobenzene doses of up to 120 mg/kg body weight and day in this chronic study (NTP 1985).

After groups of 10 male and 10 female B6C3F1 mice were given gavage doses of chlorobenzene of 0, 60, 125, 250, 500 or 750 mg/kg body weight and day on 5 days per week for 13 weeks, 4 male and 6 female animals died at 250 mg/kg body weight and day and survival was even lower at the higher doses. In addition, liver and kidney damage as well as the loss of erythropoietic precursor cells in the bone marrow and spleen occurred at 250 mg/kg body weight and day and above. In the chronic study with groups of 50 male and 50 female B6C3F1 mice, in which chlorobenzene concentrations of 0, 30 or 60 mg/kg body weight and day were administered, no substance-related changes were evident (NTP 1985).

In a 13-week study, groups of 4 male and 4 female beagle dogs were given chlorobenzene doses of 0, 0.025, 0.050 or 0.250 ml/kg body weight and day (0, 28, 55 or 277 mg/kg body weight and day) on 5 days per week in gelatine capsules. The low and the middle doses were tolerated by all animals without signs of toxicity. At the highest dose tested of 277 mg/kg body weight and day, 2 dogs died and 2 became comatose and were sacrificed between the third and fifth weeks of the study. The surviving animals were killed after 13 weeks of treatment. The dogs in the high dose group had become emaciated and cachectic and also suffered from jaundice. In the serum, the activities of alanine aminotransferase and alkaline phosphatase were increased as were the number of immature leukocytes and the bilirubin and cholesterol levels. In the liver, a greyish-yellow discoloration of the parenchyma was observed, the liver weights in the male animals were increased by 21% and the gallbladder was distended. Discoloration of the renal medulla was observed (Knapp et al. 1971).

Conclusions: The studies with repeated oral exposure yielded a NOAEL (no observed adverse effect level) of 60 mg chlorobenzene/kg body weight and day in rats and mice, and a LOAEL (lowest observed adverse effect level) of 120 mg/kg body weight and day in rats because of a significant increase in the frequency of neoplastic nodules in the liver. A similar NOAEL of 55 mg chlorobenzene/kg body weight and day was obtained with beagle dogs; a LOAEL of 277 mg/kg body weight and day was obtained for liver damage.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

Chlorobenzene was moderately irritating to the skin of rabbits (documentation "Chlorobenzene" 1999). In another study in accordance with OECD Test Guideline 404 in three New Zealand White rabbits, the mean irritation index for erythema obtained after 24, 48 and 72 hours was 2.7 on a scale of a maximum of 4. On a per animal basis, the scores for erythema after 24, 48 and 72 hours were 3 in two animals and 2 in one animal. The oedema score in each animal and at every time point was evaluated as 1 on a scale up to 4. Chlorobenzene was irritating to the skin also in this study (ECHA 2017 a).

Conclusions: Chlorobenzene is irritating to the skin of rabbits.

5.3.2 Eyes

Chlorobenzene was not irritating to the eyes of rabbits (documentation "Chlorobenzene" 1999), which was confirmed by two further studies. Irritation indices were given in only one of these studies. The mean scores after 48 and 72 hours were 0.1 on a scale of a maximum of 4 for corneal opacity, 0.9 of a maximum of 3 for conjunctival erythema, 0.4 of a maximum of 4 for corneal oedema and 0 of a maximum of 2 for iritis. In all animals, the signs of irritation had disappeared after seven days (ECHA 2017 a).

Conclusions: Chlorobenzene is not irritating in the eyes of rabbits.

5.4 Allergenic effects

In vitro

Negative findings were obtained in vitro in the direct peptide reactivity assay as well as in the cell-based systems of the KeratinoSens assay, the U937-CD86 test (MUSST assay) (Natsch et al. 2013) and in the skin model of the SENS-IS system (Cottrez et al. 2016). The human cell line activation test (h-CLAT) yielded positive results for the activation of the CD86 cells, but not for that of the CD54 cells (Nukada et al. 2011).

In vivo

Chlorobenzene was not sensitizing in a maximization test with guinea pigs (documentation "Chlorobenzene" 1999). Intradermal and topical induction was carried out using 1% and 50% chlorobenzene in a 2% Cremophor EL preparation, respectively. One day after challenge treatment with a 25% test preparation, there was weak erythema formation in 10 of 20 animals and in one of 20 animals after 48 hours. A repeated challenge produced reactions in 4 animals after 24 hours and in one animal after 48 hours. One of 10 controls reacted to the first challenge and 6 animals to the second challenge at both reading times. A positive control was not included (ECHA 2017 a).

A local lymph node assay (LLNA) in mice using 5%, 10% or 25% chlorobenzene in acetone/olive oil (4:1) yielded stimulation indices of 1.1, 1.7 and 1.6, respectively, and therefore, at these concentrations, a negative result (Ashby et al. 1995).

In a more recent LLNA in female CBA/J mice, chlorobenzene was tested in acetone/olive oil (4:1) at concentrations of 25% and 50%, and additionally in undiluted form. The stimulation indices for ^{3}H-thymidin incorporation were 1.91, 3.23 and 5.30, respectively. The lymph node cell count was increased by factors of 0.97, 1.34 and 1.61 compared with the values for the vehicle controls, while the lymph node weights increased by 1.2, 1.37 and 1.79 times and the ear weights by 1.08, 1.18 and 1.25 times, respectively. Flow-through cytometry did not reveal any substance-related effects on the B220+, CD3+, CD4+ and CD8+ cells; the B/T cell ratio was, however, increased 1.55 times in the high concentration group (ECHA 2017 b).

Conclusions: Chlorobenzene does not cause skin sensitization in guinea pigs and mice.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a 2-generation study, groups of 30 male and 30 female Sprague Dawley rats inhaled chlorobenzene concentrations of 0, 50, 150 or 450 ml/m^3 (0, 234, 702 or 2105 mg/m^3) for six hours per day, on seven days per week. Exposure began ten weeks prior to mating in the F0 generation and one week after weaning in the F1 generation. In both generations, exposure was continued during mating, gestation and lactation. The systemic findings from the final examination at the end of exposure are presented in Section 5.2.1. In particular, a dose-dependent increase in absolute and relative liver weights, hepatocellular hypertrophy and kidney changes were observed in the male rats of the F0 and F1 generations (see Table 1). In the testes, bilateral degeneration of the germinal epithelium was found in the F0 generation at 450 ml/m^3 and unilateral degeneration in the F1 generation at 150 ml/m^3 and above. There was no evidence of impaired reproduction (Nair et al. 1987). As chlorobenzene does not cause changes in lung function, testicular degeneration cannot be produced by hypoxia on a secondary basis and is therefore regarded as substance-related.

Conclusions: In this 2-generation study with rats, the NOAEC for degeneration of the testicular germinal epithelium was 50 ml chlorobenzene/m^3 and the LOAEC 150 ml/m^3.

5.5.2 Developmental toxicity

Groups of 32 to 33 F344 rats were exposed to chlorobenzene concentrations of 0, 75, 210 or 590 ml/m^3 for 6 hours per day from gestation days 6 to 15. At 590 ml/m^3, skeletal variations of the vertebrae and delayed ossification were observed in the foetuses, and the relative and absolute liver weights in the dams were increased by about 12%. No embryotoxic or teratogenic effects were found (John et al. 1984). The NOAEC for developmental toxicity in rats is 210 ml chlorobenzene/m^3.

Likewise, no treatment-related embryotoxic or dose-dependent teratogenic effects in the foetuses were found in groups of 32 to 33 New Zealand White rabbits exposed from gestation days 6 to 18 for 6 hours daily to chlorobenzene concentrations of 0, 75, 210 or 590 ml/m³ in the first study and to 0, 10, 30, 75 or 590 ml/m³ in the subsequent second study. At the concentration of 590 ml/m³, an increased incidence of resorptions was found only in one study. In both studies, chlorobenzene increased the liver weights in the dams at concentrations of 210 ml/m³ and above and at 590 ml/m³, respectively (John et al. 1984). The NOAEC for developmental toxicity in rabbits is 590 ml chlorobenzene/m³.

The effects on the liver and the kidneys observed in the F1 offspring in the 2-generation study (Nair et al. 1987) are not regarded as evidence of developmental toxicity. The NOAEC for foetotoxicity is the highest concentration tested of 450 ml chlorobenzene/m³.

Conclusions: In the studies of the developmental toxicity of chlorobenzene, a LOAEC of 590 ml chlorobenzene/m³ and a NOAEC of 210 ml/m³ were obtained for prenatal developmental toxicity and maternal toxicity, respectively, in rats. In rabbits, the NOAEC for prenatal developmental toxicity is 590 ml chlorobenzene/m³ and the NOAEC for maternal toxicity 75 ml/m³. From the 2-generation study (see Section 5.5.1), a NOAEC of 450 ml/m³, the highest concentration tested, can be derived for foetotoxicity.

5.6 Genotoxicity

5.6.1 In vitro

The results of the Salmonella mutagenicity tests carried out with chlorobenzene were all negative. In CHO cells (a cell line derived from Chinese hamster ovary), chlorobenzene did not induce chromosomal aberrations, but sister chromatid exchange was observed at the concentration of 1 mg/ml. In the UDS test (for unscheduled DNA synthesis) with rat hepatocytes, chlorobenzene did not induce DNA damage. A $TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells with up to 200 µg chlorobenzene/ml (no other details) yielded positive results. However, no differentiation was made between small and large colonies (documentation "Chlorobenzene" 1999).

5.6.2 In vivo

Somatic cells

In studies of the genotoxicity of chlorobenzene in mice considered to be valid, the substance did not induce sister chromatid exchange or micronuclei in the bone marrow after oral doses of up to 400 mg/kg body weight (documentation "Chlorobenzene" 1999).

Groups of 3 female C57BL/6 mice received either a single intraperitoneal injection or injections on 3 consecutive days of chlorobenzene (in olive oil) at the dose level of 750 mg/kg body weight. For the comet assay, the lymphocytes were obtained from the peripheral blood and the cells from the bone marrow 16 hours after the final injection. Although the administration of 750 mg chlorobenzene/kg body weight caused severe intoxication (no other details), there was no toxicity to the bone mar-

row. An increase in DNA strand breaks was found in the peripheral lymphocytes only after the administration of three doses (Vaghef and Hellman 1994).

In another study, rats received intraperitoneal injections of chlorobenzene of 0, 750, 1000 or 1250 mg/kg body weight and the bone marrow cells were evaluated 12, 24 or 48 hours after the treatment. A significant increase in micronuclei formation was found only at the high dose 24 hours after administration. No cytotoxicity occurred (Siddiqui et al. 2006).

Germ cells

A recessive lethal test with Drosophila melanogaster and a dominant lethal test in mice with oral administration of chlorobenzene yielded negative results (documentation "Chlorobenzene" 1999).

Conclusions: In bacteria, chlorobenzene was not mutagenic. DNA damage in mammalian cells was found only in the higher concentration range (0.2 to 1 mg/ml). In genotoxicity studies in mice considered to be valid, oral chlorobenzene doses did not induce sister chromatid exchange or micronuclei in the bone marrow up to 400 mg/kg body weight. After intraperitoneal injection of high chlorobenzene doses, the frequency of DNA strand breaks in the peripheral lymphocytes of mice was increased at 750 mg/kg body weight as was the incidence of micronuclei in the bone marrow cells of rats at 1250 mg/kg body weight. No chromosomal aberrations or dominant lethal mutations occurred in mice.

Overall, chlorobenzene is not considered to be genotoxic.

5.7 Carcinogenicity

In a two-year study with oral administration of chlorobenzene to groups of 50 male and 50 female rats and mice (see also Section 5.2.2), a significantly increased number of neoplastic nodules in the liver was found only in the male rats of the high dose group given 120 mg/kg body weight and day (see Table 2). No liver tumours or other carcinomas occurred (NTP 1985).

Conclusions: The significantly increased incidence of neoplastic nodules in the liver which occurred only in male rats is not regarded as evidence of a carcinogenic effect (documentation "Chlorobenzene" 1999).

Table 2 Incidences of neoplastic nodules in the liver of male rats of the carcinogenicity study with chlorobenzene

Author:	NTP 1985			
Substance:	chlorobenzene (purity > 99%)			
Species:	**rat**, F344/N, 50 ♂, 50 ♀			
Administration route:	gavage			
Dose:	0 (untreated), 0 (corn oil), 60 or 120 mg chlorobenzene (in corn oil)/kg body weight and day			
Duration:	2 years, 5 days/week			
Toxicity:	–			
	Dose (mg/kg body weight and day)			
	untreated	0 (corn oil)	60	120
Liver: neoplastic nodules				
total	4/50 (8%)	2/50 (4%)	4/49 (8%)	8/49 (16%)
adjusted	10.4%	4.5%	12.5%	29.3%
terminal	2/34 (6%)	3/50 (6%)	4/32 (13%)	7/26 (27%)*

*$p \leq 0.05$ Fisher's exact test

6 Manifesto (MAK value/classification)

The critical effects of chlorobenzene in humans are its effects on the central nervous system. In animal studies, the main effects caused by chlorobenzene are changes in the liver and the kidneys.

MAK value. From a 2-generation study with Sprague Dawley rats, a LOAEC of 150 ml chlorobenzene/m³ and a NOAEC of 50 ml/m³ were obtained based on kidney changes, the increase in relative liver weights and hepatocellular hypertrophy. From this NOAEC, by taking into account the 7-day exposure compared with the 5-day exposure at the workplace and by applying factors of 2 each for the possible increase in effects over time, for the increased respiratory volume at the workplace, and for the extrapolation of the data from a study with animals to humans, a value of 8.8 ml chlorobenzene/m³ is calculated. Using the Preferred Value Approach, a MAK value of 5 ml/m³ can be established from this. This MAK value is supported by data obtained from a study with volunteers in which sleepiness as well as headaches and pain in the eyes occurred after exposure to 60.2 ml chlorobenzene/m³ for several hours. With the corresponding NOAEC of 11.8 ml chlorobenzene/m³, at which no evidence of acute neurotoxic or chemosensory effects were found, an adjusted NAEC (no adverse effect concentration) of 5.9 ml chlorobenzene/m³ can be determined taking into consideration the higher respiratory volume at the workplace compared with that of volunteers in studies under resting conditions. The MAK value of 5 ml/m³ is thus supported also by the results from studies with volunteers.

In a 2-year study with gavage administration of chlorobenzene, a LOAEL of 120 mg/kg body weight and day in rats was obtained due to neoplastic nodules in the liver of the males. After extrapolation to a concentration in the workplace air taking into consideration toxicokinetic data such as the species-specific correction value (1:4) for the rat, the assumed oral and respiratory absorption (100%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person, this LOAEL corresponds to a concentration in air of 210 mg/m^3 $\triangleq$ 44 ml/m^3 and is thus markedly higher than the MAK value of 5 ml chlorobenzene/m^3.

Peak limitation. As the MAK value is derived on the basis of systemic effects, chlorobenzene remains assigned to Peak Limitation Category II. The initial half-lives of the metabolites 4-chlorocatechol and 4-chlorophenol in humans are 2 and 3 hours, respectively. Therefore, the previous excursion factor of 2 for peak limitation is confirmed. This excursion factor is in agreement with the results from the study with volunteers described above, in which no complaints were recorded at a concentration of 11.8 ml/m^3.

Prenatal toxicity. In a developmental toxicity study with rats, an increase in skeletal vertebral variations occurred at 590 ml/m^3 in the foetuses, but no malformations. In rats, the NOAEC for prenatal developmental and maternal toxicity is 210 ml/m^3. In rabbits, the NOAEC for prenatal developmental toxicity is 590 ml/m^3 and the NOAEC for maternal toxicity is 75 ml/m^3. In a 2-generation study with rats, the NOAEC for foetotoxicity was the highest concentration tested of 450 ml/m^3 and the NOAEC for paternal toxicity was 50 ml/m^3. Taking into consideration the higher respiratory volume at the workplace (1:2), there is a 21 and 59-fold difference between the NOAECs obtained in the developmental toxicity studies with rats and rabbits, respectively, and the MAK value of 5 ml/m^3. Even if the increased resorptions at 590 ml/m^3 are given special consideration, the difference between the next-lower concentration and the MAK value is sufficiently great. In the 2-generation study with rats, in addition to the increased respiratory volume, the 7-day exposure compared with the 5-day exposure at the workplace is taken into account, and a 63-fold difference between the NOAEC for foetotoxicity and the MAK value is obtained. As, in rats and rabbits, no increase in malformations occurred and the differences between the NOAEC and the MAK value are sufficiently great, the assignment of chlorobenzene to Pregnancy Risk Group C has been retained.

Sensitization. No clinical or clearly positive experimental findings are available which would justify the classification of chlorobenzene as a skin sensitizer. There are also no data for respiratory sensitization, so that chlorobenzene is not designated with either "Sh" or with "Sa" (for substances which cause sensitization of the skin or airways).

Carcinogenicity and germ cell mutagenicity. The results of more recent studies of genotoxicity published since the supplement of 1995 are not sufficient to evaluate chlorobenzene as genotoxic, particularly as genotoxic effects occurred only after intraperitoneal injection at high doses. The significantly increased incidence of

neoplastic nodules induced in the liver of male rats only is not regarded as evidence of a carcinogenic effect. Therefore, chlorobenzene is not classified in one of the categories for carcinogens or germ cell mutagens.

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completed 22 March 2017