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Methanol

MAK Value Documentation, supplement – Translation of the German version from 2019

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the maximum concentration at the workplace (MAK value) of methanol [67-56-1] of 200 ml/m^3, considering all toxicity end points. Available publications and unpublished study reports are described in detail. Uptake of larger amounts of methanol depresses the central nervous system and leads to developmental toxicity as direct effects of methanol followed by metabolic acidosis and ocular toxicity as formate effects. No neurobehavioral effects were observed in subjects exposed 4 hours to 200 ml/m^3 at rest leading to a concentration of 6.5 mg methanol/l blood. The steady state concentration of methanol after exposure to 100 ml/m^3 with physical activity is calculated to be 6 mg methanol/l blood and is reached after 8 hours. Therefore, taking into account the increased respiratory volume at the workplace (see List of MAK- and BAT Values, Sections Ib and Ic), the MAK value has been lowered to 100 ml/m^3. Due to the half-life for methanol of 1.4 hours in humans, no accumulation of methanol is expected during the work week. Since a systemic effect is critical, Peak Limitation Category II is retained. As the half-life in humans is 1.4 hours, the excursion factor has been set to 2. No irritation was observed in volunteers at 200 ml/m^3, the permissible peak concentration. Taking into consideration the data for methanol and the metabolite formate, damage to the embryo and foetus is unlikely when the MAK value for methanol is not exceeded. Therefore, methanol remains classified in Pregnancy Risk Group C. Methanol is not genotoxic in vitro at concentrations which are not cytotoxic. No clastogenic effects were observed in vivo. No increased tumour incidence occurred in long-term inhalation studies in mice and rats as well as in a long-term study in rats with administration in the drinking water. Uptake via the skin can lead to systemic effects and methanol remains designated with “H”.

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MAK value (2018)	**100 ml/m³ (ppm) ≙ 130 mg/m³**
Peak limitation (2018)	**Category II, excursion factor 2**
Absorption through the skin (1969)	**H**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (1995)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value (2018)	**15 mg/l urine**
CAS number	67-56-1
1 ml/m³ (ppm) ≙ 1.33 mg/m³	**1 mg/m³ ≙ 0.752 ml/m³ (ppm)**

For methanol, an assessment of its developmental toxicity from 1995, documentation from 1999 assessing all end points (Greim 2001), and a supplement on peak limitation from 2002 (Greim 2002, available in German only) have already been published.

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 or vapour with a blood:air partition coefficient < 5 (see List of MAK and BAT Values, Sections Ib and Ic). The blood:air partition coefficients determined for methanol are 1349 and 1517 (Greim 2000). This supplement evaluates whether the MAK value and the pregnancy risk group need to be changed as a result of the higher respiratory volume at the workplace. Furthermore, new data for the toxicity of methanol after repeated exposure, for its carcinogenicity and reproductive toxicity are included. In addition, the data for germ cell mutagenicity are evaluated. New data for sensitization are not available.

At the same time as the re-evaluation of the MAK value, a re-evaluation of the BAT value was carried out (Kreis et al. 2021).

Toxicokinetics and Metabolism

Data for the toxicokinetics and metabolism of methanol were provided in detail in the documentation of 1995 and 1999 (Greim 2001).

Absorption, distribution, elimination

Methanol is absorbed well orally (100%; Pollack and Brouwer 1996), via inhalation (50%; Ernstgård et al. 2005) and through the skin, and is distributed evenly in all organs and tissues of the body in a direct relationship to their water content.

In rats, absorption takes place after inhalation mainly in the upper respiratory tract and depends on the concentration, the exposure duration and the respiratory rate of the animals. The blood methanol concentration has no effect on absorption, unlike the respiration rate, which decreases with the increasing blood methanol concentration. Details of the study can be found in DECOS (2010). During inhalation, due to the good solubility of methanol in water, a wash-

in, wash-out effect takes place; that is, during inspiration, part of the methanol is adsorbed by or dissolves into the respiratory tract and is desorbed during expiration (DECOS 2010). This evidently results in the fact that the systemic availability is not proportional to the respiration rate.

After the exposure of both hands and forearms (about 2000 cm^2) for 1 hour, the percutaneous uptake of 16 200 mg methanol was determined (Greim 2001). In an in vitro study with human skin, the uptake of 12 690 mg over 1 hour was observed (Korinth et al. 2012).

Humans

In healthy humans, the background concentration of methanol is between 0.25 and 5.2 mg/l blood (US EPA 2013).

The absorption, distribution and elimination of methanol was investigated in 4 male and 4 female volunteers after exposure to 0, 100 or 200 ml/m^3 during physical exercise (2 hours at 50 watts on a bicycle ergometer) for 2 hours. At different times during the exposure and up to 23 hours after the start, samples of blood, saliva, urine and exhaled air were taken. The background levels of methanol were 9–76 µM in the blood (0.3–2.4 mg/l), 4–76 µM in the saliva, 13–86 µM in the urine and 0.0005–0.01 µM in the exhaled air. The background levels of methanol in urine and saliva were higher in men than in women. The amount of methanol absorbed after inhalation was approximately 50% at both exposure concentrations. The concentration of methanol in the blood increased to 116 and 244 µM (3.7 and 7.8 mg methanol/l) after exposure for 2 hours to 100 and 200 ml methanol/m^3 during physical exercise, respectively. The calculated steady state concentrations were 186 µM (6 mg/l) and 393 µM (12.6 mg/l), respectively. According to further calculations, the steady state concentration of methanol during physical exercise, that is, after about 8 hours exposure, is nearly twice as high as after 2 hours. The area under the concentration/time curve (AUC) of 0 to 6 hours for methanol in the blood increased in linear fashion with the exposure concentration. This applied also to the AUC of methanol in urine, saliva and exhaled air after termination of the exposure, which suggests non-saturated first-order kinetics in this concentration range. The half-life of methanol in the blood was 1.4 hours. The excretion of formic acid per minute was increased markedly, but not statistically significantly. However, there was no decrease (sometimes even an increase) of the pH of the urine (Ernstgård et al. 2005).

In numerous studies with volunteers, the concentrations of methanol in the blood or of formate in the serum after exposure to 100 to 800 ml/m^3 for up to 8 hours were determined. The results are shown in Table 1.

Tab. 1 Concentrations of methanol and formate in blood or serum after inhalation by volunteers

Concentration [ml/m^3]	Exposure/respiratory volume	Duration [h]	Methanol [mg/l blood]	Formate [mg/l serum]	References
100	50 watts physical exercise	control 2	0.64 (venous) 3.72 (capillary blood)	not determined	Ernstgård et al. 2005
191	at rest	control 1.25	0.55 ± 0.31 (not specified) 1.88 ± 0.47 (not specified)	3.8 3.6	Cook et al. 1991; Greim 2001
200	50 watts physical exercise	control 2	0.64 (venous) 7.91 (capillary blood)	not determined	Ernstgård et al. 2005
200	at rest	control 4	0.9 ± 0.6 (serum) 6.5 ± 2.7 (serum)	12.7 ± 6.4 14.3 ± 8.9	d'Alessandro et al. 1994; Chuwers et al. 1995; Greim 2001; Osterloh et al. 1996
200	at rest: 10 l/min	control 6	1.8 ± 1.2 (venous) 7.0 ± 1.2 (venous)	9.0 ± 1.3 8.7 ± 2.4	Greim 2001; Lee et al. 1992
	exposure: 18.6 l/min	control 6	1.9 ± 0.9 (venous) 8.1 ± 1.5 (venous)	8.8 ± 1.8 9.5 ± 1.0	
400	at rest	control 8	2.65 ± 1.8 (not specified) 13.4 ± 4.8 (not specified)	not determined	Franzblau et al. 1995; Greim 2001

Tab. 1 *(continued)*

Concentration [ml/m³]	Exposure/respiratory volume	Duration [h]	Methanol [mg/l blood]	Formate [mg/l serum]	References
800	at rest	control	1.8 ± 0.7 (venous)	not determined	Batterman et al. 1998
		0.5	5.3 ± 1.4 (venous)		
		1	6.6 ± 1.2 (venous)		
		2	14.0 ± 1.5 (venous)		
		8	30.7 ± 6.9 (venous)		

Dependence of the methanol concentration in the blood on the respiratory activity and exposure duration

From comparisons with other toxicokinetic studies, it can be concluded that physical exercise at 50 watts, corresponding to an approximate doubling of the respiratory volume per minute compared with that under resting conditions, results also in a doubling of the methanol concentration in the blood (Ernstgård et al. 2005).

After continuous exposure for 2 and 8 hours, 68% and 98%, respectively, of the steady state level of the methanol concentration in the blood was attained (Batterman et al. 1998).

As shown in Table 1, the methanol concentration in the blood increases after 8-hour exposure to 800 ml/m³ at rest by a factor of 2 compared with that found after 2-hour exposure at rest (14 and 30.7 mg methanol/l, respectively; Batterman et al. 1998). According to the calculations by Ernstgård et al. (2005), during physical exercise, the methanol steady state concentration, that is, after exposure for about 8 hours, is also about twice as high as after 2 hours (see above). The steady state concentration of 6 mg methanol/l blood after exposure to 100 ml/m³ during physical exercise (Ernstgård et al. 2005) corresponds to about that at which no behavioural effects were observed (6.5 mg methanol/l blood, see Tables 1 *and* 2; Chuwers et al. 1995) in volunteers.

Monkey

The daily 2.5-hour exposure of female cynomolgus monkeys to 200, 600 or 1800 ml methanol/m³ on 7 days per week before and during pregnancy and of the dams after parturition resulted in concentrations of about 5, 10 or 35 mg methanol/l blood. About 3 mg/l was determined in the control group or prior to exposure. Methanol was passed into the inhalation chambers for 2 hours, following which the animals remained in the chambers for about 30 minutes. Four minutes after the onset of the methanol flow, the concentrations in the chambers were between 60% and 70% of the target concentration. Four minutes following the end of the methanol flow there was a decline by over 80% in the chamber concentration. The methanol concentrations in the blood were determined 10 minutes after removal of the animals from the inhalation chambers. As, in this study, the half-life in the blood at both high concentrations was about 80 to 90 minutes (prior to mating), it may be assumed that the blood methanol concentrations were higher during exposure than the given values. The plasma formate concentrations in the controls and in the treated groups were about the same (Burbacher et al. 2004).

Species differences

The blood methanol concentrations (experimental or estimated) given in numerous toxicological data summaries/reviews are at about the same level in rats, monkeys and humans after exposure to up to 1200 ml/m³ for 6 hours (rats: 26.6; monkeys: 37.6; humans about 25 mg/l). At higher methanol concentrations, the blood levels in rats increased in a non-linear fashion, while the gradient was less steep in monkeys and linear in humans. In mice, the blood concentrations increase more steeply, as a result of their more rapid breathing rate and thus higher absorption (DECOS 2010; Greim 2001; NTP 2003).

The distribution volumes of methanol are 0.92, 0.77 and 0.70 l/kg body weight for rats, monkeys and humans, and those of formate 6.4, 4.6 and 4.2 l/kg body weight, respectively. The higher values for formate might be an indication of protein binding (Bouchard et al. 2001; DECOS 2010).

Metabolism

In the liver, methanol is metabolized by a series of oxidation reactions to formaldehyde, formate and CO_2; rodents and primates differ as regards metabolic pathways and the degradation rates. In primates, the formation of formaldehyde takes place via alcohol dehydrogenase, in rodents mainly via the catalase-peroxidase system. The reaction rates of methanol to formate under non-saturated conditions is of a similarly high level in rodents and primates (30 and 48 mg/kg body weight and hour, respectively). In rodents, the catalase reaction is the rate limiting step, with methanol accumulation and simultaneous saturation of the enzymatic system as sequels. In primates, the oxidation of formate to CO_2 is the rate limiting step. Therefore, following the ingestion of larger methanol quantities, formate may accumulate in the blood in humans (≥ 210 mg/kg body weight; DECOS 2010) and monkeys (see DECOS 2010; Greim 2001; OECD 2007; US EPA 2013).

Methanol can also react non-enzymatically with hydroxyl radicals to form formaldehyde (US EPA 2013).

With the aid of a biologically based dynamic model it is possible to estimate that methanol metabolism is saturated at lower concentrations in rats than in monkeys and humans. In rats exposed to 2000 ml methanol/m^3 for 6 hours, a Michaelis-Menten affinity constant (K_M) for methanol metabolism of 36.6 mg/l blood and a maximum metabolic rate (V_{max}) of 19.4 mg/l/h were estimated, whereas no saturation of the methanol metabolism was apparent in monkeys following similar exposure. In humans, after 2-hour exposure to 800 ml/m^3 or 8-hour exposure to 229 ml/m^3, saturation is likewise not to be expected. The rate constants of metabolism of methanol to formaldehyde are 0.53, 0.96 and 0.4 h^{-1}, those of the metabolism of formaldehyde to formate are 14.6, 7.2 and 7.2 h^{-1} in rats, monkeys and humans, respectively. The whole body to exhaled air coefficients combined with the metabolism rate constants of formate to CO_2 are given as 0.32, 0.81 and 0.81 h^{-1} for rats, humans and monkeys, respectively. These values indicate that formate is cleared about half as rapidly in monkeys and humans than in rats (Bouchard et al. 2001).

To clarify the question whether the metabolism in humans or monkeys is more similar to that of rodents or to that of rabbits, male CD-1 mice, New Zealand White rabbits and cynomolgus monkeys received a single intraperitoneal injection of 0, 500 (mice and rabbits only) or 2000 mg methanol/kg body weight. The plasma concentrations of methanol and formate were determined at different times after administration. In the mice, 3 to 6 animals (up to 24 hours after administration), in rabbits (up to 48 hours after administration) and monkeys (only control and high dose; up to 6 hours after administration) only 3 animals were used per time point. It was found that rabbits are more similar to primates than are mice as regards methanol metabolism and formate accumulation. The authors suggest that rabbits represent a better animal model for teratogenicity studies than rodents (Sweeting et al. 2010).

In numerous studies with volunteers, after exposure to 200 ml methanol/m^3 at rest for up to 6 hours, no accumulation of formate in the blood could be determined (see Greim 2001).

Another pharmacokinetic model based on published kinetic data for different animal species and volunteers predicts that 8-hour exposures to 500 to 2000 ml methanol/m^3 without physical activity are needed to increase blood formate concentrations and the urinary formic acid concentrations above the background values (4.9–10.3 mg/l blood and 6.3–13 mg/l urine, respectively) (Bouchard et al. 2001). These estimates are based on a respiratory minute volume of about 10 l/min, so that only 250 to 1000 ml/m^3 are necessary at a level of 10 m^3 (21 l/min) during 8 hours. This assumption has been confirmed in a study with volunteers in which a moderate (2-fold) increase in the urinary formate concentration was attained after 8 hours exposure to 400 ml/m^3 during physical exercise (Franzblau et al. 1997).

Effects in Humans

Single exposures

The studies carried out with volunteers under controlled conditions are shown in Table 2. The studies published after the documentation of 1999 (Greim 2001) are described in detail below.

The volunteers in the study already described in the Section "Toxicokinetics and Metabolism" were questioned before, during and after exposure regarding irritation or effects on the central nervous system, difficulties in breathing and perception of a solvent odour. During exposure to 0, 100 or 200 ml methanol/m³ the symptoms were assessed as slight at the most, and there were no differences between the volunteers in the methanol group and those in the control group. Nevertheless, after exposure to 200 ml/m³ the female volunteers reported three symptoms significantly more often than the males: fatigue, nausea, and headaches or irritation of the throat or airways (the publication does not make clear which of the symptoms – headache or irritation – was significantly different, as the descriptions of symptoms are contradictory in two places) (Ernstgård et al. 2005).

In a study with 12 healthy male volunteers, neurophysiological effects were investigated using the crossover design following exposure to 20 ml/m³ (control) or 200 ml/m³ for 4 hours. The electroencephalogram (EEG) was recorded before (as a baseline reference) and at the end of each exposure with open or closed eyes and recorded during the performance of a colour word stress test. The spectral power was calculated by fast Fourier transformation (FFT). The evaluation of acute symptoms (headache, nausea, dizziness, perception of an unpleasant odour, an unpleasant taste, a sensation of weakness or dizziness, difficulties in breathing, irritation of the skin and mucous membranes) revealed no differences between the two concentration groups. In the evaluations with open eyes and during the colour word stress test, no significant changes were found. With closed eyes the spectral power of the theta-band and of some electrodes of the delta-band was significantly reduced (Muttray et al. 2001). As, in this publication, merely the EEG spectrum is given, its usefulness is thus limited, so that no conclusions on behavioural disorders in the volunteers can be made.

In addition, in the volunteers in the study by Muttray et al. (2001), also the effects on the inflammation parameters interleukin-8 (IL-8), IL-1β, IL-6 and prostaglandin E2 in nasal secretions, on mucociliary transport (saccharin transport time test) and the ciliary beat frequency of nasal epithelial cells were investigated. Exposure to 200 ml/m³ produced a significant increase in IL-8 and IL-1β which, together with the other unchanged parameters, was assessed by the authors as a subclinical inflammatory reaction (Mann et al. 2002). The release of IL-1β, mediated by NF-κB, should result in the increased formation of PGE_2, but this was not observed. In addition, only one single determination was carried out after the exposure, so that a pre–post comparison was not possible. In addition, the concentrations of IL-8 in two volunteers and of IL-1ß in one volunteer deviated markedly from the values in the remaining volunteers (very steep increase after exposure), so that the meaning of this effect is unclear.

Tab. 2 Studies with volunteers after single inhalation exposures

Number of volunteers	Exposure	Study scope	Findings	References
12 ♂ (22–32 years, average 26) healthy non-smokers, no alcohol own control	0, 187 ml/m³ (250 mg/m³) 4 × 75 min (0, 187, 0, 187 ml/m³ or 187, 0, 187, 0 ml/m³) whole-body exposure at rest	before, during and after exposure: a large number of clinical, psychological and physiological end points	most end points unaffected, except: Sternberg reaction time ↑, latency of P200 components of event-related potentials ↑, greater fatigue after exposure, tendency towards poorer concentration and less vigour	Cook et al. 1991; Greim 2001
15 ♂, 11 ♀ (26–51 years, average 35.7) smokers, no alcohol abstinence, own control	0, 200 ml/m³ 4 hours whole-body exposure at rest	methanol and formate concentration in blood and urine, a large number of behavioural-toxicological, neurophysiological and visual performance tests	no noticeable findings in tests for memory, attention, interference, contrast sensitivity and colour discrimination to detect visual function changes, but: P300 amplitude ↓, no learning effect in symbol digit substitution test pre and post-exposure to methanol	Chuwers et al. 1995; Greim 2001

Tab. 2 *(continued)*

Number of volunteers	Exposure	Study scope	Findings	References
12 ♂ (26.8 ± 2.1 years) healthy non-smokers, own control	20 (control exposure), 200 ml/m³ 4 hours whole-body exposure at rest	questionnaire with 17 items on irritation (eyes, nose, throat, skin), breathing difficulties, pre-narcotic symptoms (including headache, nausea, dizziness, perception of an unpleasant odour and taste, feeling of weakness or dizziness), severity scores 0–5; EEG with open or closed eyes and during colour word stress test	no difference in assessment of symptoms, spectral power (EEG) in the theta-band and some electrodes of the delta-band ↓ with closed eyes (indication of weak excitatory effect)	Muttray et al. 2001
		concentrations of interleukin(IL)-8, IL-1β, IL-6, prostaglandin E2 in nasal secretions, mucociliary transport (saccharin transport time test), ciliary beat frequency of nasal epithelial cells	no differences in assessment of symptoms (see Muttray et al. 2001) IL-8, IL-1β ↑	Mann et al. 2002
4 ♂, 4 ♀ (20–50 years)	0, 100, 200 ml/m³ 2 hours during 50 watts physical exercise whole-body exposure	methanol concentration in blood, urine, saliva and exhaled air, formic acid concentration in urine; assessment of irritation (eyes, nose, throat, airways), assessment of CNS symptoms (headache, fatigue, nausea, dizziness, feeling of intoxication), difficulties in breathing, solvent odour immediately before, during (10, 50, 80, 104 min) and after the end (126, 210 min) of exposure	no difference between control and methanol exposure for symptoms cited, ♀ at 200 ml/m³ significantly more frequent reports of irritation of throat or airways, fatigue and nausea or headache compared with ♂ (controls: ♀ significantly more frequent nasal irritation compared with ♂)	Ernstgård et al. 2005
6 ♂ (29–55 years)	200 ml/m³ 6 hours without and with 50 watts physical exercise whole-body exposure	methanol and formate concentration in blood, pulmonary ventilation, respiratory and cardiac frequency, respiratory minute volume	no irritation of eyes, no headaches, nausea or other symptoms during exposure	Greim 2001; Lee et al. 1992

According to a personal communication, the volunteers in the toxicokinetic studies by Franzblau et al. (1995) and Batterman et al. (1998) did not report any neurotoxic symptoms (Franzblau 2018).

Conclusion: In the studies in which the effects of methanol were investigated using established behavioural toxicity tests (Chuwers et al. 1995; Cook et al. 1991), no effects were found at the concentration of 200 ml/m³. Clear irritation could likewise not be found at this concentration. The electrophysiological effects (prolonged latency of the P200 component of event-related potentials, reduced P300 amplitude) cannot be interpreted conclusively due to the methodological approach. The subjective symptoms described in the questionnaires (Cook et al. 1991) are difficult to categorize properly, as the extent of the symptoms was not quantified, and in other studies either none were reported or they were not significantly changed (Ernstgård et al. 2005; Lee et al. 1992; Muttray et al. 2001). Summarizing the available studies with volunteers carried out with small groups of young, healthy volunteers, it can be concluded that 200 ml methanol/m³ is a NOAEC (no observed adverse effect concentration) for acute neurotoxic effects and subjective irritation of the mucous membranes.

Reproductive and developmental toxicity

No data for the effects of methanol on human fertility are available.

An epidemiological study reported by the NTP (2003) in summary form investigated whether there was any relationship between the occurrences of cleft lips, orofacial clefts or cleft palates in the offspring and the occupational

activities of the women during the first trimester of pregnancy and related exposure to a large number of substances including methanol. In the authors' opinion there is no relationship between the exposure of the women to methanol during pregnancy and the occurrence of cleft lips, orofacial clefts or cleft palates in the offspring. Shortcomings of this study include the small number of women exposed to methanol, the lack of individual determinations of exposure, and confounding exposure to other chemicals (NTP 2003).

Numerous studies dealing with the relationship between the intake of multivitamins containing folic acid prior to siring and malformations in the offspring (for example neural tube defects) arrived at the conclusion that folic acid deficiency in humans is capable of enhancing such damage. Women with a reduced folic acid status are for this reason possibly more sensitive to the developmental toxicity of methanol than women with a higher folic acid status (NTP 2003).

Animal Experiments and in vitro Studies

Acute toxicity

RD_{50} values of 33 649 or 55 214 mg/m³ (25 300 or 41 520 ml/m³) were reported; the values were obtained with different mouse strains and methods (DECOS 2010).

Subacute, subchronic and chronic toxicity

Inhalation

In the documentation of 1999 (Greim 2001) the results of a 24-month inhalation study in F344 rats and an 18-month inhalation study in B6C3F1 mice (NEDO 1987) could not be fully evaluated, as the available summary reports were considered to be inadequate. In the meantime, a translation into English of the original study reports has been made available to the Commission. The results of these studies are shown in Table 3. Exposure of the animals was to concentrations of 0, 10, 100 or 1000 ml methanol/m³ for 19 hours daily on 7 days per week (Cruzan 2009; NEDO 1985 a, b). Table 3 also includes the inhalation studies described in the documentation of 1999 (Greim 2001).

Tab. 3 Effects of methanol after repeated inhalation exposure

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 5 ♂, 5 ♀	**28 days,** 6 hours/day, 5 days/week, 0, 500, 2000, 5000 ml/m³	**500 ml/m³ and above:** nasal discharge ↑ in a concentration-dependent manner, results of gross-pathological, histological and ophthalmological examinations normal	Andrews et al. 1987; Greim 2001
rat, Sprague Dawley, 4 ♂, 4 ♀	**6 weeks,** 6 hours/day, 5 days/week, 0, 200, 2000, 10 000 ml/m³	**up to 10 000 ml/m³:** histological and biochemical parameters (surfactant, proteins, DNA, enzymes) in lungs not changed (other organs not examined)	Greim 2001; White et al. 1983
rat, Sprague Dawley, 52 ♂, 52 ♀	**104 weeks,** 19.5 hours/day, 7 days/week, 0, 10, 100, 1000 ml/m³	**100 ml/m³ and above:** ♀: urinary bilirubin ↑ **1000 ml/m³:** ♀: urinary pH ↓; ♂: feed consumption ↓ in weeks 30–52, urinary glucose ↑	NEDO 1985 b
mouse, B6C3F1, 52 ♂, 53 ♀	**78 weeks,** 19.1 hours/day, 7 days/week, 0, 10, 100, 1000 ml/m³	**1000 ml/m³:** ♂: absolute and relative testis weights ↓, ♀: feed consumption (months 7–12) ↓, absolute kidney weights ↑	NEDO 1985 a
monkey, cynomolgus, 3 ♂, 3 ♀	**4 weeks,** 6 hours/day, 5 days/week, 0, 500, 2000, 5000 ml/m³	**up to 5000 ml/m³:** no effects on body weights, organ weights, no histopathological or ophthalmological data	Andrews et al. 1987; Greim 2001

Tab. 3 *(continued)*

Species, strain, number per group	Exposure	Findings	References
monkey, cynomolgus, 3, sex not specified	**29 months,** 22 hours/day, 7 days/week, 0, 10, 100, 1000 ml/m³	**10 ml/m³ and above:** slight effects on the central nervous system **inadequate documentation of effects**	Greim 2001; NEDO 1987

No evidence of systemic toxicity was found in different species up to the respective highest concentrations tested of 5000 to 10 000 ml/m³. Also in a study in dogs dating from 1944, in which the animals were exposed to about 500 ml/m³ eight hours daily for 379 days, no adverse effects were observed in the clinico-chemical, histological and ophthalmological examinations. In a study with cynomolgus monkeys exposed to concentrations of 10, 100 or 1000 ml/m³ for 22 hours daily for 29 months, slight effects on the central nervous system were found even at 10 ml/m³. Due to inadequate documentation of the effects, this study could not be used, however, for the assessment of toxicity after repeated exposure (Greim 2001; NEDO 1987). It is also not clear whether a concurrent control was used, as no control group is mentioned in the table listing neurological effects in the animals. Only very few animals per concentration were used, with examinations after 7 (2 animals per group), 19 (3 animals per group) and 26 to 29 months (3 animals per group). The sex of the monkeys was also not specified.

Oral administration

Methanol is a metabolite of the sweetener aspartame. Acute or 14-day oral administration of up to 1000 mg aspartame/kg body weight did not induce significant behavioural toxicity in male F344 rats (Magnuson et al. 2007). From each mole aspartame, one mole methanol is cleaved (Humphries et al. 2008). A dose of 1000 mg aspartame/kg body weight is metabolized to 3.4 mmol methanol/kg body weight. The maximum methanol concentration in the blood is 3.4 mM or 109 mg/l (ten Berge 2018). As it is not known whether humans and rats are equally sensitive to the behavioural toxicity of methanol, a NOAEL (no observed adverse effect level) for humans cannot be derived from the NOAEL for rats.

The studies in rodents cannot be used to derive a MAK value due to the differences in metabolism.

Reproductive and developmental toxicity

Fertility

In a 2-generation study in Sprague Dawley rats, no effects on the reproduction parameters examined in the F0 and F1 generations were found up to a concentration of 1000 ml/m³. In male rats, the concentrations of sex hormones were not affected after exposure to 5000 ml/m³ for 6 hours or to 200 ml/m³ for 7 days (6 hours/day). Exposure to 200 ml/m³ for 13 weeks had no effect on testis weights in this species and histopathology did not reveal testicular effects in rats after exposure to 800 ml/m³. In mice given oral methanol doses of 1000 mg/kg body weight, daily for 5 days, no morphologically altered sperm were detected (Greim 2001).

Groups of 11 to 12 female cynomolgus monkeys from two cohorts were exposed for 2.5 hours daily to methanol concentrations of 0, 200, 600 or 1800 ml/m³. Exposure lasted for about 350 days: before and during mating (about 120 and 65 days, respectively) and during pregnancy (about 163 days). The offspring were examined regularly for growth and end points related to behavioural toxicity during the first nine months after birth. The results of this study are shown in Table 4. Neither the menstrual cycle nor the ability to conceive were affected by exposure to methanol. Although the incidence of complications during pregnancy (for example, vaginal bleeding) and birth (for example, prolonged unproductive labour) was higher in the groups exposed to methanol, there was no statistically significant difference between this and the incidence in the controls. The duration of pregnancy in the dams exposed to methanol was shorter by six to eight days, but was not concentration-dependent (see Table 4). The authors suggested that the reason for this could be the participation of the foetal hypothalamus/pituitary/adrenal cortex axis, as this determines the length of pregnancy in most species (Burbacher et al. 1999, 2004). However, as this effect was not concentration-dependent, it

is not considered to be relevant for the evaluation. Consequently, from this study, a NOAEC for fertility and maternal toxicity of 1800 ml/m³ would be obtained. It must additionally be taken into account that, with a treatment duration of 2.5 hours per day, the steady-state was not attained at any time given that the half-life for methanol is 80 to 90 minutes.

Tab. 4 Inhalation study of reproductive toxicity with cynomolgus monkeys

Species, strain, number per group	Exposure	Findings	References
monkey cynomolgus 11–12 ♀ (2 cohorts with 24/cohort)	**one-generation study,** 0, 200, 600, 1800 ml methanol/m³ about 350 days (before and during mating (about 120 and 65 days) and during pregnancy (about 163 days)) 2.5 hours/day, 7 days/week, testing up to 9 months after birth prenatal and postnatal growth, health status of newborn offspring, reactions in the behaviour of the newborn, visual, sensory motor, cognitive development, development of social behaviour	**200 ml/m³ and above:** dams: pregnancy duration shortened by 6–8 days, not concentration-dependent; 5 × caesarean section due to complications (vaginal bleeding, prolonged unproductive labour), not concentration-dependent (0 in controls); blood methanol concentration ↑ (0, 200, 600, 1800 ml/m³: 3, 5, 10 and 35 mg methanol/l); offspring: ♂, ♀: visual recognition memory ↓ (62%, 53%, 49%, 57%, n = 7, 9, 8, 9 – controls, increasing concentrations); ♂: sensorimotor development ↓ (target attained at the age of 24, 32, 43, 41 days, n = 3, 5, 3, 2 – controls, increasing concentrations); **600 ml/m³:** foetuses: 1 × stillbirth (1 × controls, 1 × 600 ml/m³); **up to 1800 ml/m³:** dams: no effects on body weights and clinical parameters, plasma formate concentration unaffected, no effects on menstruation cycle, conception rate, number of live-born; offspring: growth, weight at birth, crown-rump length, head circumference, head length and width and other parameters (heart-beat and respiration rate, muscle tone, activity, colour, temperature) unaffected; **1800 ml/m³:** foetuses: 1 hydrocephalus (in utero †); offspring: wasting syndrome (2 ♀, at the age of 12 months – growth delay, severe malnutrition, gastroenteritis); Comment by the Commission: the steady state was not attained as the treatment duration was only 2.5 hours/day and the half-life of methanol is 80–90 min; only a low number of offspring, absence of historical controls; the study is not suitable as a basis for the assessment of developmental (neuro)toxic effects	Burbacher et al. 1999, 2004

Developmental toxicity

Prenatal developmental toxicity

Inhalation

The prenatal toxicity of methanol after inhalation and ingestion has been demonstrated in rodents. The studies discussed below were already described in detail in the documentation of 1995 and 1999 (combined in one translation: Greim 2001) and are only briefly summarized (see Table 5). These are studies with inhalation exposure and, as such, relevant for exposure at the workplace.

In Sprague Dawley rats, the NOAEC for developmental toxicity after **7-hour inhalation exposure per day** from gestation days 1 to 19 was 5000 ml/m³. At 10 000 ml/m³, the body weights of the foetuses were reduced, without any visible effects in the dams. When the dams were exposed to 20 000 ml/m³ from gestation days 7 to 15, malformations (additional cervical ribs, defects of the cardiovascular system and urinary tract, encephalocele, exencephaly) occurred, and the dams displayed an unsteady gait. The maternal methanol concentrations in the blood were 1580 mg/l at 5000 ml/m³ (NOAEC for developmental toxicity), and 2040 mg/l at the LOAEC (lowest observed adverse effect concentration) of 10 000 ml/m³ (Nelson et al. 1985, 1990).

Following **continuous** inhalation exposure from gestation days 7 to 17, maternally toxic effects occurred at 5000 ml/m³ in Sprague Dawley rats. A reduced number of live foetuses and reduced body weights of the foetuses were found on postnatal day 4, skeletal and visceral malformations were observed, and postnatal development was delayed in

the newborn as regards incisor eruption and eye-opening. Furthermore, in the male offspring, *descensus testis* was early and decreased absolute and relative brain, thyroid, thymus and testis weights as well as increased absolute and relative pituitary weights were observed at the age of 8 weeks. In the female animals, only the absolute brain weights were decreased. No histological changes were found. After further mating of the offspring, no effects on fertility or developmental toxicity were observed. In this study, the NOAEC for developmental toxicity was 1000 ml/m³ (Greim 2001; NEDO 1987). The methanol concentrations in the blood were not determined in this study. After continuous daily 20-hour exposure, which is not relevant for the workplace, initial developmental toxicity was found at 5000 ml/m³, whereas in the study by Nelson et al. (1985) with a 7-hour workplace-relevant exposure, the concentration of 5000 ml/m³ was the NOAEC for developmental toxicity.

After inhalation exposure to concentrations of 0, 1000, 2000, 5000, 7500, 10 000 or 15 000 ml/m³ for 7 hours daily from gestation days 6 to 15, the NOAEC for developmental toxicity was 1000 ml/m³ in CD-1 mice. At this concentration the mean methanol concentration in the blood was about 97 mg/l (mean values of gestation days 6 and 10 according to US EPA (2013). At the next-higher concentration of 2000 ml/m³, an increased incidence of additional cervical ribs was found. Teratogenic effects (cleft palates, defects of the sternum and the internal organs) occurred at and above 5000 ml/m³. The methanol concentrations in the blood of the dams were about 537 mg/l at 2000 mg/m³ and 1650 mg/l at 5000 mg/m³. No effects were found in the dams up to the highest concentration tested of 15 000 ml/m³ (Rogers et al. 1991, 1993; US EPA 2013). It could be demonstrated that methanol itself and not its metabolite formate is responsible for the teratogenic effect (Greim 2001).

The higher sensitivity of mice compared with that of rats can possibly be explained by the accumulation of methanol in the blood of mice due to their higher respiration rate and the greater amount thus absorbed (OECD 2007).

Tab. 5 Studies in rats and mice relevant for the assessment of developmental toxicity

Concentration [ml/m³]	NOAEC/LOAEC and findings	Methanol in blood of the dams [mg/l]	References
	SD rat, GD 1–19, 7 hours/day, whole-body **0, 5000, 10 000 ml/m³**		Nelson et al. 1985, 1990
5000	**NOAEC developmental toxicity**	1580	
10 000	**NOAEC maternal toxicity** foetuses: body weights ↓	2040	
	SD rat, GD 7–15, 7 hours/day, whole-body **0, 20 000 ml/m³**		
20 000	dams: unsteady gait foetuses: malformations (additional cervical ribs, cardiovascular and urinary tract defects, encephalocele, exencephaly)	6950	
	SD rat, GD 7–17, continuous, whole-body **0, 200, 1000, 5000 ml/m³**		NEDO 1987
1000	**NOAEC maternal toxicity and prenatal and postnatal developmental toxicity**	–	
5000	NOAEC developmental neurotoxicity dams: body weights ↓ by 5% from GD 7–20 ↓; mortality, food consumption ↓ foetuses: number of live foetuses ↓; number of late resorptions ↑; body weights ↓, skeletal and visceral malformations, delayed prenatal and postnatal development (incisor eruption, eye opening, early *descensus testis*), ♂: absolute and relative brain, thymus and testis weights ↓, weight of pituitary gland ↑; ♀: absolute brain weights ↓	–	

Tab. 5 *(continued)*

Concentration [ml/m³]	NOAEC/LOAEC and findings	Methanol in blood of the dams [mg/l]	References
	SD rat, 2-generation study, 20 hours/day, continuous, whole-body **0, 10, 100, 1000 ml/m³**		NEDO 1987
0		2.4	
10		3.2	
100	**NOAEC postnatal developmental toxicity**	2.5	
1000	**NOAEC foetotoxicity; NOAEC developmental neurotoxicity** offspring: absolute brain, pituitary, thymus weights ↓, early *descensus testis*	76	
	LE rat, GD 6–PND 21, 6 hours/day, whole-body **0, 4500 ml/m³**		Stern et al. 1996
4500	**no developmental neurotoxicity**	–	
	CD mouse, GD 6–15, 7 hours/day, whole-body **0, 1000, 2000, 5000, 15 000 ml/m³**		Rogers et al. 1991, 1993
0		1.6	
1000	**NOAEC developmental toxicity**	97[a] [63/131][b]	
2000	foetuses: number of cervical ribs ↑	537[a] [487/641][b]	
5000	foetuses: malformations (cleft palates, defects of the sternum and internal organs) ↑	1650[a] [2126/1593][b]	
15 000	**NOAEC maternal toxicity**	7330[a]	

GD: gestation day; PND: postnatal day
[a] mean values from US EPA (2013)
[b] [GD 6/GD 10]

Additional inhalation studies in rodents and also studies with monkeys have been carried out to investigate the developmental toxicity of methanol.

In the study with cynomolgus monkeys described under "Fertility" and in Table 4 in which the animals were exposed for 2.5 hours daily for about a year, methanol concentrations of up to 1800 ml/m³ did not produce any clear signs of systemic toxicity in the dams. The shortened duration of pregnancy had no effect on the size of the offspring. No effects on mortality in the foetuses or malformations were found; 34 of the offspring of 37 pregnant animals survived, 2 were stillborn (1 × controls, 1 × 600 ml/m³). In the high concentration group, one case of hydrocephalus was found in a foetus that had died in utero. At the age of 12 months, in 2 female offspring of the same treatment group, a wasting syndrome, characterized by growth delay, was diagnosed. In these animals, necropsy revealed severe malnutrition and gastroenteritis. In Burbacher et al. (1999), the authors were only able to attribute the wasting syndrome to methanol with difficulty, as the number of animals was limited and no historical control data on this effect exist; in contrast, in Burbacher et al. (2004), the effect is considered to be substance-related. Also for the remaining parameters no historical control data are given. The Commission regards the hydrocephalus that occurred as substance-related, as this malformation is an infrequent spontaneous malformation. The occurrence of the wasting syndrome one year later in 2 offspring can hardly be seen as a sequel of the in utero treatment. As the duration of treatment was only 2.5 hours per day, it may be assumed that, with a half-life of 80 to 90 minutes for methanol, the steady-state was not attained at any time. All in all, the study is not suitable as a basis for assessing the developmental (neuro)toxicity.

Oral administration

In CD-1 mice given gavage doses of methanol of 4000 mg/kg body weight, effects were observed like those seen after the inhalation of a methanol concentration of 10 000 ml/m³, at which comparable blood levels were attained (Greim 2001; Rogers et al. 1993).

Intraperitoneal injection

In new studies with intraperitoneal injection, species differences in sensitivity or modes of action were investigated. After intraperitoneal injection of methanol, developmental toxicity was studied in two strains of mice (C57BL/6J and C3H) and in New Zealand White rabbits, and the contribution of catalase to these effects was investigated in transgenic mice (Sweeting et al. 2011; Sweeting and Wells 2015). A comment on the latter publication pointed out that the effect on the lumbar vertebrae reported in rabbits is a variation and not, as may be understood from the title of the publication, a skeletal defect. There was further criticism that the evaluation was carried out at the foetus level and not at a litter level, the number of litters was small and no data for foetal weights were given. The reported disturbances in ossification more likely represent a developmental delay, which could have been produced by the lower weights. The statement that a direct effect of methanol was involved is regarded critically for this reason (White et al. 2016).

Postnatal developmental toxicity

As already described in the documentation of 1995 and 1999 (combined in one translation: Greim 2001), exposure of the dams to a methanol concentration of 4500 ml/m^3 for 6 hours daily beginning on day 6 of gestation and continuing until postnatal day 21 resulted in slight changes in motor activity of the offspring of Long Evans rats and slightly altered operant conditioning when they reached adulthood (Stern et al. 1996; Weiss et al. 1996).

In a 2-generation study, Sprague Dawley rats were continuously exposed from 8 weeks of age to methanol concentrations of 0, 10, 100 or 1000 ml/m^3. In none of the exposure groups could an effect on the F0 generation be detected. In the F1 and F2 generations, reduced brain weights were found after 8 weeks in the 1000 ml/m^3 group in both sexes without a histopathological correlate. In the male animals, early *descensus testis* was described. In the F2 generation, also reduced thymus and pituitary weights were found. No effects on either the parents or offspring were detected in the 100 ml/m^3 group. The behavioural toxicology tests carried out in the F1 offspring (locomotor activity, learning ability, sensory and motor functions) were not affected by the methanol exposure. At the NOAEC of 1000 ml/m^3 for developmental neurotoxicity, the methanol concentration was 76 mg/l blood (see also Greim 2001; NEDO 1987). In the prenatal developmental toxicity study with continuous inhalation exposure from gestation days 7 to 17 (NEDO 1987), effects on absolute and relative brain weights occurred without histological correlates and early *descensus testis* was not observed until the concentration of 5000 ml/m^3. Up to this concentration, no effects on developmental neurotoxicity were observed. However, the methanol concentrations in the blood were not determined. Due to the steep dose–response relationship in mice and rats it may be assumed that the methanol concentrations in the blood at 5000 ml/m^3 reach far higher levels than at 1000 ml/m^3 in the 2-generation study, and are similar to those found in the study by Nelson et al. (1985) (1580 mg/l blood).

The offspring of female Wistar rats given food with an adequate or reduced folic acid content (FAS or FAD) for 14 to 16 weeks prior to mating were treated with methanol from postnatal days 1 to 21 via the drinking water. Folic acid is necessary as a co-substrate in the oxidation of formate to CO_2. The methanol concentrations in the drinking water of 0%, 1%, 2% and 4% corresponded to daily doses of about 0, 1200, 2400 or 4800 mg/kg body weight (conversion factor 0.12 (for subacute exposure) according to EFSA 2012). In the postnatally exposed offspring, the tetrahydrofolate concentrations in the liver were investigated 21 days after birth, and behavioural and neurochemical parameters were recorded on postnatal day 45. Compared with the levels in the animals of the FAS groups, the tetrahydrofolate concentrations in the liver were reduced by 63% in the FAD dams before mating and by 67% in the FAD offspring on postnatal day 21. The NOAEC for developmental neurotoxicity is 1% (1200 mg/kg body weight and day) in the postnatally treated offspring; at 2% (2400 mg/kg body weight and day) in the FAS animals merely the spontaneous locomotor activity was affected. In the FAD offspring, effects on body weight gains and changes in numerous behavioural and neurochemical parameters (increased locomotor activity, reduced conditioned avoidance response; reduced striatal dopamine concentration, increased expression of Growth-Associated Protein (GAP-43) in the hippocampus) were already found at this dose level. The two latter effects occurred also in the FAS animals of the high dose group. This study shows that methanol exposure during the growth period adversely affects the developing brain, and that folic acid deficiency presumably plays a role in methanol-induced neurotoxicity (Aziz et al. 2002). As the treatment of the offspring began only at birth and not prenatally, this study is not suitable for evaluating effects on developmental (neuro)toxicity at the workplace.

Developmental neurotoxicity

In rats, the NOAEC for developmental neurotoxicity after continuous exposure (20 hours/day) in the prenatal developmental toxicity study was the highest concentration tested of 5000 ml/m^3 (Greim 2001; NEDO 1987). In this study, methanol concentrations in the blood were not determined. In the 2-generation study by the same laboratory, also with continuous exposure for 20 hours per day, no effects on developmental neurotoxicity were observed up to the highest concentration tested of 1000 ml/m^3, and the methanol concentrations were 76 mg/l blood (NEDO 1987). As already stated, it can be assumed that the methanol concentrations in the blood at 5000 ml/m^3 had attained far higher levels than those in the 2-generation study at 1000 ml/m^3.

In the study with cynomolgus monkeys exposed for 2.5 hours daily for about a year described in the Section "Fertility", the offspring were subjected to numerous developmental neurotoxicity tests regarding neonatal behaviour, early reflexes, development of motor activity in early life, spatial memory and social behaviour up to the age of nine months (see Table 4). Only two tests revealed effects of methanol; these were a delay in early sensorimotor development in the male offspring of all groups and a reduction in visual recognition memory in the offspring of both sexes. In the evaluation, especially of the effects on the male offspring, the small group size of 2 to 5 animals must be taken into consideration. No effects from methanol exposure were found in the remaining neuropsychological tests (early reflex responses, gross motor development, spatial and concept learning, memory, social behaviour). The authors themselves advise cautious interpretation of these effects, as only a small number of offspring was examined, and, due to the wide interindividual variability, statistical significance was achieved only in one test, but not in the other tests, and because no effects were observed in all other developmental neurotoxicity tests (Burbacher et al. 1999, 2004). The Commission agrees with this and additionally points out the absence of dose-dependency as well as the large number of similar tests with negative results, some of which covering the same end points. Therefore, these findings are not considered an indication of developmental neurotoxicity. As the treatment duration was only 2.5 hours per day, it can be assumed, as already stated, that the steady state was not attained at any time. The study is thus not suitable as a basis for the assessment of a developmental (neuro)toxic effect.

Conclusion

Due to the described methodological shortcomings, the study in cynomolgus monkeys is not suitable for evaluating developmental (neuro)toxicity. Mice are more sensitive to the developmental toxicity of methanol than rats. Embryotoxicity was found in rats after **continuous** inhalation exposure at maternally toxic concentrations of 5000 ml methanol/m^3 and above and after **exposure for 7 hours per day** at 10 000 ml/m^3 and above without maternal toxicity. In mice, embryotoxicity was observed at maternally non-toxic concentrations of 2000 ml/m^3 and above. After the exposure of rats for 7 hours a day, the maternal blood methanol concentrations were 1580 mg/l at 5000 ml/m^3 (NOAEC for developmental toxicity), and 2040 mg/l at the LOAEC of 10 000 ml/m^3 (Nelson et al. 1985, 1990). In CD-1 mice these were 97 mg/l at 1000 ml/m^3 (NOAEC for developmental toxicity), and about 537 mg/l at 2000 ml/m^3 (Rogers et al. 1993). After continuous exposure (20 hours daily) from gestation days 7 to 17, the NOAEC was 1000 ml/m^3 for developmental toxicity and 5000 ml/m^3 for developmental neurotoxicity. Methanol concentrations in the blood were not determined in this study. In a 2-generation study by the same laboratory with continuous exposure, methanol concentrations in the blood of 76 mg/l were obtained at 1000 ml/m^3, which was the NOAEC for developmental neurotoxicity (NEDO 1987).

In vitro studies

The available in vitro studies have been described in detail in several toxicological summaries (DECOS 2010; RAC 2014). To summarize, the prenatal developmental toxicity in rodents has been confirmed in cultured rat and mouse embryos treated with methanol. In a study with mouse embryos from different murine strains, including those that express human catalase, or those in which the catalase is not expressed, it could be demonstrated that reactive oxygen species participate in methanol-induced malformations, that the activity of embryonic catalase in mice correlates inversely with the developmental toxicity of methanol, and that mouse embryos expressing human catalase are protected against teratogenic effects, although a number of significant effects on growth were observed (RAC 2014).

Genotoxicity

As described in the documentation of 1999 (Greim 2001), methanol was not found to possess genotoxic potential in the in vitro and in vivo studies relevant for the evaluation. A new study of oxidative DNA damage (McCallum et al. 2011) has confirmed these findings.

In vitro, methanol was mutagenic in bacteria or aneugenic in fungi only at cytotoxic concentrations. Also, the result of a test for sister chromatid exchange in CHL cells (a cell line derived from Chinese hamster lung) was positive only at cytotoxic concentrations, whereas concentrations in the non-cytotoxic range induced neither sister chromatid exchange nor chromosomal aberrations in mammalian cells (Greim 2001). In 8-oxoguanine glycosylase 1 knockout mouse embryo fibroblasts, there was no accumulation of 8-oxodeoxyguanosine (McCallum et al. 2011). A number of mutation tests with V79 cells yielded negative results as did a $TK^{+/-}$ test with the addition of the normal S9 mix concentration. However, when the concentration of S9 mix was increased, the test result was positive (Greim 2001). All in all, methanol was found to be neither mutagenic nor clastogenic at non-cytotoxic concentrations in vitro.

In vivo, no sex-linked recessive lethal mutations were induced in Drosophila melanogaster (Greim 2001). After intraperitoneal injection, oxidative DNA damage occurred neither in the bone marrow nor in the spleen of mice, rabbits or monkeys. This was found after intraperitoneal injection of male CD-1 mice (n = 4), New Zealand White rabbits (n = 3) or cynomolgus monkeys (n = 3) with 2000 mg methanol/kg body weight. Single doses did not produce an increased concentration of 8-oxodeoxyguanosine, a marker of oxidative DNA damage, in the bone marrow and spleen in any of these species. The examination was carried out 6 hours after dosing, in the CD-1 mice in addition 24 hours after treatment. The 15-day treatment of male CD-1 mice likewise did not increase 8-oxodeoxyguanosine concentrations in the bone marrow and spleen. In knockout mice, which are unable to express oxoguanine glycosylase 1, an important enzyme in DNA repair, 8-oxodeoxyguanosine accumulated with increasing age in the bone marrow and spleen but not, however, after treatment with methanol. In the monkeys, free radical-mediated hydroxynonenal-histidine protein adducts were not enhanced by methanol in the spleen and bone marrow. The same results were obtained also in the bone marrow of New Zealand White rabbits and in the spleen of CD-1 mice. On the other hand, a slight increase was observed in the spleen of treated rabbits and in the bone marrow of treated mice (McCallum et al. 2011).

In mice, no sister chromatid exchanges, chromosomal aberrations or micronuclei were induced after inhalation exposure. Also the results of micronucleus tests with oral or intraperitoneal administration were negative. There are numerous negative clastogenicity test results, but only two tests with positive results. In a study with oral administration, the induction of chromosomal aberrations and micronuclei in the bone marrow was observed. On the other hand, in a second study with oral administration and higher doses than those used in the first, no micronuclei were induced in the same target tissue (Greim 2001). Therefore, methanol is regarded as not clastogenic in vivo.

Carcinogenicity

In the documentation of 1999 (Greim 2001) two 24-month and 18-month inhalation studies in F344 rats and B6C3F1 mice, respectively, were described (NEDO 1987); the translations of the original studies are now available. A detailed description of the studies is given below. Exposure of the rats took place for 19.5 hours daily, on 7 days per week, for 104 weeks. The concentrations were 0, 10, 100 or 1000 ml methanol/m^3 and the exposure groups consisted of 52 animals per sex. Histopathological examination was carried out as a rule in all control animals and the 1000 ml/m^3 group. The kidneys (males and females), lungs (males) and the adrenal glands (females) were examined also in the two lower concentration groups.

No statistically significant increase in tumour incidences was found (see Table 6). No data are given for historical controls of the laboratory regarding adenocarcinomas, adenomas and "adenomatosis" in the lungs (NEDO 1985 b). An external review came to the conclusion that the pathological data involving the effects in the lungs (including an evaluation of the photographs of the histopathological sections which were included with the report of the original study) indicate a proliferative change in the pulmonary alveolar epithelium (Methanol Foundation 2007 b). Methanol

was not found to be carcinogenic in rats although, with a daily exposure of 19.5 hours on all days of the week, an extreme situation had been created.

Tab. 6 Study of the carcinogenicity of methanol after inhalation by F344 rats

Author:		NEDO 1985 b			
Substance:		methanol (99.6%)			
Species:		**rat**, F344/N, groups of 52 ♂, 52 ♀			
Administration route:		inhalation			
Concentration:		0, 10, 100, 1000 ml/m³			
Duration:		104 weeks, 7 days/week, 19.5 hours/day			
Toxicity:		at and above 100 ml/m³: ♀: bilirubin in the urine ↑; 1000 ml/m³: ♀: urine pH ↓; ♂: food consumption ↓ in week 30–52, glucose in the urine ↑			
		Exposure concentration (ml/m³)			
		0	10	100	1000
Survivors	♂	36/52 (69%)	34/52 (65%)	40/52 (77%)	34/52 (65%)
	♀	31/52 (60%)	33/52 (63%)	31/52 (60%)	35/52 (67%)
Tumours and preneoplasms					
Lungs:					
swelling of the alveolar epithelium	♂	3/52 (6%)	2/50 (4%)	1/52 (2%)	1/52 (8%)
	♀	0/52 (0%)	0/19 (0%)	0/20 (0%)	0/52 (0%)
"adenomatosis"	♂	4/52 (8%)	1/50 (2%)	5/52 (10%)	4/52 (8%)
	♀	3/52 (6%)	2/19 (11%)	1/20 (5%)	1/52 (2%)
papillary adenomas	♂	1/52 (2%)	5/50 (10%)	2/52 (4%)	6/52 (12%)
	♀	2/52 (4%)	0/19 (0%)	0/20 (0%)	2/52 (4%)
adenocarcinomas	♂	0/52 (0%)	0/50 (0%)	0/52 (0%)	1/52 (2%)
	♀	0/52 (0%)	0/19 (0%)	0/20 (0%)	0/52 (0%)
Adrenal glands:					
hyperplasia	♂	0/52 (0%)	0/16 (13%)	0/10 (20%)	2/51 (4%)
adrenal cortex	♀	2/50 (4%)	3/51 (6%)	7/49 (14%)	2/51 (4%)
phaeochromocytomas	♂	7/52 (14%)	2/16 (13%)	2/10 (20%)	4/52 (8%)
	♀	2/50 (4%)	3/51 (6%)	2/49 (4%)	7/51 (14%)

The mice were exposed for 19.1 hours daily, on 7 days per week, for 78 weeks. The concentrations were again 0, 10, 100 or 1000 ml methanol/m³. The exposure groups consisted of 52 males and 53 female mice. Histopathological examination was performed in the controls and in the high exposure group. In addition, the liver was examined in all treatment groups. In the mice also, no statistically significant increase in the incidence of neoplasms was found. In the male mice, pulmonary adenomas were diagnosed in 7 of 52 animals exposed to 1000 ml/m³ compared with in 4 of 52 animals in the control group (not statistically significant). In the other groups, only 3 animals per group were examined; in none of them was a corresponding neoplasm found (NEDO 1985 a). In an external review of the pathological data (including an evaluation of the photographs of the histopathological sections included with the original study report), the tumour incidences in the lungs were assessed. As no "adenomatosis" was found, the author does not regard the slight increase in pulmonary adenomas as substance-related (Methanol Foundation 2007 a).

At the highest concentration tested of 1000 ml/m³, the metabolism of methanol to formaldehyde is saturated, so that the maximum tolerated dose (MTD) is attained as a result of the kinetics. The methanol concentration in the blood of these rats was ten times as high as that in the controls (Cruzan 2009; NEDO 1985 a, b).

Another carcinogenicity study was carried out in Sprague Dawley rats given methanol in the drinking water. The concentrations of methanol were 0, 500, 5000 or 20 000 mg/l (calculated doses (see below) of about 0, 55, 542 or 1840 mg/kg body weight and day for the male rats and about 0, 67, 630 or 2250 mg/kg body weight and day for the female rats), the exposure duration was 2 years, the recovery period lasted up to the natural death of the animals. The animals were taken from the laboratory's own breeding colony. In the publication, only the incidences of the observed tumours are given. Data for survival, body weights, food and water consumption, organ weights, methanol concentrations in the blood, clinical parameters or for the histopathological examination of non-neoplastic effects are not reported. In the high dose group, there was a statistically significant increase in the incidences of lymphoimmunoblastic lymphomas mainly in the lungs of female rats, of carcinomas of the ear duct in the males and females and the number of animals with tumours. According to the authors, a statistically significant increase in interstitial hyperplasia and adenomas of the testis and of uterine sarcomas was observed in the high dose group (Soffritti et al. 2002). In the publication, however, incidences of non-neoplastic effects are not given. As the incidences of testicular adenomas are not statistically significant, this statement cannot be verified. Also the statistically significant increase in uterine sarcomas is not verifiable. In the meantime, some additional data were made available to the US EPA by the "European Ramazzini Foundation" for verification. A publication based on these additional data evaluated and described in detail both the studies from Japan (NEDO 1985 a, b) and the study by the Ramazzini Foundation (Soffritti et al. 2002). Based on the body weights and the water consumption at 18 time points during the study, doses of 55, 542 or 1840 mg/kg body weight and day for the male rats, and 67, 630 or 2250 mg/kg body weight and day for the female rats were calculated (average water consumption × methanol concentration / mean body weight). According to the authors, the study design is not in accordance with OECD, US EPA or NTP specifications. For example, the methanol sample used is not characterized, health monitoring was not carried out, the animals were not assigned randomly to their treatment groups, the animals were not sacrificed in extremis, and there was no external examination of the pathological preparations. Doubts were also expressed about whether a concurrent control group had been used. Possibly, a common control group was used in a number of carcinogenicity studies carried out by the Ramazzini Foundation during the same year (Cruzan 2009).

A "Pathology Working Group (PWG)" mutually initiated by the NTP and US EPA investigated the results from a total of five carcinogenicity studies by the Ramazzini Institute, one of which being the methanol study. The PWG agreed with the diagnosis by the Ramazzini Institute for most of the tumour types, with the exception of the lymphomas and leukaemia in the respiratory tract or neoplasms in the inner ear and skull. It was difficult to distinguish between the lymphomas or leukaemia and pulmonary infections, and between ear and skull neoplasms and inflammatory infiltrates. Strain-specific pulmonary infections evidently occurred towards the end of life in the rats used in the studies by the Ramazzini Institute. The repeated evaluation of the data by the PWG did not reveal any treatment-specific increase in lymphomas or leukaemia in rats. Also, lower incidences of these neoplasms were diagnosed. Among other reasons, different categorization schedules were suggested as a cause of the different diagnoses of lymphomas or leukaemia (Gift et al. 2013).

The re-analysis of the inner ear neoplasms likewise failed to reveal any dose-dependent increase (EPL 2011).

Manifesto (MAK value/classification)

The critical effects of methanol are its central nervous effects and developmental toxicity from methanol itself, as well as the acidosis caused by its metabolite formate with the resultant effects in humans, including damage to nerves, especially the optic nerve.

MAK value. The experimental studies in rodents are not used for the derivation of a MAK value due to the differences in metabolism. Studies with volunteers demonstrated that exposure to 200 ml methanol/m^3 at rest for 4 hours does not cause any relevant behavioural effects. Irritation was likewise not observed. Studies with volunteers in which behavioural toxicity was tested using higher concentrations are not available. Behavioural toxicity cannot be excluded at higher concentrations or with increased respiratory activity.

At a concentration of 6.5 mg methanol/l blood (Chuwers et al. 1995) after 4-hour exposure to 200 ml/m³ at rest, no effects in the behavioural tests with volunteers were described. According to the calculations by Ernstgård et al. (2005), the blood methanol concentration after exposure to 100 ml/m³ during physical exercise at 50 watts is 6 mg/l in the steady state, that is after about 8 hours, and therefore corresponds to the concentration in the blood at which no behavioural effects were observed (see above). The half-life of methanol in the blood was calculated to be 1.4 ± 0.3 hours (Ernstgård et al. 2005) so that at this exposure concentration the accumulation of methanol over the working week is not to be expected. Therefore, the MAK value has been lowered to 100 ml/m³.

This value should also protect against the acidosis produced by its metabolite formate. In the following, it is estimated whether there is a reduction in the blood pH after exposure at the level of the MAK value of 100 ml/m³ (133 mg/m³). At a respiratory volume of about 1.25 m³ per hour and 50% absorption, 83 mg methanol (2.6 mmol) is absorbed per hour. Assuming 2.6 mmol of methanol is absorbed per hour and the volume of blood in humans is about 4.5 l, the methanol concentration obtained is 0.57 mmol/l. If it is further assumed that the metabolism of methanol to formic acid is complete and 100% dissociation to formate takes place, 0.57 mmol H^+ ions/l blood reduces the bicarbonate concentration of 24 to 23.4 mmol/l blood. According to the Henderson-Hasselbalch equation (CO_2 concentration in the blood = 1.2 mmol/l, concentration of HCO_3^- = 24 mmol/l, pKs = 6.1) the physiological pH value of 7.4 is changed to 7.39 and is therefore within the physiological variation of the pH in the blood of 7.35 to 7.45 (Jungermann and Möhler 1984). Acidosis is therefore not to be expected at a MAK value of 100 ml/m³.

Peak limitation. As the critical effect is systemic, methanol remains assigned to Peak Limitation Category II. In humans, the half-life of methanol in the blood is 1.4 hours (Ernstgård et al. 2005); according to the procedure of the Commission (see Hartwig and MAK Commission 2017) an excursion factor of 2 has been established. No irritation was observed in volunteers at the permitted peak concentration of 200 ml/m³.

Absorption through the skin. Contact with methanol of both hands and forearms (about 2000 cm²) leads to the percutaneous uptake of 12 690 to 16 200 mg (Section "Absorption, distribution, elimination") in one hour. By comparison, after inhalation exposure to about 100 ml/m³ for 8 hours (130 mg methanol/m³, 10 m³ respiratory volume, 50% retention in the lungs) 650 mg methanol is absorbed. Methanol is therefore designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Prenatal toxicity. Since 1995, methanol has been assigned to Pregnancy Risk Group C with a MAK value of 200 ml/m³ (Greim 2001). As already stated at the time, the developmental toxicity in rodents can evidently be attributed to methanol itself. In primates, the role of formate should not be neglected as the metabolism of methanol differs from that in rodents. Therefore, both aspects must be included when deciding the pregnancy risk group.

Developmental toxicity

At the teratogenic methanol concentrations of 10 000 ml/m³ in the rat and 2000 ml/m³ in the mouse, methanol concentrations of 2040 and 537 mg/l (mean concentration from three determinations), respectively, are attained in the blood. In humans, these blood concentrations result in death or effects on the central nervous system. The NOAEC for prenatal developmental toxicity was 5000 ml/m³ in rats and 1000 ml/m³ in mice after exposure for 7 hours a day from gestation days 1 to 19 (rat) or days 6 to 15 (mouse); the blood methanol concentrations were 1580 mg/l and 97 mg/l, respectively. As the mouse is the more sensitive species, the internal exposure in humans at rest and during physical exercise have to be compared with the blood methanol concentration of the mouse at rest of 97 mg/l at 1000 ml/m³ (Rogers et al. 1993). In humans, the blood methanol concentration after 8-hour exposure to 800 ml/m³ was 30 mg/l (Batterman et al. 1998); at 1000 ml/m³, levels of 37.9 mg/l blood at rest and about 75 mg/l blood after physical exercise (double the value at rest) were calculated. During physical exercise, that is with an increased respiratory volume, humans are therefore, at most, as highly exposed as mice under resting conditions. Accordingly, in this case, the increased respiratory volume is not included when calculating the margin between the external methanol concentration at the NOAEC and the MAK value.

The margin between the blood methanol concentrations at the NOAEC for developmental toxicity in mice and those in humans at the level of the MAK value of 100 ml/m³ (blood methanol concentration 6 mg/l; Ernstgård et al. 2005) is 16-fold (97/6).

After daily inhalation exposure to 5000 ml/m^3 for 7 hours, the blood methanol concentrations in rats and mice are similar. In the case of rats, the margin between the blood methanol concentration at the NOAEC for developmental toxicity after inhalation exposure for 7 hours (1580 mg/l) and that at the MAK value (6 mg/l) is 263-fold (1580/6). Therefore, these margins are sufficiently large.

Developmental neurotoxicity
In two developmental neurotoxicity studies in rats with continuous exposure (20.5 hours/day), no developmental neurotoxicity was found in either the 2-generation study or the prenatal developmental toxicity study up to concentrations of 1000 and 5000 ml/m^3, respectively (NEDO 1987). In the study with prenatal treatment, the blood methanol concentrations in the dams were not determined. The value from the 2-generation study (calculated blood methanol concentration: 76 mg/l at 1000 ml/m^3) is a worst-case estimate, as the possible exposure at the workplace is, as a rule, 8 hours. The margin between the blood methanol concentration at the NOAEC for developmental neurotoxicity in the rat with continuous exposure (76 mg/l) and that at the MAK value (6 mg/l) is 13-fold (76/6) and therefore sufficiently large.

Formate
Since formate does not accumulate in the blood of rodents after exposure to methanol (Kavet and Nauss 1990; Medinsky and Dorman 1994), methanol appears to be the actual teratogen. In primates, as the metabolism of methanol differs from that in rodents, the role of formate cannot be neglected. After the exposure of monkeys to 900 ml methanol/m^3–also with folate deficiency (folate = cosubstrate for formate oxidation)–neither greatly increased methanol nor increased formate concentrations in the blood were found. In volunteers, after 6-hour exposure to 200 ml methanol/m^3 no increase in the formate concentration was observed (Greim 2001). The formate concentration in humans does not increase until exposure to methanol concentrations of 400 ml/m^3 and above. Therefore, when the MAK value of 100 ml/m^3 is observed, embryotoxic effects of methanol are unlikely even in the case of pregnancy-associated folate deficiency resulting from an increased folic acid demand during pregnancy.

Taking developmental toxicity, developmental neurotoxicity and the estimated formate concentrations into account, Pregnancy Risk Group C has been retained for methanol.

Germ cell mutagenicity. Germ cell mutagenicity tests are not available. In vitro, methanol is neither mutagenic nor clastogenic at non-cytotoxic concentrations. In vivo, the substance was not found to have any clastogenic effects. Methanol has therefore not been classified in one of the categories for germ cell mutagens.

Carcinogenicity. In an inhalation study in F344 rats with almost continuous exposure for 104 weeks, a statistically significant increase in tumour incidences was not observed. The data indicate an increase in proliferative changes in the pulmonary alveolar epithelium of male animals. In the female rats, the incidence of phaeochromocytomas in the adrenal glands was slightly increased in the high dose group compared with that in the control group, although this was not statistically significant. An inhalation study in mice with almost continuous exposure over a period of 78 weeks yielded a negative result. The MTD as regards toxic effects was not attained in either study; however, the metabolism of methanol was already saturated at the highest concentration tested of 1000 ml/m^3.

In a drinking water study in Sprague Dawley rats, increased incidences were reported for "lympho-immunoblastic lymphomas" mainly in the lungs of the females, carcinomas in the ear duct of the males and the females and for the number of animals with tumours. However, these incidences could not be confirmed by the Pathology Working Group of the US EPA and the NTP.

All in all, the available studies do not demonstrate a carcinogenic potential for methanol; therefore, the substance is not classified in one of the categories for carcinogens.

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (https://www.dfg.de/en/dfg_profile/statutory_bodies/senate/health_hazards/conflicts_interest/index.html) ensure that the content and conclusions of the publication are strictly science-based.

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