The MAK Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

2-Methyl-2-propanethiol

MAK Value Documentation – Translation of the German version from 2021

A. Hartwig[1,*] MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords

2-methyl-2-propanethiol; haematotoxicity; lung; liver; skin absorption; sensitization; toxicity; developmental toxicity; maximum workplace concentration; MAK value

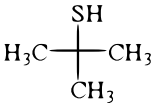
Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated data for 2-methyl-2-propanethiol [75-66-1] considering all toxicological end points. Data from a 90-day inhalation study with rats show that the critical effect of 2-methyl-2-propanethiol is its haematotoxic potential. On the basis of the NOAEC of 9 ml/m^3 and taking into account the increased respiratory volume at the workplace, the maximum concentration at the workplace (MAK value) has been set at 1 ml/m^3. As the critical effect is systemic, Peak Limitation Category II has been assigned with an excursion factor of 2. The NOAEC for developmental toxicity was 11 ml/m^3 in mice and 195 ml/m^3 in rats. When the increased respiratory volume at the workplace is taken into consideration, a sufficient margin remains between the values determined for rats and the MAK value. In mice, the LOAEC for developmental toxicity was 99 ml/m^3 and the NAEC is assumed to be above 11 ml/m^3; thus, there is a sufficient margin between these values and the MAK value. Accordingly, damage to the embryo or foetus is unlikely when the MAK value is not exceeded and 2-methyl-2-propanethiol has been classified in Pregnancy Risk Group C. According to skin absorption models, percutaneous absorption is expected to contribute significantly to systemic toxicity. Therefore, 2-methyl-2-propanethiol has been designated with an “H”. 2-Methyl-2-propanethiol can cause sensitization of the skin in animals and has therefore been designated with “Sh”. 2-Methyl-2-propanethiol is not mutagenic in vitro or clastogenic in vivo and no studies in germ cells are available. No carcinogenicity studies have been performed.

Citation Note:
Hartwig A, MAK Commission. 2-Methyl-2-propanethiol. MAK Value Documentation – Translation of the German version from 2021. MAK Collect Occup Health Saf. 2023 Mar;8(1):Doc015. https://doi.org/10.34865/mb7566e8_1or

Manuscript completed:
04 May 2021

Publication date:
30 Mar 2023

MAK value (2020)	**1 ml/m³ (ppm) ≙ 3.7 mg/m³ [a)]**
Peak limitation (2020)	**Category II, excursion factor 2**
Absorption through the skin (2020)	**H**
Sensitization (2020)	**Sh**
Carcinogenicity	**–**
Prenatal toxicity (2020)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	*tert*-butanethiol *tert*-butyl mercaptan
Chemical name	2-methylpropane-2-thiol
CAS number	75-66-1
Structural formula	
Molecular formula	$C_4H_{10}S$
Molar mass	90.18 g/mol
Melting point	−0.5 °C (OECD 2010)
Boiling point at 1013 hPa	64 °C (OECD 2010)
Density at 25 °C	0.794 g/cm³ (OECD 2010)
Vapour pressure at 20 °C	241 hPa (NLM 2020)
log K_{OW}	2.14 (calculated) (OECD 2010)
Solubility	1470 mg/l water (OECD 2010)
1 ml/m³ (ppm) ≙ 3.742 mg/m³	**1 mg/m³ ≙ 0.267 ml/m³ (ppm)**

[a)] Even if the MAK value is observed, "odour-associated" symptoms cannot be ruled out in individual cases.

2-Methyl-2-propanethiol is added to odourless gases as a warning substance. The quantities used for this purpose result in concentrations in the air of 4.1 to 63 µl/m³ (IIT Research Institute 1982). Furthermore, the substance is used as a catalyst in pesticide synthesis and as an anticatalyst in hydrocarbon and metal transfer reactions (OECD 2010).

1 Toxic Effects and Mode of Action

In rats, after inhalation exposure for 90 days, there was a decrease in the erythrocyte count and an increase in alveolar macrophages in the lungs at concentrations of 97 ml/m³ and above. After oral administration for 6 weeks, hepatocellular centrilobular hypertrophy, hepatic periportal fatty degeneration, and increased absolute and relative liver weights were observed in males at dose levels of 50 mg/kg body weight and day and above. These effects occurred also in the females of the 200 mg/kg group. In both sexes, delayed body weight gains and reduced food intake, as well as changes in haematological and clinico-chemical parameters occurred in this dose group. Haemosiderin deposits were found in the spleen. In male rats, sex and species-specific alpha-2u globulin nephropathy was observed in the kidneys after both inhalation and oral exposure.

2-Methyl-2-propanethiol is not irritating to the rabbit skin; it is slightly irritating in the rabbit eye.

No findings in humans are available for the skin sensitization potential of 2-methyl-2-propanethiol. A positive result was obtained in the local lymph node assay, indicating a low sensitization potential. Data for the respiratory sensitization potential of 2-methyl-2-propanethiol are not available.

In rats, no developmental toxicity was observed up to the highest concentration tested of 195 ml/m^3. By contrast, an increased incidence of spinal abnormalities was observed in mice at 99 ml/m^3; according to current evaluation criteria, these are to be interpreted as variations.

The substance is not mutagenic in bacteria and mammalian cells. 2-Methyl-2-propanethiol does not induce sister chromatid exchange (SCE) in vitro. No clastogenic effects were observed in mouse bone marrow after oral doses of up to 5000 mg/kg body weight. Studies of carcinogenic effects of the substance are not available.

2 Mechanism of Action

As was already described in the documentation for 1-butanethiol from 2000 (Greim 2005), thiols can contribute to the formation of reactive oxygen species through autoxidation in the presence of suitable metal ions. The resulting disulfides are reduced again to thiols. This redox cycling leads to oxidative stress. The haemolytic effect typical of aliphatic thiols can be seen, among other things, in the formation of Heinz bodies (clumping of irreversibly denatured haemoglobin). As a result, the number of erythrocytes decreases as they lose their deformability and are destroyed in the reticulohistiocytic system. Erythroclasia occurs mainly in the spleen, recognizable by its enlargement and dark discoloration. A decrease in circulating erythrocytes stimulates compensatory erythropoiesis, but anaemia occurs if there is too little new formation (Munday 1989). This effect can be assumed also for 2-methyl-2-propanethiol and is confirmed by the findings in the spleen and a reduced erythrocyte count from the studies in rats with repeated inhalation exposure (see Section 5.2.1) and repeated oral administration (see Section 5.2.2).

The bioactivity of 2-methyl-2-propanethiol was investigated in 235 in vitro assays during the US EPA's ToxCast/Tox21 testing program and the results were negative in all of them (US EPA 2020).

Thiols have a very high affinity to olfactory receptors (Li et al. 2016). Therefore, their odour threshold is very low and due to the sulfur content in these molecules, the odour quality is very similar to that of hydrogen sulfide, which smells like rotten eggs (Schiffman and Williams 2005). For this reason, these chemicals are added to odourless gases as odorants. This takes advantage of the biological function of such odours, which is based primarily on hazard prevention (Stevenson 2010). This association is learned (Hatt 2019), and therefore individuals from different cultures sometimes react very differently to odours (Ayabe-Kanamura et al. 1998).

Some substances can directly trigger "odour-associated" symptoms such as nausea or headaches in some individuals. There is generally no information in the scientific literature about physiological mechanisms that trigger these symptoms, but it is mainly very odour-intensive substances that can cause such reactions in individual cases (DFG 2021).

3 Toxicokinetics and Metabolism

On the basis of toxicity studies in animals, absorption via the respiratory and gastrointestinal tract can be assumed. However, quantitative data are not available.

According to the formula of Buist et al. (2012), the blood:air partition coefficient of 2-methyl-2-propanethiol is 6.6.

Calculations based on the IH SkinPerm model (Tibaldi et al. 2014) and according to Fiserova-Bergerova et al. (1990) yield dermal penetration rates of 0.034 and 0.49 mg/cm^2 and hour, respectively, for a saturated aqueous solution. Exposure of both hands and forearms (2000 cm^2 of skin) for 1 hour would thus result in absorbed amounts of 68 and 980 mg, respectively.

Several metabolic pathways are known for simple thiols in mammals: *S*-methylation leads to the formation of a methyl thioether or sulfide with subsequent oxidation to the corresponding sulfoxides and sulfones. Furthermore, thiols can react with glutathione to form mixed disulfides. Especially in the case of thiols with a low molar mass, oxidative desulfurization can take place with the formation of carbon dioxide and sulfate (WHO 2000). These metabolic pathways can therefore be assumed also for 2-methyl-2-propanethiol.

4 Effects in Humans

There are no data available for the end points repeated exposure, reproductive toxicity, genotoxicity, carcinogenicity and sensitization.

The odour detection threshold (ED_{50}) and the odour recognition threshold (R_{50}) at which half of the volunteers perceived an odour or recognized the substance to be smelled were determined with 10 female volunteers trained in odour perception and aged on average in their mid 40s (no other details). These thresholds were 0.9 ± 0.5 and 2.2 ± 1.6 µl/m³ for 2-methyl-2-propanethiol, respectively (IIT Research Institute 1982).

Another part of the study dealt with adaptation to the odour of the tested substances. Two experiments were conducted per day, one with a rapid, the next with a slow increase in the 2-methyl-2-propanethiol concentration in an exposure chamber, reaching the target concentration of 65 µl/m³ after half an hour and after 1 hour, respectively. Each experiment consisted of 4 stages: 20 minutes odorant-free, a 30 or 60-minute rise in the odorant concentration to about 65 µl/m³, a 30 or 60-minute decrease in the odorant concentration, and the last 20 minutes were again odorant-free. Ten "untrained" volunteers remained in the exposure chamber throughout the experiment. Their task was to record their odour perception at 2-minute intervals. Every 20 minutes, the volunteers used a subjective linear scale to record their evaluation of the strength of the odour and to indicate whether it was acceptable or not. This group consisted of men and women, on average in their early 30s (no other details). The group of female subjects from the first experimental stage (establishing odour detection and recognition thresholds) breathed the air of the exposure chamber outside through several outlets of the exposure chamber for the duration of 2 minutes. They recorded odour perception in 4 different ways: (1) identification of the sampling station with the odorant, (2) quantification of the odorant intensity using the butanol wheel (*n*-butyl alcohol as a comparison), (3) quantification of the odorant intensity using a subjective linear scale, and (4) characterization of the quality of the odorant as acceptable or unacceptable (defined as the concentration leading to a "negative reaction"). The experiments were conducted three times with rapid concentration rises and three times with slow concentration rises. The untrained subjects rated odour quality as unacceptable after a smaller number of experiments than did the trained subjects; however, the proportion of untrained subjects rating an odour as unacceptable decreased with increasing concentration. This effect can be explained by the strong adaptation in this group, which was continuously exposed to the odour. The trained subjects who were not continuously exposed to the increasing concentration of 2-methyl-2-propanethiol adapted to a lesser extent to the odour they had evaluated at the outlet of the exposure chamber (IIT Research Institute 1982). These experiments illustrate that 2-methyl-2-propanethiol can be perceived very well by the sense of smell. The substance triggers unpleasant sensations. However, the exposed persons adapted in some cases rapidly to the odour (IIT Research Institute 1982). Data for sensory irritation were not collected. Therefore, this study cannot be included in the derivation of the MAK value.

The highest concentrations tested in the IIT Research Institute study (1982) were 65 µl/m³, which is only 6.5% of the MAK value of 1 ml/m³. At the same time, they were more than a factor of 70 above the odour threshold of 2-methyl-2-propanethiol. The most significant decrease in odour intensity was observed in the persons who were continuously exposed to the odour ("untrained" volunteers), despite the rapid or slow rise in concentration to 65 µl/m³. The available data do not allow a precise extrapolation to the concentration range of the MAK value. Generally, adaptation to an odour is based on the calcium-dependent regulation of the responsiveness of cAMP-gated ion channels (CNG channels). Activation of the olfactory receptors by an odorant leads to an increase in the cAMP concentration in the nerve cell, which triggers the opening of these CNG channels and allows Ca^{2+} ions to enter the cell. This leads to the action potential and thus to the perception of odour in the brain. The inflowing Ca^{2+} ions bind intracellularly to calmodulin, which in

turn blocks the further influx of Ca^{2+} ions, so that odour perception decreases despite constant activation of the olfactory receptors by the odorant (Hatt 2019). In principle, this mechanism is to be expected also for higher concentrations.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

The 4-hour LC_{50} of 2-methyl-2-propanethiol is very high compared with that of other thiols. Values of 22 200 to 26 643 ml/m³ were obtained in the rat and of 16 500 ml/m³ in the mouse. Lacrimation, prostration, tremor, staggering gait, muscular weakness, cyanosis and sedation, and also mucosal irritation (rubbing of eyes and nose, closed eyes, watering of eyes, corneal opacity) and retraction of the head (amongst others) were observed. The dead animals were found to have red-coloured lungs (OECD 2010).

For the structurally related 1-butanethiol, 4-hour inhalation LC_{50} values of 4020 and 6060 ml/m³ in the rat and of 2500 ml/m³ in the mouse were reported. They are thus within the same order of magnitude as those for ethanethiol of 4420 ml/m³ and 2770 ml/m³ in rats and mice, respectively. In the case of 1-propanethiol, the values are somewhat higher at 7300 or > 8170 ml/m³ in the rat and 4010 ml/m³ in the mouse (OECD 2010).

5.1.2 Oral administration

LD_{50} values in the rat of 4729 and 8400 mg/kg body weight, respectively, are reported. The animals exhibited inactivity and sedation. Gross-pathological examination did not reveal unusual findings (Fairchild and Stokinger 1958; Farr and Kirwin 1994).

5.1.3 Dermal application

The dermal lethal dose of 2-methyl-2-propanethiol in the rabbit is reported to be 20 800 mg/kg body weight. The animals displayed marked inactivity and weakness up to 3 days after the end of treatment. Gross-pathological examination did not reveal unusual findings (no other details; Farr and Kirwin 1994).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Inhalation studies were conducted with 2-methyl-2-propanethiol in rats. The results are shown in Table 1.

Tab. 1 Inhalation studies with 2-methyl-2-propanethiol in rats

Species, strain, number per group	Exposure	Findings	References
rat, CD, 10 ♂, 10 ♀	**14 days**, 0, 201, 1086, 1990 ml/m³, 6 hours/day, 7 days/week, **dose-finding**, (histopathological examination of liver only, only 0, 1990 ml/m³)	**201 ml/m³ and above**: ♂, ♀: absolute liver weights ↑; ♂: absolute spleen weights ↑, absolute kidney weights ↑; **1900 ml/m³**: ♂, ♀: hepatocellular hypertrophy (weak to moderate)	IRDC 1981
rat, Sprague Dawley, 15 ♂, 15 ♀	**90 days**, 0, 9, 97, 196 ml/m³, 6 hours/day, 5 days/week, (histopathological examination at first only 0, 196 ml/m³, follow-up examination of kidneys and lungs: 9 and 97 ml/m³, lungs: additional repeat examination: 0, 196 ml/m³)	**9 ml/m³**: ♂, ♀: NOAEC for local and systemic effects; ♂: chronic nephrosis (all kidney findings due to alpha-2u-globulin nephropathy) (3/15); **97 ml/m³**: ♂, ♀: alveolar macrophages ↑ (5/15 ♂, 3/15 ♀); ♀: erythrocytes ↓ (week 6); **97 ml/m³ and above**: ♂: absolute and relative kidney weights ↑, chronic nephrosis (13/14); ♀: erythrocytes ↓ (week 12); **196 ml/m³**: ♂, ♀: alveolar macrophages ↑ (14/15 ♂, 12/15 ♀), "interstitial pulmonary fibrosis" diagnosed in follow-up examination as an artefact from tissue preparation; ♂: chronic nephrosis (14/15), inflammation in nasal turbinates (1/15)	IRDC 1982 b, 1983, 1984

90-Day inhalation studies were conducted in Sprague Dawley rats with **2-methyl-2-propanethiol** and the structurally related **1-butanethiol** (see also supplement to 1-butanethiol, Hartwig and MAK Commission 2020). For this purpose, 15 rats per sex and group were exposed to 0, 9, 97 or 196 ml **2-methyl-2-propanethiol**/m³ or 0, 9, 70 or 150 ml **1-butanethiol**/m³ for 6 hours daily, on 5 days per week.

Changes in haematological parameters occurred in both studies, but were interpreted by the study authors as not biologically relevant. Thus, in the female animals exposed to 2-methyl-2-propanethiol, there was a decrease in the erythrocyte count in week 6 (97 ml/m³) and week 12 (at 97 ml/m³ and above). However, since haematological effects, especially the decrease in the erythrocyte count are characteristic of thiols (see Section 2), they are considered by the Commission to be treatment-related. The absolute and relative kidney weights of the males were increased, probably due to chronic nephrosis (see below). Histopathological examination was initially performed only in the controls and the high treatment group (IRDC 1982 b). In a follow-up examination, histopathological sections of kidney and lung were evaluated for the 9 and 97 ml/m³ groups. In the case of the lungs, a re-evaluation of the control and high concentration groups was performed. Chronic nephrosis was observed in the kidneys of the males in all groups exposed to 2-methyl-2-propanethiol (0, 9, 97, 196 ml/m³: 0/15, 3/15, 13/14, 14/15) (IRDC 1983, 1984), which was due to alpha-2u-globulin nephropathy. Since this is sex and species-specific, it is not relevant to humans (Hard et al. 1993). In the study itself, no immunohistochemical detection of alpha-2u-globulin was performed, but this was carried out in the screening study described in Section 5.2.2 (MHLW 2006). In the lungs, an increase in alveolar macrophages was observed at concentrations of 97 ml/m³ and above. The interstitial pulmonary fibrosis diagnosed in the original evaluation was interpreted as an artefact from tissue preparation (IRDC 1983, 1984). Because 1 of 15 animals in the high treatment group exhibited inflammation in the nasal turbinates and many more animals displayed pulmonary effects (males: 14/15, females: 12/15), it can be assumed that the lungs are more sensitive than the upper respiratory tract. Thus, in summary, the NOAEC (no observed adverse effect concentration) for local and systemic effects in the 90-day inhalation study was 9 ml 2-methyl-2-propanethiol/m³.

5.2.2 Oral administration

In a screening study according to OECD Test Guideline 422, groups of 12 male and 12 female rats were given gavage doses of 2-methyl-2-propanethiol of 0, 10, 50 or 200 mg/kg body weight and day on 7 days per week. Treatment began 14 days before mating and lasted a total of 6 weeks for the males and 7 weeks for the females, until day 4 after birth.

Satellite groups for the control and high dose groups, consisting of 5 females that were not pregnant and 5 males, were examined 14 days after the end of treatment. Renal effects were observed in the males of all treatment groups, which were due to demonstrated alpha-2u-globulin nephropathy and thus not relevant to humans. At 50 and 250 mg/kg body weight and day, the male animals exhibited hepatocellular centrilobular hypertrophy, hepatic periportal fatty degeneration, and increased absolute and relative liver weights. In the high dose group, in addition to delayed body weight gains and reduced food intake, changes in haematological and clinico-chemical parameters were observed in both sexes (see Table 2). Haemosiderin deposits were observed in the spleen. In the liver of the female animals, hepatocellular centrilobular hypertrophy was found, and the absolute and relative liver weights were increased compared with those of the controls. The alpha-2u-globulin nephropathy observed in male rats at 10 mg/kg body weight and day and above is not relevant to humans. The NOAEL (no observed adverse effect level) in males was 10 mg/kg body weight and day, and that in females 50 mg 2-methyl-2-propanethiol/kg body weight and day (MHLW 2006).

Tab. 2 Studies with oral administration of 2-methyl-2-propanethiol in rats

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 12 ♂, 12 ♀	**6–7 weeks**, 0, 10, 50, 200 mg/kg body weight and day in corn oil, 7 days/week, gavage, **OECD Test Guideline 422**	**10 mg/kg body weight**: ♂, ♀: **NOAEL**; ♂: swollen and pale kidneys (1/12); **10 mg/kg body weight and above**: ♂: absolute and relative kidney weights ↑, hyaline droplets in the proximal tubular epithelium (degree of severity increased dose-dependently), basophilic renal tubules ↑ (demonstrated alpha-2u-globulin nephropathy); **50 mg/kg body weight**: ♀: NOAEL; ♂: absolute and relative liver weights (+ 29% and + 35%, respectively, compared with the control values) ↑; **50 mg/kg body weight and above**: ♂, ♀: cholesterol ↑; ♂: hepatocellular, centrilobular hypertrophy, periportal fatty degeneration of the hepatocytes, swollen and pale kidneys (3/12), MCHC ↓, phospholipids ↑; **200 mg/kg body weight**: ♂, ♀: body weight gains ↓, food intake ↓, erythrocytes ↓, glucose ↓, α1-globulin ↓, albumin ↑, phospholipids ↑, haemosiderin deposits in spleen; ♂: absolute thymus weights ↓, absolute and relative liver weights (+ 41% and + 64%, respectively, compared with the control values) ↑, Hb ↓, haematocrit ↓, thrombocytes ↓, alpha-2u-globulin ↑, γ-GTP ↑, swollen liver (2/12), swollen and pale kidneys (4/12); ♀: reticulocytes ↑, total protein content ↑, A/G ↑, hepatocellular centrilobular hypertrophy, absolute and relative liver weights (+ 26% and + 38%, respectively, compared with the control values) ↑; no changes found in FOB	MHLW 2006

A/G: albumin to globulin ratio; FOB: Functional Observational Battery; γ-GTP: γ-glutamyltranspeptidase; Hb: haemoglobin; MCHC: mean corpuscular haemoglobin concentration

5.3 Local effects on skin and mucous membranes

After occlusive application of 0.5 ml of undiluted test substance for 4 hours, all 6 albino rabbits exhibited moderate redness (score 2) that was reversible overnight. The irritation index (24, 48 and 72 hours averaged) was 0 (no other details; ECHA 2018; OECD 2010).

During the determination of the dermal LD_{50}, faint redness and discoloration occurred at the site of application (no other details; Farr and Kirwin 1994).

In a study carried out according to OECD Test Guideline 405, the undiluted test substance was slightly irritating to the eyes of rabbits. The effects were reversible within 7 days (ECHA 2018). The primary stimulation index according to the Draize scheme calculated as the mean value after 24, 48 and 72 hours was 7.73/110.

After application of the test substance to the eyes of rabbits, mild to moderate conjunctival irritation occurred, which regressed after 7 days. When rats and mice were exposed to the substance by inhalation, likewise mucosal irritation of the eye occurred (no other data available; Farr and Kirwin 1994).

5.4 Allergenic effects

A local lymph node assay (LLNA) carried out according to OECD Test Guideline 429 with groups of 4 female CBA/J mice yielded a positive result for 2-methyl-2-propanethiol (purity 98.71%). The substance was used in 5%, 10%, 25% and 50% preparations in acetone/olive oil (4:1) and undiluted. The corresponding stimulation indices were 1.73, 1.77, 3.62, 4.26 and 30.43, respectively. Thus, the 25%, 50% and 100% preparations increased the simulation index by more than threefold; an increase in ear thickness was not detected. The EC3 value (concentration which leads to a threefold increase in lymphocyte proliferation) is 20% (CIT 2011).

In a Buehler test in 10 female and 10 male Hartley guinea pigs, undiluted 2-methyl-2-propanethiol was used for the induction treatment. The challenge treatment was performed with a 75% preparation of the test substance in mineral oil. After both 24 and 48 hours, all 20 animals produced weak to marked reactions (score 1 to 3). In the 10 control animals, 10 and 9 weak reactions occurred after 24 and 48 hours, respectively (Elf Atochem North America Inc 1995).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In the screening study according to OECD Test Guideline 422 already described in Section 5.2.2 and Table 2, no effects on the reproductive parameters mating index, duration of mating, duration of pregnancy, implantation index, number of pups, number of live pups, survival index on day 4 after birth and sex ratio were observed in male and female Sprague Dawley rats treated with 2-methyl-2-propanethiol at dose levels of 0, 10, 50 or 200 mg/kg body weight and day. Histopathological examination of the reproductive organs did not reveal any unusual substance-related findings. The NOAEL for fertility in this study was 200 mg/kg body weight and day, the highest dose tested (MHLW 2006).

5.5.2 Developmental toxicity

In the screening study carried out according to OECD Test Guideline 422 in Sprague Dawley rats already described in Section 5.2.2 and Table 2, the body weights of the offspring were decreased in a statistically significant manner in the 200 mg/kg group on day 4 after birth (♂: 8.6 ± 0.9 g compared with 10.3 ± 0.8 g in the controls, ♀: 8.2 ± 0.9 g compared with 9.8 ± 0.9 g in the controls), but not on the day of birth. Exencephaly, an open eyelid, and a protruding tongue were observed in one pup in this dose group; there were no malformations in the other groups (MHLW 2006). The incidence of malformations is within historical control values (historical controls, data in % (range), for exencephaly: study laboratory: 0, other laboratories: 0.03 (0–0.72), 0.03 (0–0.38), 0.01 (0–0.28), 0.04 (0–0.33), 0.01 (0–0.36) etc., for open eyelid: study laboratory: 0, other laboratories: 0.04 (0–1.01), 0.03 (0–0.29), 0.07 (0–0.25), 0.01 (0–0.27), 0.08 (0–0.33); Nakatsuka et al. 1997). Therefore, these malformations should be considered incidental rather than substance-related. The NOAEL for perinatal toxicity in this study was 200 mg/kg body weight and day based on the fact that body weights were decreased only on day 4 after birth and not on the day of birth. A full investigation of teratogenicity is not included in a study conducted according to OECD Test Guideline 422.

In a preliminary study, groups of 5 pregnant COBS CD rats were exposed to 2-methyl-2-propanethiol concentrations of 0, 201, 1086 or 1990 ml/m^3 for 6 hours per day. The animals were exposed from day 6 to day 10 and from day 13 to day 17 of gestation and investigated on day 20 of gestation. Body weight gains were delayed even in the low concentration group and the mean number of postimplantation losses was increased (control value and ascending concentrations: 0.5, 2.0, 8.6, 13.8). At concentrations of 1086 ml/m^3 and above, the number of dams with resorptions was increased, whereas the number of live foetuses per dam was decreased. The mean number of corpora lutea or total implantations in all groups were similar to those in the untreated control group and the historical controls. Based on these results, 200 ml/m^3 was selected as the highest concentration to be tested for the developmental toxicity study (IRDC 1981).

In a developmental toxicity study similar to OECD Test Guideline 414, groups of 25 pregnant COBS CD rats were exposed whole-body by inhalation to 2-methyl-2-propanethiol concentrations of 0, 11, 99 or 195 ml/m^3 for 6 hours daily from gestation days 6 to 19. In the treatment groups, there was an increase in the number of rats with hair loss during

exposure compared with the number in the control group. No other signs of maternal toxicity occurred. There were no biologically relevant or statistically significant differences in the mean number of live foetuses, total number of implantations, corpora lutea or sex ratio compared with the control values. Up to the highest concentration tested of 195 ml/m^3, no increase in the number of malformations was found in the foetuses compared with the control values. The NOAEC for developmental toxicity and maternal toxicity in rats was 195 ml/m^3, the highest concentration tested (IRDC 1982 a).

In another developmental toxicity study, similar to OECD Test Guideline 414, groups of 25 pregnant CD-1 mice were exposed whole-body by inhalation to 2-methyl-2-propanethiol concentrations of 0, 11, 99 or 195 ml/m^3 for 6 hours daily from gestation days 6 to 16. No signs of maternal toxicity were observed in the dams of the treated groups. At 99 and 195 ml/m^3, there was an increase in the sum of all malformations representing spinal anomalies in the foetuses, which on a litter basis amounted to incidences of 47.8% at 99 ml/m^3 and 28.6% at 195 ml/m^3 compared with 16.7% in the control group. There was no difference on a foetus basis and no concentration dependency. Compared with the control values, there were no differences as regards the mean number of live foetuses, the total number of implantations, corpora lutea or the sex ratio. The authors consider the NOAEC for developmental and maternal toxicity in mice to be 195 ml/m^3 (IRDC 1982 a). The anomalies of the spine were unclearly described findings, the incidence of which was high even in the controls and was not concentration-dependent. The study was conducted in a period when these changes, which are now judged to be variations, were still regarded as malformations. Nevertheless, the Commission takes these findings seriously and, unlike the authors, considers the NOAEC for developmental toxicity in the mouse to be 11 ml/m^3 and for maternal toxicity to be 195 ml/m^3.

5.6 Genotoxicity

5.6.1 In vitro

A mutagenicity test in the Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of a metabolic activation system yielded negative results up to a concentration of 10 000 µg/plate (OECD 2010).

In an assay for the induction of SCE in CHO cells (a cell line derived from Chinese hamster ovary cells), a statistically significant increase in the number of SCEs per cell was observed at the concentrations of 450 and 1350 µg/ml in the presence of a metabolic activation system (10.66 and 10.88, respectively, compared with 7.58 in the control); the other concentrations did not reach statistical significance. The highest concentration tested of 1350 µg/ml resulted in growth inhibition. Since none of the concentrations led to a doubling of SCE, the test result was considered negative (ECHA 2018; OECD 2010). The concentrations used were not reported, only the two concentrations causing a statistically significant increase in SCE per cell.

In a TK$^{+/-}$ mutation assay in L5178 mouse lymphoma cells using concentrations of 0, 61, 90, 135, 202, 300, 449, 670 or 1000 µg/ml without the addition of a metabolic activation system, the induction of mutations was observed at 202 and 1000 µg/ml with a maximum twofold increase in mutation frequency compared with the control value. The test results with the other concentrations were negative. Thus, the findings were not dose-dependent. The result was negative also in the presence of a metabolic activation system. Without the addition of a metabolic activation system, survival of the cells was 93.5% at 202 µg/ml, 10.6% at 670 µg/ml and 3% at the high concentration, respectively. In the presence of metabolic activation, survival was 10.9% at 1000 µg/ml (no other details; ECHA 2018; OECD 2010). It is not reported whether the evaluation distinguished between small and large colonies. Due to the strong cytotoxicity at 1000 µg/ml, the lack of concentration dependency, and the maximum twofold increase in mutation frequency compared with the control values, this test result is considered negative.

5.6.2 In vivo

A micronucleus test in groups of 15 male and 15 female ICR mice (20 males and 20 females in the high dose group; 5 of each sex for the positive control) yielded negative results after single oral doses, whereas the positive control

cyclophosphamide produced the expected results. The bone marrow of the animals was examined 24, 48 and 72 hours after treatment with 0, 1250, 2500 or 5000 mg/kg body weight. The doses to be used were defined by a preliminary study in which no deaths occurred at 5000 mg/kg body weight. In the main study, the treated animals were lethargic; mortality did not occur. In some of the animals there was a decrease in the ratio of polychromatic erythrocytes to total erythrocytes (up to −20% compared with the control value), which demonstrated that the bone marrow was reached (Microbiological Associates Inc 1995).

5.7 Carcinogenicity

There are no data available.

6 Manifesto (MAK value/classification)

The critical effects of 2-methyl-2-propanethiol are effects on the erythrocytes, spleen and lungs, and mucosal irritation.

MAK value. As with all thiols, 2-methyl-2-propanethiol is a substance with a strong, unpleasant odour. Data in humans that can be used to derive a MAK value are not available.

For 2-methyl-2-propanethiol, the MAK value is derived based on systemic effects. The starting point is the NOAEC from a 90-day inhalation study in rats of 9 ml/m^3. Haematological effects were observed at the next-higher concentration of 97 ml/m^3. After extrapolation of the experimental animal data to humans (1:2), taking into account the increased respiratory volume of humans at the workplace compared with that of test animals at rest (1:2), and assuming a possible increase in effects with chronic exposure (1:2), a concentration of 1.1 ml/m^3 is obtained from this NOAEC. Using the preferred value approach, the MAK value is 1 ml/m^3.

The oral screening study in rats with a NOAEL of 10 mg/kg body weight and day does not contradict the MAK value of 1 ml/m^3. At the next-higher dose of 50 mg/kg body weight and day, hepatocellular hypertrophy was observed. The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL to a concentration in workplace air: a possible increase in effects with chronic exposure (1:4), the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding species-specific correction value for the rat (1:4), the assumed oral absorption (100%), the body weight (70 kg) and respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentration calculated from this is 6.13 mg/m^3. Extrapolating the data from animal experiments to humans (1:2) and using the preferred value approach, a MAK value of 0.5 ml/m^3 is obtained. Hepatocellular hypertrophy, which was the most sensitive adverse effect in this study, is enhanced by gavage administration because of the first-pass effect. Therefore, this route of administration should be considered the worst case compared with inhalation exposure. The MAK value for 2-methyl-2-propanethiol should therefore be derived from the inhalation study.

Similar systemic effects were observed in several studies with inhalation exposure of rats to 2-butanethiol, 2-methyl-2-propanethiol and 1-butanethiol. The limit values derived from these studies are of the same order of magnitude and are lower than the limit values that would be derived on the basis of local effects. Thus, the local effects of 2-methyl-2-propanethiol are avoided if the MAK value of 1 ml/m^3 is observed (Hartwig and MAK Commission 2020, 2023).

Not only are acute annoyance reactions or disgust triggered by odours, but some individuals may also experience "odour-associated symptoms" such as headaches or nausea (no other details regarding thiols; Shusterman 1999). However, studies with 2-methyl-2-propanethiol are not available. Therefore, it cannot be ruled out that 2-methyl-2-propanethiol causes reversible "odour-associated symptoms" in individual cases even if the MAK value of 1 ml/m^3 is observed. Pathophysiological mechanisms for these symptoms are not described in the scientific literature. For comparison, after 3 hours of daily exposure (5 and 10 days) of subjects (n = 2 and n = 1, respectively) to ethanethiol, irritation of the oral

and nasal mucous membranes, nausea and changes in the sense of taste occurred at the concentration of 3.9 ml/m^3, but not at 0.39 ml/m^3 (Blinova 1965).

Peak limitation. Since the MAK value for 2-methyl-2-propanethiol is derived on the basis of a systemic effect, the substance has been assigned to Peak Limitation Category II. Information on the half-life of the substance is not available. Therefore, the default excursion factor of 2 for substances with systemic effects has been established. Thus, the permissible short-term concentration is also below the locally effective concentration.

Prenatal toxicity. Two prenatal developmental toxicity studies in rats and mice with inhalation exposure are available. In rats, there was no developmental or maternal toxicity up to the highest concentration tested of 195 ml/m^3. In mice, the NOAEC for developmental toxicity was 11 ml/m^3. Taking into account the increased respiratory volume (1:2), the margins between the NOAECs for developmental toxicity for rats and mice and the MAK value of 1 ml/m^3 are 98-fold and 6-fold, respectively. The LOAEC (lowest observed adverse effect concentration) in mice is 10 times as high as the NOAEC. This suggests that the NAEC (no adverse effect level) for developmental toxicity in mice may be higher.

A screening study in rats carried out according to OECD Test Guideline 422 with oral administration of 2-methyl-2-propanethiol yielded a NOAEL for perinatal toxicity of 200 mg/kg body weight and day, the highest dose tested. After extrapolation of this NOAEL of 200 mg/kg body weight and day using the toxicokinetic parameters mentioned above, a concentration of 131 ml/m^3 (490 mg/m^3) is obtained (see "MAK value").

Due to the sufficient margin between the MAK value of 1 ml/m^3 and the NOAEC from the inhalation study in rats, and in view of the presumably higher NAEC and thus greater margin between the concentration in air calculated from the inhalation study in mice and the MAK value, 2-methyl-2-propanethiol has been assigned to Pregnancy Risk Group C.

Absorption through the skin. The acute toxicity of 2-methyl-2-propanethiol after dermal application is low. Apart from this, no experimental data are available to assess absorption through the skin. Calculations based on models yielded dermally absorbed amounts of 68 and 980 mg (see Section 3). An 8-hour exposure (10 m^3 respiratory volume) at the level of the MAK value, assuming complete absorption, would correspond to an absorbed amount of 37 mg of 2-methyl-2-propanethiol via the respiratory tract. Thus, dermal exposure may result in systemic toxicity even if the MAK value is observed. Therefore, 2-methyl-2-propanethiol has been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There are no data available for skin sensitizing effects of 2-methyl-2-propanethiol in humans. A positive result in a valid local lymph node assay indicates that 2-methyl-2-propanethiol has a low sensitization potential. The substance has therefore been designated with "Sh" (for substances which cause sensitization of the skin), but not with "Sa" (for substances which cause sensitization of the airways).

Germ cell mutagenicity and carcinogenicity. Studies with germ cells are not available. The substance is not mutagenic in bacteria and mammalian cells. 2-Methyl-2-propanethiol does not induce SCE in vitro. In vivo, no clastogenic effects were observed in mouse bone marrow. There are no data available for mutagenic effects in vivo and the question as to whether the germ cells are reached. Based on the structure and the data for other structurally similar thiols, germ cell mutagenicity is not to be expected. In view of the data available, 2-methyl-2-propanethiol has not been classified in one of the categories for germ cell mutagens.

Carcinogenicity studies are not available. Therefore, 2-methyl-2-propanethiol has not been classified in one of the categories for carcinogens.

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.

References

Ayabe-Kanamura S, Schicker I, Laska M, Hudson R, Distel H, Kobayakawa T, Saito S (1998) Differences in perception of everyday odors: A Japanese-German cross-cultural study. Chem Senses 23(1): 31–38. https://doi.org/10.1093/chemse/23.1.31

Blinova EA (1965) [Experimental data on the mutagenic action of toluene, isopropanol and sulfur dioxide]. Gig Sanit 30: 18–22

Buist HE, de Wit-Bos L, Bouwman T, Vaes WHJ (2012) Predicting blood:air partition coefficients using basic physicochemical properties. Regul Toxicol Pharmacol 62(1): 23–28. https://doi.org/10.1016/j.yrtph.2011.11.019

CIT (Centre International de Toxicologie) (2011) Evaluation of skin sensitization potential in mice using the local lymph node assay (LLNA), tertiobutylmercaptan. Study No. 37688, 2011, Evreux: CIT, unpublished

DFG (Deutsche Forschungsgemeinschaft), editor (2021) List of MAK and BAT Values 2021. Maximum Concentrations and Biological Tolerance Values at the Workplace. Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, report 57. Düsseldorf: German Medical Science. https://doi.org/10.34865/mbwl_2021_eng

ECHA (2018) 2-Methylpropane-2-thiol (CAS Number 75-66-1). Registration dossier. Joint submission, first publication 03 Mar 2011, last modification 13 Nov 2018. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/2129, accessed 06 Nov 2020

Elf Atochem North America Inc (1995) Initial submission: letter from Elf Atochem No Amer Inc to USEPA regarding dermal sensitization study in guinea pigs with t-butyl mercaptan by modified Buehler design dated 010495. NTIS/OTS0556419. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0556419.xhtml, accessed 18 Oct 2019

Fairchild EJ, Stokinger HE (1958) Toxicologic studies on organic sulfur compounds. I. Acute toxicity of some aliphatic and aromatic thiols (mercaptans). Am Ind Hyg Assoc J 19(3): 171–189. https://doi.org/10.1080/00028895809343573

Farr CH, Kirwin CJJ (1994) Organic sulfur compounds. In: Clayton GD, Clayton FE, editors. Patty's industrial hygiene and toxicology, 4th ed. Volume 2F. New York, NY: John Wiley & Sons. p. 4311–4372

Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. Am J Ind Med 17(5): 617–635. https://doi.org/10.1002/ajim.4700170507

Greim H, editor (2005) Butanethiol. MAK Value Documentation, 2000. In: The MAK Collection for Occupational Health and Safety, Part I: MAK Value Documentations. Volume 21. Weinheim: Wiley-VCH. p. 161–170. Also available from https://doi.org/10.1002/3527600418.mb10979e0021

Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE, Valcovic LR (1993) Hazard evaluation of chemicals that cause accumulation of alpha 2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ Health Perspect 99: 313–349. https://doi.org/10.1289/ehp.9399313

Hartwig A, MAK Commission (2020) 1-Butanethiol. MAK Value Documentation – Translation of the German version from 2019. MAK Collect Occup Health Saf 5(4): Doc079. https://doi.org/10.34865/mb10979e5_4ad

Hartwig A, MAK Commission (2023) 2-Butanethiol. MAK Value Documentation – Translation of the German version from 2021. MAK Collect Occup Health Saf 8(1): Doc012. https://doi.org/10.34865/mb51353e8_1or

Hatt H (2019) Geruch. In: Brandes R, Lang F, Schmidt RF, editors. Physiologie des Menschen mit Pathophysiologie. Springer-Lehrbuch, 32nd ed. Berlin, Heidelberg: Springer-Verlag. p. 781–788. https://doi.org/10.1007/978-3-662-56468-4

IIT Research Institute (1982) Odor recognition and fatigue. NTIS PB83153882. Chicago, IL: IIT Research Institute. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB83153882.xhtml, accessed 04 Feb 2019

IRDC (International Research and Development) (1981) Initial submission: two week range-finding and teratological toxicity studies of t-butyl mercaptan in rats with cover letter dated 08/24/92. NTIS/OTS0555472, EPA/OTS Doc ID 88-920009419. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0555472.xhtml, accessed 12 Sep 2020

IRDC (International Research and Development) (1982 a) Thirteen week inhalation toxicity study of n-butyl and t-butyl mercaptan in rats. Report No. 409-054, 1982, Mattawan, MI: IRDC, unpublished

IRDC (International Research and Development (1982 b) Inhalation teratology study of n-butyl mercaptan and t-butyl mercaptan in rats and mice. Report No. 409-052, 1982, Mattawan, MI: IRDC, unpublished

IRDC (International Research and Development) (1983) Addendum to the final report: thirteen week inhalation toxicity study of n-butyl and t-butyl mercaptan in rats. Report No. 409-054, 1983, Mattawan, MI: IRDC, unpublished

IRDC (International Research and Development (1984) Amendment to the addendum to the final report: thirteen week inhalation toxicity study of n-butyl and t-butyl mercaptan in rats. Report No. 409-054, 1984, Mattawan, MI: IRDC, unpublished

Li S, Ahmed L, Zhang R, Pan Y, Matsunami H, Burger JL, Block E, Batista VS, Zhuang H (2016) Smelling sulfur: Copper and silver regulate the response of human odorant receptor OR2T11 to low-molecular-weight thiols. J Am Chem Soc 138(40): 13281–13288. https://doi.org/10.1021/jacs.6b06983

MHLW (Japan Ministry of Health Labor and Welfare) (2006) [Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test]. Tokyo: MHLW. https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF75-66-1d.pdf, accessed 09 Aug 2020

Microbiological Associates Inc (1995) Micronucleus cytogenetic assay in mice, t-butyl mercaptan. Study No. G95AT24.122, 1995, Bethesda, MD: Microbiological Associates, Inc., unpublished

Munday R (1989) Toxicity of thiols and disulphides: involvement of free-radical species. Free Radic Biol Med 7(6): 659–673. https://doi.org/10.1016/0891-5849(89)90147-0

Nakatsuka T, Horimoto M, Ito M, Matsubara Y, Akaike M, Ariyuki F (1997) Japan Pharmaceutical Manufacturers Association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. Congenit Anom (Kyoto) 37(1): 47–138. https://doi.org/10.1111/j.1741-4520.1997.tb00547.x

NLM (National Library of Medicine) (2020) t-Butyl mercaptan. ChemIDplus Data Bank. https://chem.nlm.nih.gov/chemidplus/number/75-66-1, accessed 21 Apr 2010

OECD (Organisation of Economic Co-operation and Development) (2010) C2–C4 aliphatic thiols category, 1-ethanethiol (CAS No. 75-08-1), 1-propanethiol (CAS No.107-03-9), 1-butanethiol (CAS No.109-79-5), 2-propanethiol, 2-methyl (CAS No. 75-66-1). OECD SIDS Initial Assessment Report. Paris: OECD. https://hpvchemicals.oecd.org/UI/handler.axd?id=236e6893-6b1b-48d3-895c-e31db1f9f55d, accessed 28 Jan 2019

Schiffman SS, Williams CM (2005) Science of odor as a potential health issue. J Environ Qual 34(1): 129–138

Shusterman D (1999) The health significance of environmental odour pollution: revisited. J Environ Med 1(4): 249–258. https://doi.org/10.1002/jem.38

Stevenson RJ (2010) An initial evaluation of the functions of human olfaction. Chem Senses 35(1): 3–20. https://doi.org/10.1093/chemse/bjp083

Tibaldi R, ten Berge W, Drolet D (2014) Dermal absorption of chemicals: estimation by IH SkinPerm. J Occup Environ Hyg 11(1): 19–31. https://doi.org/10.1080/15459624.2013.831983

US EPA (US Environmental Protection Agency) (2020) Chemical dashboard. t-Butyl mercaptan (CAS Number 75-66-1). https://comptox.epa.gov/dashboard/chemical/invitrodb/DTXSID0026418, accessed 16 Apr 2020

WHO (World Health Organization) (2000) Safety evaluation of simple aliphatic and aromatic sulfides and thiols. In: Safety evaluation of certain food additives. WHO food additives series, No. 44. Geneva: WHO. http://www.inchem.org/documents/jecfa/jecmono/v44jec09.htm, accessed 18 Feb 2020