Ethanol

MAK Value Documentation

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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 work place (MAK value) and the Pregnancy Risk Group of ethanol [64-17-5].
Ethanol is produced endogenously. Uptake of greater amounts depresses the central nervous system and is carcinogenic especially for the liver. The former MAK value of 500 ml/m³ was derived from a study in exposed subjects at rest. It was calculated that at 500 ml/m³ the AUC (product of the blood ethanol concentration and time of exposure) was similar to the standard deviation of the lifetime AUC of endogenous ethanol. At 50 W physical activity, which corresponds to a respiratory volume of 10 m³ per day, the blood concentration of ethanol is about twice as high as compared with that of subjects in rest. Therefore, taking into account the increased respiratory volume at the workplace (see List of MAK- and BAT Values, Sections I b and I c), the MAK value is now lowered to 200 ml/m³. Since a systemic effect is critical, Peak Limitation Category II is retained. As irritation was observed at 1900 ml/m³, the excursion factor is now set to 4.
After oral uptake, ethanol causes developmental toxicity. The lowest reported concentration of ethanol in the blood in pregnant rats causing effects in the F1-generation was 300 mg/l with a NOAEC of 70 mg/l. In an inhalation study, the NOAEC for developmental neurotoxicity in rats was 10 000 ml/m³ which according to a PBPK model corresponds to a concentration of ethanol in the blood of about 65 mg/l. These NOAEC are more than 10 times as high as the ethanol concentration of 1 mg/l in humans exposed to 200 ml/m³ even considering the increased respiratory volume at the workplace. Therefore, ethanol remains assigned to Pregnancy Risk Group C.

Keywords

ethanol; ethyl alcohol; toxicokinetics; metabolism; reproductive toxicity; developmental toxicity; peak limitation; prenatal toxicity; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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Ethanol

[64-17-5]

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MAK value (2017)	**200 ml/m³ (ppm) ≙ 380 mg/m³**
Peak limitation (2001)	**Category II, excursion factor 4**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity (1998)	**Category 5**
Prenatal toxicity (1994)	**Pregnancy Risk Group C**
Germ cell mutagenicity (2002)	**Category 5**
BAT value	**–**
1 ml/m³ (ppm) ≙ 1.911 mg/m³	**1 mg/m³ ≙ 0.523 ml/m³ (ppm)**

Documentation for ethanol was published in 1998 (documentation "Ethanol" 1999), followed by a supplement reviewing the peak limitation category in 2001 (supplement "Ethanol" 2010) and a supplement reviewing germ cell mutagenicity in 2002 (supplement "Ethanol" 2010).

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. This applies to gases or vapour with a blood:air partition coefficient greater than 5 (see List of MAK and BAT Values, Sections I b and I c). The blood:air partition coefficient of ethanol is 1265 (documentation "Ethanol" 1999). This supplement evaluates whether the MAK value and the pregnancy risk group for ethanol need to be re-assessed as a result of the higher respiratory volume at the workplace.

Toxicokinetics and metabolism

The data for toxicokinetics and metabolism were already discussed in detail in the documentation published in 1998 (documentation "Ethanol" 1999). Ethanol is read-

ily absorbed, particularly after oral administration and after inhalation exposure. It is absorbed also through the skin. Primarily, ethanol diffuses throughout the aqueous compartments of the body. It is rapidly converted to acetaldehyde in the liver. Ethanol is formed also endogenously. The MAK value was derived from the endogenous ethanol levels (documentation "Ethanol" 1999).

Humans

In a more recent study, 5 male and 5 female non-smokers aged 22 to 32 years were exposed at rest to ethanol concentrations of 125, 250, 500, 750 and 1000 ml/m^3 for 4 hours in an 18 m^3 exposure chamber as soon as the concentration in the chamber was constant. In addition, test persons were exposed to concentrations of 750 ml/m^3 for 4 hours, during which time the subjects were asked to exercise on a cycle ergometer at 50 watts for 12 minutes each hour. Each day of exposure was followed by 7 exposure-free days and the test persons did not drink alcohol for 48 hours before each exposure period. The ethanol concentrations in the blood and exhaled air were determined before the beginning of exposure and after 1, 3, 4, 4.5 and 5 hours. The steady-state concentration in the blood was always reached within 1 hour and increased linearly with the exposure concentration. The values in men and women were not found to differ significantly. One hour after the end of exposure, the ethanol concentration in the blood had almost returned to the level at the beginning of exposure. Blood ethanol concentrations were 2 to 3 times higher during the interim periods of physical activity on the cycle ergometer than they were during periods of rest. The experimental values agreed better with the predicted values if the previously adjusted PBPK (physiologically based pharmacokinetic) model (see documentation "Ethanol" 1999) was refined by adding a further compartment with extra-hepatic metabolism of low capacity and high affinity in richly perfused tissues to the brain, fatty tissue, liver, and well and poorly perfused tissue compartments (Dumas-Campagna et al. 2014). The findings conclusively demonstrate that the body burden of ethanol increases during physical exercise as a result of the increased respiratory volume.

Animals

PBPK models were developed from published data for adult, pregnant and neonatal rats after inhalation exposure and oral and intravenous administration and confirmed based on in vivo studies. The models predicted the concentrations in the blood and tissues in all three stages of life with relatively good accuracy (Martin et al. 2014). Maximum blood ethanol concentrations of 23 and 65 mg/l were calculated for pregnant rats after 6-hour exposure to 5000 and 10 000 ml/m^3, respectively (Beasley et al. 2014). These maximum blood ethanol concentrations were reached within the first 2 hours and remained at the respective level in a steady state. At 21 000 ml/m^3, the blood ethanol concentration only gradually increased, and the maximum blood ethanol concentration of 1920 mg/l was reached only at the end of the 6-hour exposure period. A steady state was not achieved. The respiratory minute volume decreased within the first hour of exposure at the two low concentrations and then remained constant, like the blood ethanol concentration. In contrast, at 21 000 ml/m^3, the respiratory minute volume decreased slowly but steadily, while

the blood ethanol concentration slowly increased (Martin et al. 2014). A saturation of metabolism was suggested as the cause of the large difference between the estimated blood ethanol concentration of 1920 mg/l after exposure to 21 000 ml/m^3 and the very low blood ethanol concentrations of 23 and 65 mg/l after exposure to 5000 and 10 000 ml/m^3, respectively (Beasley et al. 2014).

After comparing a single inhalation exposure (6.33 hours per day, 21 000 ml/m^3) with a single gavage dose (2000 mg/kg body weight) given to rats on gestation day 20, it was found that in both cases the modelled maximum concentrations in the blood and brain of the dams and foetuses were of about the same level. After oral administration, the maximum blood ethanol concentrations were about 2000 mg/l and after inhalation exposure, they were about 1900 mg/l. However, in the case of inhalation exposure, the maximum blood ethanol concentrations were reached only at the end of the 6-hour exposure period, while they were reached within the first hour after bolus administration. After 6-hour inhalation exposure, the blood ethanol concentration decreased more rapidly after the end of exposure than after oral bolus administration (estimated from the figure: basal values were reached after about 1.5 hours and 2 hours, respectively) (Figure 8; Martin et al. 2014).

Effects in Humans

Reproductive and developmental toxicity

There are no controlled inhalation studies available for ethanol at the workplace that recorded exposure data and investigated offspring. As described in the documentation from 1998 (documentation "Ethanol" 1999), prenatal toxicity was induced in humans and animals after oral administration of ethanol.

The "foetal alcohol spectrum disorders" (FASDs) comprise the entire continuum of effects caused by prenatal exposure to ethanol. The "foetal alcohol syndrome" (FAS) refers to the most severe form at the end of the spectrum (Dörrie et al. 2014; Mattson et al. 2011). Qualitatively similar neuropsychological and behavioural disorders occur across the entire spectrum (Mattson et al. 2011). Recognition, motor coordination, attention, language development, executive functions, memory, social perception and emotion processing are impaired to a variable extent. In its most severe form, the disorder is also manifest in structural changes in the cerebrum and cerebellum (Dörrie et al. 2014).

In 2013 it was estimated that 130 to 5400 children with severe FAS are born in Germany each year. This figure was calculated from data published in international publications during the previous ten years that reported a prevalence of 0.2 to 8.2 per 1000 births and an annual number of births in Germany of about 678 000 (Landgraf et al. 2013).

There is extensive evidence that the consumption of ethanol doses of about 6 g/kg body weight and day during pregnancy has a harmful effect on the foetus. However, the effects of small amounts of ethanol of about 1 to 2 g/kg body weight and day are unclear. The data suggests that even small amounts of ethanol may lead to behavioural problems such as hyperactivity (Poon and Leibowitz 2016).

Animal Experiments and in vitro Studies

Developmental toxicity

As reported in the documentation from 1998 (documentation "Ethanol" 1999), prenatal toxicity was induced in humans and animals after oral administration of ethanol. However, it is difficult to reach harmful blood ethanol concentrations by inhalation exposure.

The lowest reported maternal blood concentration at which effects were observed in the offspring of pregnant rats that had been given oral doses of ethanol was 300 mg/l. One-trial learning on a moving platform version of the Morris water maze and activity-dependent potentiation of evoked D-aspartate release from hippocampal slices were diminished. These effects were no longer observed at a blood concentration of 70 mg/l (Savage et al. 2002).

There are a large number of recent publications that studied the effects of oral administration. The following describes only those publications with inhalation exposure relevant to the workplace in which an exposure–effect relationship was observed and the findings in the offspring were clearly caused by ethanol.

These studies are shown in Table 1.

In a study of the toxic effects of ethanol on prenatal development, groups of 18 pregnant Long Evans rats were exposed to concentrations of 0, 5000, 10 000 and 21 000 ml/m^3 from gestation days 9 to 20 for 6.5 hours per day. Behavioural tests were performed on the offspring up to postnatal day 180. The horizontal motor activity of male and female offspring was assessed together, as no statistical differences between the sexes had been determined with respect to the ethanol concentration. Motor activity was slightly increased on postnatal day 62 at 5000 and 21 000 ml/m^3, but not at 10 000 ml/m^3. The grip strength in the hind limbs was decreased on postnatal day 29 after exposure to 5000 and 21 000 ml/m^3, but not after exposure to 10 000 ml/m^3. In contrast, grip strength had increased on postnatal day 62 after exposure to 10 000 ml/m^3, but not after exposure to the other two concentrations. As no systematic concentration–effect relationship could be established, the directional changes observed in the effects across the concentrations varied and the changes in effects at high concentrations were slight, little biological significance is attributed to the changes observed at high concentrations. Blood samples were not taken to keep the exposure conditions in the chambers constant (Beasley et al. 2014). According to a PBPK model, the maximum blood concentration after exposure to an ethanol concentration of 21 000 ml/m^3 is 1920 mg/l (see Section "Toxicokinetics and metabolism"; Martin et al. 2014).

In the male offspring of Long Evans rats exposed to ethanol concentrations up to 21 000 ml/m^3 from gestation days 9 to 20 (whole-body exposure, vapour, 6.5 hours per day), no changes were detected during functional examination of the peripheral, somatosensory, auditory and visual nervous systems (Boyes et al. 2014).

In two different tests using the same test conditions, the same research group observed impaired learning in female animals at the low concentration and above. However, this effect was not concentration-dependent, which means that the findings may have been affected by confounding through maternal care or altered anxiety levels in the offspring. After exposure to 21 000 ml/m^3, an increased incidence

Table 1 Studies with prenatal inhalation exposure to ethanol

Species	Exposure	Findings	References
rat, Long Evans, 18 ♀	**GD 9–20**, 0, 5000, 10 000, 21 000 ml/m³, analysed concentrations: 0, 4858 ± 265, 10 252 ± 460, 20 998 ± 741 ml/m³, calculated BEC: 23, 65, 1920 mg/l (using PBPK model of Martin et al. (2014)), vapour, whole-body, 6.5 hours/day (during the first 30 minutes the concentration was adjusted), purity: 95%, standardization of litter size on PND 3 to 6 ♂ and 4 ♀ (if not enough ♂ in a litter, as many ♂ as possible were used and the litter was then filled with ♀ until 10 animals/litter was achieved), separation of offspring from dams on PND 22, examination: up to PND 180	**10 000 ml/m³ and above**: dams: feed consumption ↓ (including the calories from inhaled ethanol: similar caloric intake in all groups); **> 21 000 ml/m³: NOAEC developmental and maternal toxicity**; no detectable changes: dams: body weights, clinical observations, pregnancy, offspring: litter size, sex ratio, body weights on PND 0 and 29, systolic blood pressure on PND 90 ↑ but not in a dose-dependent manner (only at 10 000 ml/m³), but not on PND 180, blood: GHbA1c on PND 90 or 180 (parameter for determining glucose status), lipoprotein profile, standard liver function test, urine: standard analysis on PND 90 or 180, maturation of the immune system, humoral and cellular immune response in PNW 6, FOB, grip strength in hind limbs ↓ on PND 29 and ↑ on PND 62 but not in a dose-dependent manner, motor activity ↑ on PND 62 but not in a dose-dependent manner	Beasley et al. 2014
rat, Long Evans, 16 ♀	**GD 9–20**, 0, 5000, 10 000, 21 000 ml/m³, analysed concentrations: 0, 4858 ± 265, 10 252 ± 460, 20 998 ± 741 ml/m³, vapour, whole-body, 6.5 hours/day, purity: 95%, examination: PND 106–PND 128, ♂ only	**> 21 000 ml/m³: NOAEC developmental neurotoxicity and maternal toxicity**; no detectable changes: functional examination of the peripheral (motor unit action potential, nerve conduction velocity), somatosensory (cortical and cerebral evoked potentials), auditory (auditory evoked brainstem potentials) and visual nervous system (pattern VEPs elicited, VEP contrast sensitivity, electroretinogram)	Boyes et al. 2014

Table 1 (continued)

Species	Exposure	Findings	References
rat, Long Evans, 16 ♀	**GD 9–20**, 0, 5000, 10 000, 21 000 ml/m^3, see Beasley et al. (2014)	**5000 ml/m^3 and above**: ♀ offspring: impaired cue learning after fear conditioning and absence of bias for the correct quadrant after place training during a reference memory probe in the Morris water maze (both not dose-dependent, therefore confounding through maternal care or altered anxiety levels); **10 000 ml/m^3: NOAEC developmental neurotoxicity of offspring;** **21 000 ml/m^3: NOAEC maternal toxicity,** ♂ offspring: anticipatory response during a preparatory period in the choice reaction time test ↑ (dose-dependent, evidence of deficiency in response inhibition, i.e. impulsive response ↑, ♀ not tested for this task); no detectable changes: spatial learning and working memory in the Morris water maze and the delayed match to position test, ♂ (♀ not tested): only at 5000 and 10 000 ml/m^3: decision-making time in the choice reaction time test transiently ↑	Oshiro et al. 2014

BEC: blood ethanol concentration; FOB: Functional Observational Battery; GD: gestation day; GHbA1c: glycosylated haemoglobin A1c; NOAEC: no observed adverse effect concentration; PBPK: physiologically based pharmacokinetic; PND: postnatal day = days post natum; PNW: postnatal week; VEP: visual evoked potential

of anticipatory responses in the choice reaction time test was observed in male offspring. The incidence was dependent upon the concentration and the increase was statistically significant in the high concentration group. This behaviour was observed early on and lasted throughout the examination period. The effect was interpreted as an increase in impulsive responses. The authors pointed out that permanent effects are induced in the offspring as from blood concentrations of about 2000 mg/l, which is reached at 21 000 ml/m³. The fact that not many pervasive cognitive deficits were observed after exposure to concentrations of 21 000 ml/m³, as was the case at the same blood concentration after oral administration, is considered evidence of route-dependent differences in the toxicokinetics of ethanol (Oshiro et al. 2014). The NOAEC (no observed adverse effect concentration) for developmental neurotoxicity was 10 000 ml/m³.

Two studies with inhalation exposure of rats during pregnancy (Maciejewski-Lenoir 1993; Zink et al. 2009) are not relevant for this assessment because they did not include data for the concentrations in the air.

A publication discussed the construction of whole animal vapour chambers for studying the toxic effects of prenatal exposure of mice to ethanol (Morton et al. 2014). This study is not included in the evaluation because it focuses primarily on the measuring procedure and no effects are described.

Manifesto (MAK value/classification)

The critical effect of ethanol is its effect on the central nervous system and, after long-term administration, its carcinogenic potential.

MAK value. A MAK value of 500 ml/m³ was derived from a study of volunteers at rest. Under these conditions, the AUC (product of the blood ethanol concentration and time of exposure) was 10.5 mg/l × years and thus within the standard deviation of the lifetime AUC of endogenous ethanol of 13.6 mg/l × years (documentation "Ethanol" 1999). While carrying out physical activity at 50 watts, the blood ethanol concentration of humans is 2 to 3 times as high as that of subjects at rest (Dumas-Campagna et al. 2014). This is to be expected because of the high blood:air partition coefficient of ethanol. Therefore, after exposure to 500 ml/m³, the AUC for workplace exposure (20–30 mg/l × years) is outside the standard deviation of the lifetime AUC of endogenous ethanol (documentation "Ethanol" 1999). The AUC for exposure to 200 ml/m³ under resting conditions was 4.2 mg/l × years. At an increased respiratory volume, the AUC was about 8 to 12 mg/l × years and thus within the standard deviation of the lifetime AUC of endogenous ethanol. For this reason, the MAK has been lowered to 200 ml/m³.

Peak limitation. Ethanol remains classified in Peak Limitation Category II. The MAK value was derived from the AUC, which means that the systemic effects are not determined by the concentration. As irritation was observed in test subjects at concentrations of 1900 ml/m³ and above, but not at 1000 ml/m³ as was described in the documentation from 1998 (documentation "Ethanol" 1999), an excursion factor of 4 has been established.

Prenatal toxicity. As described in the documentation published in 1998 (documentation "Ethanol" 1999), prenatal toxicity is induced in humans and animals after oral administration of ethanol. It was also pointed out in the documentation from 1998 (documentation "Ethanol" 1999) that the maternal blood ethanol concentrations that are thought to cause these effects are within an order of magnitude that can never be reached by inhalation exposure at the MAK value.

There are 3 new studies with inhalation exposure of pregnant rats in which offspring were examined for different end points of developmental neurotoxicity (Beasley et al. 2014; Boyes et al. 2014; Oshiro et al. 2014). At the concentration of 21 000 ml/m^3, an increase in anticipatory responses in the choice reaction time test was observed in male offspring; the females were not tested for this task. This effect was interpreted as an increase in impulsive response and as a permanent pervasive cognitive deficit. The NOAEC for this effect was 10 000 ml/m^3 (Oshiro et al. 2014). According to a PBPK model, the maximum blood ethanol concentration after inhalation exposure to concentrations of 21 000 ml/m^3 was 1920 mg/l (Martin et al. 2014). This is equivalent to the blood ethanol concentration at which severe developmental toxicity was induced after oral administration. Differences in the severity of the effects after inhalation exposure or oral bolus administration reflect route-dependent differences in the toxicokinetics of ethanol (Oshiro et al. 2014). Using the above PBPK model, blood ethanol concentrations of 65 mg/l were determined at the NOAEC of 10 000 ml/m^3 (Oshiro et al. 2014). The lowest reported maternal blood concentration at which effects were recorded in the offspring of pregnant rats given oral doses of ethanol was 300 mg/l. No effects were observed at a blood concentration of 70 mg/l (Savage et al. 2002). The estimated blood concentration at the NOAEC of 10 000 ml/m^3 is thus within the range of the NOAEL (no observed adverse effect level) of the oral exposure study. There is therefore extensive evidence that after exposure to concentrations up to 10 000 ml/m^3, maternal blood ethanol concentrations do not reach levels that are thought to cause the toxic effects on prenatal development observed in oral exposure studies. Therefore, even if the increased respiratory volume is taken into consideration, prenatal toxicity is not to be expected if exposure remains at the MAK value of 200 ml/m^3. For this reason, ethanol remains classified in Pregnancy Risk Group C.

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