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Ammonia

MAK Value Documentation, supplement – Translation of the German version from 2020

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

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

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

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

Keywords

short-term exposure; irritation; maximum workplace concentration; MAK value; toxicity; hazardous substance; peak limitation; fertility; developmental toxicity

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated ammonia [7664-41-7] considering all toxicological end points. Available publications are described in detail. The critical effect is irritation of the mucous membranes in humans. From three acute studies in humans, the highest concentrations tested of 50 ml/m^3, 25 ml/m^3 and 20 ml/m^3 were evaluated as NOAECs for sensory irritation. The concentration of 20 ml/m^3 (average of 0–40 ml/m^3) was found to be close to the NOAEC as reversible increases in eyeblink frequency were observed in female subjects. The apparent discrepancy between the NOAECs is due to the concentrations applied and the higher sensitivity of women for alterations in eye-blink frequency as compared to men. Therefore, the maximum concentration at the workplace (MAK value) of 20 ml/m^3 is retained. As the critical effect is local, Peak Limitation Category I is confirmed. The excursion factor of 2 is retained because a study found unspecific and slight redness of the eyes in three persons at 50 ml/m^3, which was not regarded as adverse. The increased eye-blink frequency after short-term exposure to 40 ml/m^3 is considered to still fall within the physiologically normal range and therefore does not contradict the excursion factor of 2. A high detoxification capacity of the liver can be assumed and blood ammonia concentrations were not increased in exposed animals at concentrations in the range of the MAK value. Therefore, damage to the embryo or foetus is unlikely when the MAK value is not exceeded and the classification of ammonia in Pregnancy Risk Group C is retained. There are no data for sensitization in humans or animals. Due to the high vapour pressure and the low boiling point, prolonged dermal contact is unlikely; this applies also for aqueous solutions. Compared with the local toxicity, the systemic toxicity is low. Therefore, skin contact is not expected to contribute significantly to the systemic toxicity.

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MAK value (1996)	**20 ml/m³ (ppm) ≙ 14 mg/m³**
Peak limitation (2000)	**Category I, excursion factor 2**
Absorption through the skin	–
Sensitization	–
Carcinogenicity	–
Prenatal toxicity (1986)	**Pregnancy Risk Group C**
Germ cell mutagenicity	–
BAT value	–
CAS number	7664-41-7
1 ml/m³ (ppm) ≙ 0.707 mg/m³	**1 mg/m³ ≙ 1.415 ml/m³ (ppm)**

For ammonia, there is documentation from 1973 (Henschler 1993) and supplements from 1986 (included in the translation Henschler 1993), from 1996 (Greim 1999) and for peak limitation from 2000 (Greim 2000, available in German only).

Numerous new studies have been published; for this reason, the MAK value has been re-evaluated.

Ammonia forms ammonium hydroxide in aqueous systems. At physiological pH, 98.3% of the ammonia is present in the blood in the form of the ammonium ion and 1.7% in the form of ammonia (US EPA 2016 a, b). Since ammonium salts in aqueous solution likewise dissociate into the ammonium ion and the corresponding anion, studies of such salts are used to assess the reproductive toxicity of ammonia.

Toxicokinetics and Metabolism

In humans and other mammals, ammonia is produced endogenously in all tissues, including foetal tissue. Endogenous ammonia formation in the intestine is about 4.2 g per person and day (Henschler 1993). Ammonia is essential for nucleic acid and protein biosynthesis, it is an essential component of nitrogen homoeostasis and is necessary for maintaining the acid-base balance. The concentration of ammonia in the blood is homoeostatically controlled to keep it low enough to avoid toxic effects. In healthy adults, it is between 0.1 and 0.8 µg/ml in venous blood, and in the umbilical cord blood at birth the ammonia concentration is about 50% to three times higher than the level in maternal blood (US EPA 2016 a).

Absorption

In humans, 83% to 92% of the inhaled ammonia was retained in the nasal mucosa after exposure for up to 120 seconds to ammonia concentrations of 40 to 354 mg/m³ (28 to 250 ml/m³). Prolonged exposure for 10 to 27 minutes to an ammonia concentration of 354 mg/m³ resulted in a decrease in retention to 4% to 30%, with expired breath concentrations of 247–283 mg/m³ observed by the end of the exposure period, which suggests the saturation of absorption into the nasal mucosa. Since only irritation of the nose and pharynx is observed, but not of the trachea, it can be assumed that ammonia is retained in the upper respiratory tract due to its very good solubility in water. The unchanged levels for blood urea nitrogen and non-protein nitrogen as well as urea and ammonia in urine after these acute inhalation exposures indicate a very low systemic uptake (US EPA 2016 b).

Data in rabbits and dogs provide supporting evidence for high-percentage nasal retention, resulting in a lower fraction of the inhaled dose reaching the lower respiratory tract (US EPA 2016 b). While exposure of male Crl:COBS CD(SD)

rats (5 animals per concentration and time point; 15, 32, 310, 1157 ml/m³) to ammonia concentrations of up to 32 ml/m³ did not result in increased blood ammonia concentrations after 8, 12 and 24 hours, exposure to 310 and 1157 ml/m³ significantly increased the blood ammonia concentrations 8 hours after the start of exposure, but not after 12 and 24 hours (Schaerdel et al. 1983). In female Wistar rats (5 animals per concentration; 0, 25, 300 ml/m³) that were exposed by inhalation to an ammonia concentration of 25 ml/m³ for 6 hours daily for 5, 10 or 15 days, no statistically significant increases in the blood ammonia concentrations (0.021 to 0.057 mmol/l) were determined at these 3 points in time compared with those in the controls (0.032 to 0.043 mmol/l). The ammonia concentrations of the animals exposed to 300 ml/m³ were increased 3-fold after 5 days of exposure, but not after 10 and 15 days (Manninen et al. 1988). The decrease in the blood ammonia concentrations after prolonged exposure in both studies suggests compensation resulting from increased ammonia metabolism (US EPA 2016 b).

Distribution

In female Wistar rats exposed to ammonia concentrations of 300 ml/m³ by inhalation for 6 hours daily, in addition to the increased blood ammonia concentration, a 48% increase in the glutamine concentration in the brain was found after 5 days of exposure, but not after 10 and 15 days (Manninen et al. 1988), demonstrating transient distribution of ammonia to the brain (US EPA 2016 b).

Metabolism

In most tissues, ammonia is metabolized by glutamine synthetase to glutamine in the glutamine cycle and predominantly to urea in the urea cycle of the liver (US EPA 2016 b).

Elimination

Ammonia is excreted mainly in the form of urea with the urine, small amounts are excreted unchanged with faeces, sweat and expired air. In the breath of humans, the ammonia concentrations were between 0.009 and 2 mg/m³ (US EPA 2016 a).

The high detoxification capacity of the liver for inhaled ammonia at the MAK value of 50 ml/m³ valid at the time was already pointed out in the 1986 supplement (Henschler 1993). If this estimation is applied to the current MAK value of 20 ml/m³ (14 mg/m³), about 140 mg ammonia per person and day is absorbed assuming complete absorption. Assuming complete metabolism to urea, this would result in about 250 mg urea. In comparison, the daily urinary excretion of urea in a healthy adult is 20 to 35 g (Henschler 1993).

Effects in Humans

There are numerous case reports describing severe to severest irritation of the skin and respiratory tract after inhalation and dermal exposure (e. g. Amshel et al. 2000; Brautbar et al. 2003; Cruz and Fonseca 2009; Latenser and Lucktong 2000; Perry et al. 2016; White et al. 2007). These studies are not included in the evaluation due to the lack of exposure data.

The new studies with inhalation exposure of volunteers to ammonia are shown in detail in Table 1.

In a 4-hour study with constant whole-body exposure to 2.5 ml/m³ (odour threshold) or exposure to varying concentrations between 0 and 40 ml/m³, that changed every half an hour (with a time-weighted average concentration of 20 ml/m³), concentration-dependent chemosensory perceptions and acute symptoms occurred. The study included 19 volunteers with seasonal allergic rhinitis and 18 control subjects. The study took place outside the pollen season, so that overlapping effects caused by the respective allergens could be excluded. Compared with that at the 2.5 ml/m³ exposure level, the blinking frequency was significantly increased at 20 ml/m³ in both groups of test persons. Especially

the exposure peaks of 40 ml/m^3 were effect-enhancing, since the highest blinking frequency was determined during these periods. The blinking frequencies of the subjects with seasonal allergic rhinitis were higher at 2.5 and 20 ml/m^3 than those in the control subjects, but this difference was not statistically significant. No evidence of an increased response to ammonia was found in the 19 volunteers with seasonal allergic rhinitis compared with the response in 18 subjects without this allergy (Pacharra et al. 2017). The study provides physiologically demonstrable indications of sensory irritation, which, however, were not found in the biochemical parameters studied. In absolute terms, the values of the increased blinking frequency are considered to still fall within the physiologically normal range. In the exposure scenario investigated, the average concentration of 20 ml/m^3 can be regarded as being close to the NOAEC (no observed adverse effect concentration), with marked odour effects and probably reversible sensory irritation.

In a study, 37 healthy women were exposed in five 15-minute exposure steps to ascending ammonia concentrations of 0, 1.25, 2.5, 5 and 10 ml/m^3. It was found that individual olfactory acuity (measured as the sum of the subtests of the Sniffin' Sticks: odour threshold, discrimination and identification (TDI)) modulated the ratings for hedonic valence (pleasant/unpleasant odour) and working memory performance. Thus, subjects with a lower olfactory acuity (TDI value below the median) evaluated the odour of 10 ml ammonia/m^3 as significantly more unpleasant than the subjects with a higher olfactory acuity and also produced more "false alarm" reactions in this phase in the working memory test (Pacharra et al. 2016 b). However, this result is a singular effect in only one test and with a single performance parameter, so that this finding cannot be interpreted as a distraction caused by the ammonia odour.

This question was further investigated in another study with whole-body exposure by the research group. Here, 13 healthy women with and 13 without self-reported chemical intolerance were exposed as in the above study. Two neuropsychological tests, which assessed cognitive performance in the areas of working memory and reaction inhibition, were used to investigate possible distraction effects caused by the chemosensory perception of ammonia. During the 75-minute exposures with increasing ammonia concentrations, performance improved in both groups; this was explained by the authors as the effect of learning through performing repeated tasks. There were no indications of distraction effects, such as reduced test performance. The physiological responses (biochemical inflammation parameters in the nasal lavage fluid) did not reveal differences between the two groups nor effects caused by acute exposure to ammonia. The participants with self-reported chemical intolerance generally rated the pungency of the different ammonia concentration levels as stronger (Pacharra et al. 2016 a).

In a study in 43 healthy male volunteers with whole-body exposure to ammonia concentrations of 0, 10, 20, 20/40 (two 30-minute concentration peaks of 40 ml/m^3 on day 4 of the study), or 50 ml/m^3 (in vapour form) for 4 hours daily on 5 consecutive days, 9% (3/33) of the unaccustomed, that is neither occupationally nor privately exposed volunteers were found to have reversible, slight conjunctival reddening at 50 ml/m^3. A slit lamp was used for the examination. No such findings were seen in the 10 occupationally exposed persons. Up to the highest concentration tested of 50 ml/m^3, the lung function measurements, the tests for cognitive performance and the determination of interleukins in nasal lavage fluid did not yield any unusual findings. The exposure peaks, following a baseline exposure of 20 ml/m^3, were perceived sensorily by all subjects, regardless of their habituation. However, no significant changes in physical findings were reported for the 30-minute short-term exposures of 40 ml/m^3. Even though subjective complaints increased with increasing exposure levels, no acute habituation effect was observed during a 4-hour exposure day (Hoffmann et al. 2004). From this study, a NOAEC of 50 ml/m^3 for local irritation was obtained.

Another study of the same collective investigated the relationship between personality traits and the number of symptoms reported. Subjects with high positive affectivity reported fewer respiratory and irritative complaints, while subjects with negative affectivity reported more olfactory and respiratory symptoms. This correlation decreased with higher exposure levels and was no longer statistically significant at 50 ml/m^3 (Ihrig et al. 2006).

In a study, 12 healthy volunteers were exposed whole-body to 0, 5 or 25 ml/m^3 for 3 hours, half the time exercising on a 50 watt bicycle ergometer. At 25 ml/m^3 there was a statistically significant increase in the ratings for irritant and systemic symptoms compared with those given for the control exposure group. Irritant effects on the eyes, nose and throat as well as the rating for nausea did not reach the "somewhat" level (corresponding to 26 out of 100) with an average of about 15 out of 100. The cumulative dose of methacholine causing a 20% decrease in the forced expiratory

volume in 1 second (FEV_1) was significantly lower after the exposure than before the exposure, but not compared with that in the control group with sham exposures. Furthermore, ammonia did not significantly influence lung function or the exhaled NO concentrations (Sundblad et al. 2004). Due to the insignificance of the reported effects, the NOAEC for local effects is 25 ml/m³.

Data for lateralization thresholds (sensory irritation thresholds)

In a study in which 8 volunteers were exposed to increasing concentrations of ammonia vapour of 60 to 350 ml/m³, there was no difference between the lateralization thresholds in humid (181 ml/m³) and dry air (172 ml/m³) (Monsé et al. 2016).

In a study in 6 subjects, the lateralization thresholds were between 37 and 67 ml ammonia/m³ (Wise et al. 2005).

In 24 healthy women, the lateralization thresholds were 31.7 ml/m³ after static olfactometry and 60.9 ml/m³ after dynamic olfactometry. The difference between these values was not statistically significant ($p = 0.07$) (Smeets et al. 2007).

Likewise, in subjects with and without asthma who were exposed to ammonia by inhalation there was no statistically significant difference in the lateralization thresholds and 1-second capacity (Petrova et al. 2008).

Tab. 1 Effects of ammonia after inhalation exposure in studies with volunteers

Exposure	Number of exposed test persons	Concentration: findings	References
2.5 ml/m³ (constant); 0–40 ml/m³ (starting with 40 ml/m³, alternating between 0 and 40 ml/m³ every 30 minutes, average concentration: 20 ml/m³); 4 hours, single, ammonia vapour, whole body	19 subjects (10 men and 9 women; age: 25.1 ± 3.9 years) with seasonal allergic rhinitis, 18 control subjects (8 men and 10 women; age: 23.6 ± 2.5 years)	**20 ml/m³: NOAEC for local irritation** (♂, ♀); **20 ml/m³ (0–40 ml/m³)**: significant odour effects and reversible sensory irritation, but no changes in biochemical parameters; concentration-dependent increase in chemosensory perception (LMS) and acute symptoms (SPES), blinking frequency increased compared with that at 2.5 ml/m³ (effects in controls with a lower initial blinking frequency much more pronounced compared with subjects with allergy, absolute values still considered "normal"), but no effects of allergic rhinitis; irritation was rated on average as "moderate"; no cortisol stress response, no objective nasal congestion (measured by anterior active rhinomanometry), no inflammatory response in nasal lavage fluid (substance P, tumour necrosis factor, high mobility group protein 1); no evidence that seasonal allergic rhinitis increases response to ammonia	Pacharra et al. 2017
increasing concentrations after every 15 minutes: 0–1.25–2.5–5–10 ml/m³, total time: 75 minutes, determination of olfactory acuity: TDI, before exposure and after each increase in concentration: query ratings (LMS) and tasks (3-back, flanker), ammonia vapour, whole body	37 healthy women (non-smokers), not pregnant (age: 22.2 ± 2.0 years)	**10 ml/m³**: test subjects with lower olfactory acuity (TDI value < median): ammonia odour at 10 ml/m³ evaluated as significantly more unpleasant and more "false alarm" reactions in the working memory test than in test subjects with better olfactory acuity	Pacharra et al. 2016 b

Tab. 1 *(continued)*

Exposure	Number of exposed test persons	Concentration: findings	References
increasing concentrations after every 15 minutes: 0–1.25–2.5–5–10 ml/m³, total time: 75 minutes, before exposure and after each increase in concentration: query ratings (LMS) and tasks (working memory: 3-back, reaction inhibition: flanker), ammonia vapour, whole body	13 healthy women (non-smokers) each with or without self-reported chemical intolerance (using TMS; CSS-SHR or negOAS) from online surveys with 88 ♂ and 233 ♀ participants	**0–10 ml/m³**: female subjects with self-reported chemical intolerance: higher number of reports of burning sensation; no difference in cognitive performance and physiological response between the two groups	Pacharra et al. 2016 a
on 5 consecutive days: 0, 10, 20, 20/40 (2 concentration peaks for 30 minutes on day 4), 50 ml/m³, 4 hours/day, ammonia vapour, whole body	43 healthy men (age: 21–47 years), 10 with occupational exposure to ammonia and 33 with neither occupational nor private exposure to ammonia, 6 participants with non-specific bronchial hyperreactivity	**50 ml/m³: NOAEC for local irritation (♂)**; **50 ml/m³**: 3/33 (9%) of the volunteers unaccustomed to ammonia had slight conjunctival reddening, probably of unspecific nature (ventilators in exposure chamber), examination by slit lamp; **up to 50 ml/m³: no noticeable changes**: pulmonary function measurements (anterior rhinomanometry, whole-body plethysmography, spirometry, flow volume curve, bronchial responsiveness, R_{tot}, FEV_1, MEF_{25-75}); nasal secretion: IL-1β, IL-6, IL-8; cognitive functions (concentration, attention, reaction time), severity of symptoms (SPES): concentration-dependent increase, but not statistically significant compared with values at the start of exposure; volunteers with ammonia habituation: on average lower complaint values for irritation than unaccustomed volunteers: accustomed volunteers between "not at all" and "hardly", unaccustomed volunteers just below "hardly", the most sensitive persons of both groups: "somewhat"	Hoffmann et al. 2004; Ihrig et al. 2006
		statistically significant correlation between personality traits (recorded with PANAS and FPI) and number of symptoms (recorded with SPES including irritant, olfactory and respiratory symptoms): volunteers with high positive affectivity were found to have fewer respiratory and irritative symptoms; persons with negative affectivity had more olfactory and respiratory symptoms; the correlation decreased as the concentration increased from 20 ml/m³ onwards	Ihrig et al. 2006
0 (sham exposure), 5, 25 ml/m³ (0, 3.5, 17.7 mg/m³), 3 sessions: 3 hours each (half the time seated, half on a bicycle ergometer at 50 watts), between sessions at least a 1-week interval; whole body	5 men, 7 women (average age: 25 years, range: 21–28 years), without allergies or respiratory diseases	**25 ml/m³: NOAEC for local irritation**; **up to 25 ml/m³**: statistically significant increase in symptoms, degree of local irritation (eye, nose, throat, breathing difficulties) and "nausea" on average around 15/100; decrease in $PD20FEV_1$ compared with the value before exposure, but no statistically significant difference to control group; no noticeable changes: VC, TLC, FEV_1, PEF; exhaled NO; nasal fluid: total cell count and IL-8 concentration; plasma: complement factor 3b; blinking frequency and conjunctiva not examined	Sundblad et al. 2004
ascending concentrations: 60–350 ml/m³, 4 series of 5 minutes each, dry and humid ammonia vapour	5 men, 3 women (age: 35–50 years), non-smokers	no difference between lateralization thresholds in humid (181 ml/m³) and dry air (172 ml/m³)	Monsé et al. 2016
olfactometry: 37, 52, 67, 97, 131, 289 ml/m³, ammonia vapour	4 men (age: 26–52 years), 2 women (age: 27 and 28 years)	lateralization thresholds: 37–67 ml/m³	Wise et al. 2005

Tab. 1 *(continued)*

Exposure	Number of exposed test persons	Concentration: findings	References
static olfactometry: 0.03–154 ml/m³, dynamic olfactometry: 0.1–615 ml/m³, ammonia vapour, 2-second stimuli, intervals of 30–60 seconds	24 healthy women (age: 29.9 ± 8.9 years), non-smokers, not pregnant	no statistically significant difference between the two methods; median odour threshold: static, dynamic: 2.6 ml/m³; lateralization thresholds: static: 31.7 ml/m³; dynamic: 60.9 ml/m³ (p = 0.07)	Smeets et al. 2007
start: 1000 ml/m³, 20 dilution steps: 500 to 2 ml/m³, 20-second stimuli	25 healthy persons (age: 29.7 ± 10.8 years), 15 mild to moderate asthmatics (age: 29.1 ± 9.6 years), gender not specified	lateralization thresholds of healthy volunteers: ocular lateralization thresholds: 127 ± 22 ml/m³, nasal lateralization thresholds: 179 ± 24 ml/m³, combined nasal/ocular with and without velopharyngeal closure: 87 ± 20 ml/m³ or 102 ± 14 ml/m³, no significant difference between groups; FEV_1: no significant difference between both groups, decrease in FEV_1 in no subject > 5%	Petrova et al. 2008

CSS-SHR: Chemical Sensitivity Scale for sensory hyperreactivity; FEV_1: forced expiratory volume in 1 second; FPI: Freiburg Personality Inventory; IL: interleukin; LMS: Labelled Magnitude Scales; $MEF_{25–75}$: maximum expiratory flow at 25% to 75% of vital capacity; negOAS: negative awareness subscale of the Odour Awareness Scale; PANAS: Positive and Negative Affectivity Schedule; $PD20FEV_1$: cumulative dose of methacholine causing a 20% decrease in FEV_1; PEF: peak expiratory flow; R_{tot}: respiratory resistance; SPES: Swedish Performance Evaluation System; TDI: Sniffin' Sticks total score; TLC: total lung capacity; TMS: trigeminally-mediated sensitivity; VC: vital capacity

In a cross-sectional study conducted at a petrochemical plant in Iran, 124 male employees engaged in producing ammonia were investigated. They were divided into workers subjected to high exposure (responsible for operational work) and low exposure (repair and maintenance). For various lung function parameters (see Table 2) changes were found in the workers exposed to high levels, compared with the values in the control group at the same plant without exposure to ammonia, and in the exposed workers after the shift (Neghab et al. 2018). Exposure to other substances is to be expected in petrochemical plants. Data for other chemicals are not given in the study, however. Due to the exposure to a mixture of substances, the unclear decrease in vital capacity and the low exposure to ammonia, the study cannot be used for the derivation of the MAK value.

Tab. 2 Effects of ammonia after chronic inhalation exposure

Exposure	Number of exposed workers	Findings	References
low exposure: 0.29 ± 0.31 ml/m³ (range: detection limit–1.17 ml/m³), length of employment: 4.8 ± 3.9 years; high exposure: 1.35 ± 4.59 ml/m³ (detection limit–24.55 ml/m³), length of employment: 7.3 ± 4.8 years; determined during 12-hour shifts; peak concentrations (leakages): 94.8 ± 83.1 ml/m³ (25–290 ml/m³); reference values for control persons: below detection limit, length of employment: 7.6 ± 4.5 years; detection limit not specified	124 ♂, 67 of whom with high-level and 57 with low-level exposure; petrochemical industry, Assalouyeh, Iran, 120 ♂ control persons from the same plant; questioned about smoking, body weights and height, length of employment, education	high exposure: VC ↓, FEV_1 ↓ pre-shift compared with the values in the control group; high and low exposure: VC ↓, FVC ↓, FEV_1/FVC ↑ post-shift compared with pre-shift values, reversible	Neghab et al. 2018

FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; VC: vital capacity

Reproductive and developmental toxicity

In a retrospective study conducted at a petrochemical plant in Beijing, China, the association between exposure to petrochemicals and spontaneous abortions was investigated. Of the total of 3105 women at the plant, 2853 participated (20 to 44 years of age, married, had never smoked). The analysis was limited to the first pregnancy between 1973 and 1993. During this time, 1620 of the participants were exposed to petrochemicals (56.8%) and 97 women to ammonia

(3.4%) during the first trimester of pregnancy, according to their job history. There was an increased risk of spontaneous abortion for exposure to petrochemicals: odds ratio (OR) 2.9; 95% confidence interval (CI) 2.0 to 4.0, but not for exposure to ammonia (OR 1.2; 95% CI 0.5 to 2.6) (Xu et al. 1998). Data for exposure concentrations are not available.

Animal Experiments and in vitro Studies

Acute toxicity

Inhalation

In a comparative study of sensory irritation potency, 4 male Wistar Hsd Cpb:WU rats and 4 male OF1-ICO mice were exposed nose-only for 45 minutes to anhydrous ammonia concentrations of 0, 100, 200, 400 or 1000 mg/m^3 (0, 142, 283, 566, 1415 ml/m^3; purity ≥ 99.9%) in dry or humid air. In male rats, the RD_{50} values for ammonia were 972 and 905 mg/m^3 (1375 and 1281 ml/m^3) for dry and humid air, respectively. The corresponding values for male OF1 mice were 582 and 732 mg/m^3 (824 or 1036 ml/m^3, respectively) (Li and Pauluhn 2010).

Groups of 5 male and 5 female rats were exposed nose-only for 1 hour to anhydrous ammonia concentrations of 0, 9222, 11 015 or 14 044 mg/m^3 (0, 13 049, 15 586 or 19 872 ml/m^3) or for 4 hours to ammonia concentrations of 0, 4285, 5725, 6390 or 7150 mg/m^3 (0, 6063, 8101, 9042 or 10 114 ml/m^3; purity ≥ 99.98%). The animals were observed for 2 weeks without further exposure. In the more susceptible male animals, the LC_{50} values were 12 303 mg/m^3 (17 409 ml/m^3) for the 1-hour exposure and 4923 mg/m^3 (6966 ml/m^3) for the 4-hour exposure, and the non-lethal threshold concentrations (LC_{01}) were 10 067 mg/m^3 (14 245 ml/m^3) for the 1-hour exposure and 4028 mg/m^3 (5700 ml/m^3) for the 4-hour exposure. At sublethal concentrations, the ventilation of rats was about one third of normal breathing. Overall, it was shown that C-dependent and C × t-dependent causes of toxicity must be considered when across-species extrapolation is performed for inhalation exposure (Pauluhn 2013).

Groups of 4 to 8 male Sprague Dawley rats were exposed to anhydrous ammonia concentrations of 9000, 20 000, 23 000, 26 000, 30 000 or 35 000 ml/m^3 (purity ≥ 99.99%; head-only exposure) for 20 minutes. Depending on the concentration, increased chewing and licking, eye irritation, salivation and lacrimation, oronasal secretion and laboured breathing were observed. The 20-minute LC_{50} was 23 672 ml/m^3. At 9000 ml/m^3 pulmonary oedema occurred. At 9000 ml/m^3 and above, body weight losses were recorded. An increased number of dead cells and an increase in the total number of cells in the bronchoalveolar lavage fluid were observed at 20 000 ml/m^3 and above (Perkins et al. 2016).

In another study by the same research group, 6 male Sprague Dawley rats were exposed (head-only) to anhydrous ammonia concentrations of 0, 9000, 20 000 or 23 000 ml/m^3 for 20 minutes. The animals were subsequently observed for up to 24 hours. At all concentrations, the respiratory minute volume and tidal volume were decreased during exposure and 24 hours after exposure. The inspiratory time and expiratory time decreased during exposure and were increased 24 hours after exposure compared with the values in the controls. Histopathological examinations were carried out only in the animals exposed to 20 000 ml/m^3. Oedema, epithelial necrosis and exudate were found in the lungs and trachea 1 hour, 3 hours and 24 hours after exposure (Perkins et al. 2017).

Reproductive and developmental toxicity

Fertility

Groups of 40 gilts (young sows) (Yorkshire × Hampshire × Chester White) per group were exposed whole-body to anhydrous ammonia concentrations of 7 ml/m^3 (4 to 12 ml/m^3) or 35 ml/m^3 (26 to 45 ml/m^3) at the age of 2 to 4.5 months. Half of the animals in each concentration group were continuously treated 6 weeks prior to mating up to day 30 of gestation or killed and examined after 6 weeks of exposure. There was no control group. At the concentration of

35 ml/m^3 the body weight gains of the animals were significantly decreased after 2 weeks compared with those in the group exposed to 7 ml/m^3. After 4 and 6 weeks, however, no difference was observed. After 6 weeks of exposure, lung damage and moderate degeneration of the nasal conchae occurred in the animals of both groups. The age of the onset of puberty, and the incidence of conception and implantation did not differ between the two groups (Diekman et al. 1989, 1993). Due to the absence of a control group, the small scope of the study and the unusual animal species without historical reference values, the study is not included in the evaluation.

In the evaluation of ammonia under the OECD HPV programme, an unpublished screening study according to OECD Test Guideline 422 from 2002 with diammonium phosphate (corresponding to 17.93% N and 46.86% P_2O_5) is described. Groups of 5 male and 10 female Crl:CD(SD)IGS BR rats were given gavage doses of diammonium phosphate of 0, 250, 750 or 1500 mg/kg body weight and day on 7 days per week. The males and females were treated 2 weeks before mating, during mating and the females additionally during gestation and up to day 4 post partum. Treatment-related deaths and signs of toxicity were not observed in the parent animals. In the males, at 750 mg/kg body weight and day and above, the activated partial thromboplastin times were reduced to 74% and 76% of the control values. In addition, at 750 mg/kg body weight and day and above, a higher variability of blood chemistry parameters was found, of which the following are probably substance-related: a not dose-dependent increase in alkaline phosphatase activity to 132% and 131% of the control values and a dose-dependent decrease in total protein concentrations to 93% and 91%, respectively. In the female animals, the blood phosphorus concentration was reduced to 81% at 1500 mg/kg body weight and day. Mating performance and fertility were not affected by the treatment. No treatment-related abnormalities were found in the offspring. The NOAEL (no observed adverse effect level) for effects on fertility and the offspring was 1500 mg/kg body weight and day, the NOAEL for systemic toxicity was 250 mg/kg body weight and day (OECD 2007).

Developmental toxicity

In the study in gilts with inhalation exposure to anhydrous ammonia already described in the Section "Fertility", no statistically significant differences were found regarding the number of live foetuses and the weights and lengths of foetuses on gestation day 30 (Diekman et al. 1989, 1993). The shortcomings of this study have already been pointed out (see Section "Fertility").

Ten pregnant Sprague Dawley rats per group were given ammonium chloride in concentrations of 0 or 0.17 mol/l drinking water (3060 mg NH_4^+/l; about 367 mg NH_4^+/kg body weight and day, conversion factor 0.12 (for data from subacute studies) according to EFSA (2012)) from gestation days 7 to 10, on day 20 of gestation the foetuses were examined. No deaths occurred in either the dams or the foetuses. Other parameters were not recorded in the dams. During the external examination of the foetuses, special attention was paid to the dorsal and ventral midline and to eye defects. No skeletal abnormalities were detected by alizarin red S staining. The body weights and crown-rump lengths of the foetuses were decreased in the treated animals (Goldman and Yakovac 1964). Since only 1 dose was tested and because of the very limited scope of the study, this study is of only limited validity.

Wistar rats (at least 8 animals per group in several subgroups examined at different times) were given ammonium acetate in concentrations of 0% or 20% by weight (about 18 000 mg/kg body weight and day, conversion factor 0.09 (for data from subchronic studies) according to EFSA (2012)) with the diet from day 1 of gestation up to postnatal day 21. After weaning, the offspring continued to receive the diet containing ammonium acetate from postnatal days 120 to 150 (male animals: n = 12, female animals: n = 9) or diet without the test substance (male animals: n = 8, female animals: n = 13). The offspring of the control group consisted of 7 male and 8 female animals. In the offspring, only the body weight gains were recorded, which was lower in the exposed animals. Other parameters were likewise not reported for the dams. In the isolated cerebellar neurons of the offspring treated with ammonium acetate prenatally up to 7 to 8 days of age, long-lasting impairment of the *N*-methyl-*D*-aspartate (NMDA) receptor function was observed, and thus protection against the toxicity of glutamate and NMDA (Miñana et al. 1995). Due to the very limited scope of the study, it cannot be used to evaluate developmental toxicity.

In the unpublished screening study according to OECD Test Guideline 422 from 2002 described in the previous section, no treatment-related abnormalities were observed in the offspring up to the highest dose tested of 1500 mg diammo-

nium phosphate/kg body weight and day. The NOAEL for effects on the offspring is therefore 1500 mg diammonium phosphate/kg body weight and day (OECD 2007).

A number of in vitro studies with ammonium compounds are available, the results of which do not indicate any specific reproductive toxicity (Health Council of the Netherlands 2009). These studies are not described here further.

Manifesto (MAK value/classification)

The critical effect of ammonia is local irritation in humans.

MAK value. In a study with short-term whole-body exposure for 4 hours of 43 healthy male subjects with and without habituation to ammonia, 3 of 33 subjects (9%) without habituation experienced slight conjunctival reddening at 50 ml/m^3 (Hoffmann et al. 2004; Ihrig et al. 2006). The irritation was probably caused by the air circulation generated by ventilators and is therefore not ammonia-specific. Due to the low degree of severity on the one hand and the reversibility of the effect on the other hand, it would, in any case, not be interpreted as an adverse effect. From this study, the NOAEC for local irritation in male test persons is 50 ml/m^3. In another study with 12 subjects (5 men, 7 women) a NOAEC for local irritation of 25 ml/m^3, the highest concentration tested, was derived (Sundblad et al. 2004). In 18 volunteers without (8 men and 10 women) and 19 with seasonal allergic rhinitis (10 men and 9 women), who were exposed for a total of 4 hours to variable concentrations ranging from 0 to 40 ml/m^3 that changed every half an hour (average concentration: 20 ml/m^3), physiologically detectable signs of sensory irritation (blinking frequency) were found, but these were not reflected in the biochemical parameters (Pacharra et al. 2017). In the exposure scenario investigated, the average concentration of 20 ml/m^3 was found to be close to the NOAEC, with marked odour effects and probably reversible sensory irritation. The apparent discrepancy between the NOAECs from the 3 studies is due to the concentrations applied and the higher sensitivity of women, compared with men, for changes in blinking frequency (Ernstgård et al. 2002).

Extrapolation for time need not be taken into account here, since an increase in the blinking frequency is not to be expected for an 8-hour exposure.

Several studies of the (sensory irritation) lateralization threshold, all of which yielded values above 20 ml/m^3 (Monsé et al. 2016; Petrova et al. 2008; Smeets et al. 2007; Wise et al. 2005), also support a MAK value of 20 ml/m^3.

The data suggest that even persons with seasonal allergic rhinitis should be protected from the adverse effects of ammonia at the MAK value of 20 ml/m^3 (Pacharra et al. 2017).

Overall, the available data confirm the previous MAK value of 20 ml/m^3, which has therefore been retained.

Peak limitation. As the critical effect is local, Peak Limitation Category I has been retained.

In the study with 43 male volunteers, reversible slight conjunctival reddening, determined by slit lamp, was observed at 50 ml/m^3 (Hoffmann et al. 2004); this is regarded as non-specific and not adverse (see above). Excursion factor 2 can therefore be retained. A (repeated) acute increase in ammonia levels, following a baseline exposure of 20 ml/m^3, was perceived by all subjects, regardless of their habituation. However, a 30-minute short-term exposure to 40 ml/m^3 did not lead to significant changes in physical findings. Even though subjective complaints increased with increasing exposure levels, no acute habituation effect was observed during a 4-hour exposure day (Hoffmann et al. 2004).

The slightly increased blinking frequency during short-term exposure to 40 ml/m^3 (Pacharra et al. 2017) does not contradict the excursion factor of 2, as it was still within a range that can be regarded as physiologically "normal". All in all, adverse effects are therefore not to be expected with an excursion factor of 2, which corresponds to 40 ml/m^3.

Prenatal toxicity. Valid prenatal developmental toxicity studies according to valid test guidelines are not available for ammonia or ammonium compounds. In a prenatal developmental toxicity study, which is of only limited meaningfulness, no teratogenic effects were found in Sprague Dawley rats at the only dose tested of about 367 mg

NH_4^+/kg body weight and day, administered with the drinking water from gestation days 7 to 10 (Goldman and Yakovac 1964).

The high detoxification capacity of the liver for inhaled ammonia was already noted in the supplement of 1986 (Henschler 1993). After exposure in the range of the MAK value of 20 ml/m³ (14 mg/m³), about 140 mg of ammonia is absorbed per day assuming complete absorption. Assuming complete metabolism, about 250 mg of urea is formed. In comparison, the daily urinary excretion in a healthy adult is 20 to 35 g urea. Endogenous ammonia formation in the intestine is about 4.2 g per person and day (Henschler 1993). Experiments have demonstrated that rats do not have elevated blood ammonia levels after continuous inhalation exposure to ammonia concentrations of up to 32 ml/m³ for 24 hours (Schaerdel et al. 1983) and after inhalation exposure to ammonia concentrations of up to 25 ml/m³ for 6 hours daily for 15 days (Manninen et al. 1988).

Since the detoxification capacity can be assumed sufficient for exposure at the level of the MAK value and increased ammonia levels in the blood are not to be expected, the classification of ammonia in Pregnancy Risk Group C at the MAK value of 20 ml/m³ has been retained.

Absorption through the skin. Studies of the absorption of ammonia through the skin are not available. The MAK value for ammonia is based on the marked local irritation and not on systemic effects. The latter are only to be expected at exposure levels leading to changes in the endogenous nitrogen balance (Henschler 1993). This cannot be achieved by inhalation because of the irritant effect. Due to the high vapour pressure and the low boiling point, prolonged dermal contact is unlikely; this also applies to aqueous solutions. Therefore, the substance has not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There are no data available for sensitizing effects in humans, animals or in vitro. Ammonia has therefore not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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