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Isoflurane

MAK Value Documentation, addendum – Translation of the German version from 2022

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Keywords

isoflurane; reproductive toxicity; neurotoxicity; developmental neurotoxicity; MAK value; maximum workplace concentration; peak limitation

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) summarized and evaluated the data for isoflurane [26675-46-7] to derive an occupational exposure limit value (maximum concentration at the workplace, MAK value) considering all toxicological end points. Relevant studies were identified from a literature search. The critical effects are neurotoxicity in humans as well as liver toxicity and effects on reproductive organs in animals. Data on liver toxicity at non-anaesthetic concentrations are not available for humans. From a long-term study in male rats, a NOEC of 20 ml/m^3 can be derived for liver toxicity, cytochrome P450 (CYP) content and serum levels of alanine aminotransferase. By analogy with halothane, the estimated NAEC for pre-narcotic effects in humans is 92 ml isoflurane/m^3. Since the halothane exposure lasted 3–4 hours and the steady state for isoflurane is reached after about 100 minutes, and because a pre-narcotic effect depends only on the concentration, an effect amplification with time is not to be expected. Therefore, for the neurotoxic effect, a maximum concentration at the workplace (MAK value) of 92 ml/m^3 would be derived. The most sensitive endpoint is reproductive toxicity in male rats with a NOAEC of 50 ml/m^3. On this basis, a MAK value of 2 ml/m^3 has been set. For the derivation of the MAK value a C × T dependence was assumed for the critical effect on the testes. Therefore, an excursion factor of 8 can be set. There are no studies in neonatal or juvenile animals at non-anaesthetic concentrations from which to derive a NOAEC for developmental neurotoxic effects of isoflurane. Therefore, isoflurane is assigned to Pregnancy Risk Group D. New studies on the carcinogenic effect of isoflurane are not available. In a long-term study in mice, no increased tumour incidences were observed up to a concentration of 4000 ml/m^3. No clear genotoxic potential of isoflurane can be determined from the available data and further clarification is needed, especially in the concentration ranges present at the workplace. Skin contact is expected to lead to a relatively minor contribution to systemic toxicity. Limited data do not show clear evidence of a sensitizing potential.

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MAK value (2021)	**2 ml/m³ (ppm) ≙ 15 mg/m³**
Peak limitation (2021)	**Category II, excursion factor 8**
Absorption through the skin	–
Sensitization	–
Carcinogenicity	–
Prenatal toxicity (2021)	**Pregnancy Risk Group D**
Germ cell mutagenicity	–
BAT value	–
CAS number	26675-46-7
Molar mass	184.5 g/mol
Density at 20 °C	1.45 g/cm³ (IFA 2021)
Vapour pressure at 20 °C	320 hPa (IFA 2021)
log K_{OW}	2.06 (NCBI 2024)
Solubility	4470 mg/l water (NCBI 2024)
1 ml/m³ (ppm) ≙ 7.656 mg/m³	**1 mg/m³ ≙ 0.131 ml/m³ (ppm)**

Documentation for isoflurane was published in 1993 (Greim 1996) followed by a supplement in 2007 (Hartwig and MAK Commission 2016). As new data for toxicity have become available, it is evaluated whether a MAK value can be derived.

In clinical publications, concentrations of inhalation anaesthetics are usually expressed as a percentage by volume or as MAC (= minimum alveolar concentration, the concentration at which 50% of patients no longer react to pain stimulus). The MAC is a measure of the potency of an inhalation anaesthetic. The MAC varies depending on the age, size and weight of the patient, and the combination of the substance with other inhalation anaesthetics. In humans, the MAC for isoflurane in 100% oxygen or together with 50% to 70% nitrous oxide (N_2O) is in the range of 11 500 to 12 200 ml/m³. The concentrations are much higher if general anaesthesia is induced and maintained with isoflurane alone in ambient air. MAC values for isoflurane of 14 000 ml/m³ in rats and of 13 000 to 17 000 ml/m³ in mice were reported (Saber and Hougaard 2009).

1 Toxic Effects and Mode of Action

Isoflurane is a halogenated methyl ethyl ether with depressive effects on the central nervous system; the substance is used globally in large quantities as an inhalation anaesthetic. Isoflurane has analgetic properties and is a muscle relaxant. Postoperative neurological and psychomotor disorders can develop. In narcotic concentrations, isoflurane has marked effects on the functions of the heart and circulatory system and reduces the glomerular filtration rate by 30% to 50% (Greim 1996). The first pre-narcotic effects of isoflurane become noticeable in humans at concentrations of 1150 ml/m³ and above. In male rats, effects on the reproductive organs, lower sperm counts and decreased sperm production in addition to lower concentrations of testosterone in serum were found at isoflurane concentrations of 300 ml/m³ and above.

In rodents exposed to concentrations that induce anaesthesia (6000 ml/m^3 and above), reduced foetal weights, delayed ossification, an increased incidence of late resorptions and evidence of an increased occurrence of cleft palates in mice were found. Prenatal exposure also caused postnatal effects on behaviour in mice and rats. The findings from mechanistic studies suggest that the developing brain is vulnerable to the effects of isoflurane, responding with neurodegeneration. There is evidence indicating that isoflurane induces clastogenic effects in vitro. The studies that examined the genotoxic effects caused by inhalation exposure of animals investigated only narcotic concentrations; these reported findings of DNA damage (comet assay). To date, isoflurane has not been shown to induce carcinogenic effects.

As there are few findings relating to occupational exposure to isoflurane, it is not clear whether isoflurane induces sensitizing effects on the skin and airways. There are no data from animal studies or in vitro data available for this end point.

2 Mechanism of Action

2.1 Hepatotoxicity

Enflurane and isoflurane began to be used in place of halothane to prevent the disease known as idiosyncratic hepatitis. Unlike direct toxic liver damage, this form of liver damage is unpredictable, not dependent on the dose and the risk is increased with repeated exposure. There are 2 types of idiosyncratic reactions, metabolic and allergic (Gröger et al. 2011; Höner zu Siederdissen and Cornberg 2016). Metabolic idiosyncrasy is an abnormal, presumably genetically determined metabolic reaction to an actually non-toxic substance with the formation of toxic metabolites (Gröger et al. 2011).

An immunological mechanism is responsible for the allergic liver damage caused by halothane. This mechanism involves the formation of trifluoroacetyl adducts with proteins from the hepatocytes, leading to the development of antibodies (Gröger et al. 2011; Höner zu Siederdissen and Cornberg 2016). Trifluoroacetyl chloride is produced as a reactive intermediate when halothane undergoes oxidation to form trifluoroacetic acid; at narcotic concentrations, 15% to 20% of the halothane is metabolized. By contrast, after surgical procedures using isoflurane as an anaesthetic, less than 0.2% of the isoflurane was metabolized to fluorides and recovered in the urine (Greim 1996). Reports of severe liver damage in humans following anaesthesia with isoflurane are quite rare (see Section 4.1) (no author 2018). In rats exposed for 8 hours to various inhalation anaesthetics at 1.25 times the MAC, immunohistochemical analysis revealed trifluoroacetyl-protein adducts after exposure to halothane, enflurane and isoflurane. Halothane had the highest reactivity. Positive results were obtained in the ELISA test if the hepatocytes of rats treated with halothane or enflurane were incubated with serum from patients diagnosed with halothane hepatitis, but not if the rats had been treated with isoflurane, desflurane or oxygen (Njoku et al. 1997).

However, it was proposed that halothane hepatitis may, to some extent, be caused by the interplay of several factors such as sex, genetic factors, fasting and stress resulting from the induction of inflammatory responses in the body (Dugan et al. 2010).

2.2 Neurotoxicity

Isoflurane induces its hypnotic effect via γ-aminobutyric acid ($GABA_A$) receptors (with β3-subunit), while motionlessness is produced by proportionate modulation of *N*-methyl-D-aspartate (NMDA), glycine and $GABA_A$ receptors, and potassium-2P channels (Eckle et al. 2009). The NMDA receptor plays an important role in various physiological processes such as memory, learning and neuronal development. The inhibition of NMDA receptors during synaptogenesis interferes with the formation of neuronal networks, leading to an impairment of learning and memory processes. This mechanism forms the basis of adverse outcome pathway (AOP) 13 and is relevant for developmental neurotoxicity. The molecular initiating event is described as the chronic binding of an antagonist to NMDA receptors in neurons during

synaptogenesis in the hippocampus (one of the brain structures critical for learning and memory formation) (Sachana et al. 2018). In vitro, isoflurane was found to compete for the binding of glycine on the NMDA receptor, thereby inhibiting the receptor (Dickinson et al. 2007). Animal studies investigating the mechanism of action of isoflurane used narcotic concentrations or concentrations at which pre-narcotic effects, such as a reduced righting reflex, were clearly noticeable. After exposure of rats to isoflurane concentrations of 12 000 to 30 000 ml/m^3, interleukin-1β and tumour necrosis factor-α were increased and accumulated in the hippocampus region, suggesting neuroinflammation. At the same time, β-amyloid peptide levels increased. The activation of caspase-3 expression caused increased apoptosis of glial cells and neurons. An increase in HIF-1α (hypoxia-inducible factor-1α), VEGF (vascular endothelial growth factor), MMP-2 (matrix-metalloproteinase 2) in addition to a decrease in type IV collagen and occludin (transmembrane proteins) in the hippocampus are signs of damage to the blood–brain barrier (see Table 4). The developing brain is particularly vulnerable to neuroapoptotic effects, as shown in young rats and mice (see Section 5.5.2).

Groups of three 18-month-old mice were anaesthetized for 2 hours with 1.4% isoflurane (14 000 ml/m^3) in 100% oxygen. The control animals were exposed to pure oxygen (100%). After exposure to isoflurane, the level of phosphorylated histone protein H2A (variant X (γH2A.X)) in the prefrontal cortex of the brain was 2.4-fold in comparison with the control level (Ni et al. 2017).

2.3 Fertility disorders

In male rats, damage to the seminiferous tubules and the spermatocytes as well as reduced testosterone levels were observed at isoflurane concentrations of 300 ml/m^3 and above. A decrease in the follicle-stimulating hormone (FSH) in serum was detected after exposure to isoflurane concentrations of 1800 ml/m^3 and above (Xu et al. 2012). Another study found reduced levels of testosterone, luteinizing hormone (LH) and FSH in serum after 25 days. In addition, the levels of gonadotropin-releasing hormone (GnRH) in the brain were decreased. The expression of genes relevant for reproduction in the hypothalamus (*AR, Kiss-1, GPR54*) and in the pituitary gland (*LH-β, FSH-β, GnRH-R*) was reduced (Ding et al. 2015). Both authors propose that the effects on the morphology of the testes and on the sperm induced by exposure to isoflurane, also at non-narcotic concentrations, may be caused not only by effects on the androgen receptor-kisspeptin-GPR54 signalling pathway in the hypothalamus, but also by direct effects on the testes.

3 Toxicokinetics and Metabolism

The data for the toxicokinetics of isoflurane were presented in detail in the documentation published in 1993 (Greim 1996) and in the supplement from 2007 (Hartwig and MAK Commission 2016).

3.1 Absorption

Inhalation is the main route by which isoflurane is absorbed at the workplace. After 10 healthy, pregnant women were anaesthetized with isoflurane in a concentration of 6000 ml/m^3, isoflurane concentrations of 24 mg/l were determined in the maternal blood and of 70 mg/l in the umbilical blood (Saber and Hougaard 2009).

Exposure to liquid isoflurane is highly unlikely because isoflurane has a high vapour pressure. The fraction of isoflurane that is absorbed by rats via the skin after exposure to an external isoflurane concentration of 50 000 ml/m^3 is equivalent to 0.1% of the amount taken up by inhalation (McDougal et al. 1990).

3.2 Distribution

Only the parameters that are important for the derivation of a MAK value are reviewed again below. Isoflurane has a half-life of about 20 minutes in well-perfused organs such as the brain, liver, heart and kidneys (Greim 1996; Hartwig and MAK Commission 2016); therefore, the steady state is reached after about 100 minutes (5 half-lives).

The partition coefficients for isoflurane in humans are shown in Table 1.

Tab. 1 Partition coefficients for isoflurane in humans

Blood:air	Urine:air	Lipid:air	Tissue:air				
			brain	liver	kidneys	muscles	heart
1.42[a)]	0.72[b)]	69.68[a)]	2.23[a)]	3.12[a)]	1.75[a)]	3.01[a)]	2.2[c)]

a) Meulenberg and Vijverberg 2000
b) Accorsi et al. 2001
c) Saber and Hougaard 2009

4 Effects in Humans

4.1 Single exposures

Isoflurane has a mildly pungent musty ethereal odour (Saber and Hougaard 2009). An odour threshold is not known.

In very rare cases, mostly in women, a single or repeated anaesthesia with isoflurane impaired liver function (Hasan 1998; Malnick et al. 2002) and caused liver failure and death (Martin et al. 2001; Peiris et al. 2012; Turner et al. 2000) (see also Section 2). The effects, including a marked increase in liver enzymes in serum, yellow discoloration of the liver and necrotic areas, were very similar to those that occur with halothane hepatitis. Antibodies against trifluoroacetylated proteins were found in 3 cases (Gunaratnam et al. 1995; Martin et al. 2001; Meldrum et al. 1998). Liver enzymes were increased in the serum of patients who had received anaesthesia with isoflurane and with halothane with a long interval between the 2 exposures (Gunaratnam et al. 1995; Malnick et al. 2002; Nishiyama 2013). Cross-reactivity between the inhalation anaesthetics halothane and isoflurane was assumed. However, this was not confirmed by the findings of a retrospective study that compared the activities of various liver enzymes in patients who had been administered inhalation anaesthetics on several occasions during surgery (Nishiyama 2013).

Eight male test persons were exposed to isoflurane in such a way that subnarcotic concentrations of isoflurane of 1150, 2300 and 4600 ml/m^3 (0.1, 0.2 and 0.4 MAC; 1 MAC = 11 500 ml/m^3) in 100% oxygen were reached after 20 minutes. Each test person was exposed to isoflurane at each of the concentrations with at least 1 week in between exposures. Two lists of 15 words each were read aloud to the test persons and their response to commands was tested. After exposure to 4600 ml/m^3, the test persons failed to respond to commands and no eyelash reflex was discernible in 5 of 8 test persons. At 2300 ml/m^3, 7 of 8 test persons opened their eyes and the eyelash reflex was present in all. The response to commands was impaired to varying degrees in all test persons. After exposure to 1150 ml/m^3, the ability of 3 test persons to respond to commands was impaired. One hour after the end of exposure to a concentration of 4600 ml/m^3, none of the test persons was able to recall the terms that had been read to them. Most of the test persons showed a marked impairment of memory even at the lowest concentration (Newton et al. 1990). This study cannot be used to derive a NOAEC (no observed adverse effect concentration) for acute neurotoxic effects. The lowest concentration tested of 1150 ml/m^3 is the LOAEC (lowest observed adverse effect concentration).

Neurotoxicity – comparison with halothane

Neurotoxicity was evaluated by comparison with halothane. Halothane is the only halogenated anaesthetic for which data for neurotoxicity in humans are available after exposure to only that substance. In a study with 9 men and 1 woman (anaesthetists, anaesthetic technicians) who were exposed for 3 to 4 hours to halothane concentrations of 100 to 150 ml/m^3, no effects with statistical significance were observed in the visual response test. The test was carried out during exposure with each test person acting as his or her own control (Smith and Shirley 1977). In another study carried out by Cook et al. (1978) with 29 male students, no toxic effects on behaviour were observed in a memory test (digit span test) and in an audiovisual reaction test up to a halothane concentration of 200 ml/m^3 with an exposure period of 0.5 hours. On the basis of the NOAEC for halothane of 100 ml/m^3, a halothane concentration in the brain of 0.025 mM was calculated by applying the corresponding partition coefficients (see Table 2). Taking into consideration that about 60% of halothane is metabolized in the concentration range of 20 to 100 ml/m^3 (Dallmeier and Henschler

1981), a halothane concentration in the brain of 0.0083 mM is determined at this NOAEC. As the 2 inhalation anaesthetics have similar molecular structures and pharmacodynamic properties, it is assumed that the concentration of 0.0083 mM can likewise be regarded as the NOAEC for neurotoxicity induced by isoflurane. The concentration of 0.0083 mM in the brain is equivalent to an isoflurane concentration of 92 ml/m³ in the air. On this basis, a NOAEC for acute neurotoxic effects induced by isoflurane in humans of 92 ml/m³ has been established.

Tab. 2 Isoflurane and halothane concentrations in brain calculated from concentrations in air and partition coefficients

	Molar mass	Concentration in the air		Blood:air partition coefficient	Concentration in the blood		Brain:blood partition coefficient	Concentration in the brain	Concentration in the air at 0.0083 mM
		(ml/m³)	(mg/l)		(mg/l)	(mM)		(mM)	(ml/m³)
halothane	197.4	7700 (MAC)	63.07	2.51	158.3	0.802	2.21	1.77	
		100 (NOAEC)	0.82	2.51	2.06	0.010	2.21	0.025	
isoflurane	184.5	12000 (MAC)	91.87	1.4	128.6	0.697	1.57	1.095	92

MAC: minimum alveolar concentration

4.2 Repeated exposure

4.2.1 Hepatotoxicity and nephrotoxicity

It is stated in the supplement from 2007 that nephrotoxic effects had yet to be observed after anaesthesia with isoflurane (Hartwig and MAK Commission 2016).

The studies that were published after 2006 are described below.

Statistically significant increases in enzyme activity and in the concentrations in the blood of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γ-GT), total bilirubin and lymphocytes determined in 119 health care workers could not be attributed solely to exposure to isoflurane because there was concurrent exposure to halothane, enflurane and N_2O. The exposed workers reported headaches, weakness, arrhythmia, allergic reactions, gastritis, rhinitis, laryngitis, herpes and menstrual disorders more frequently than the 184 control persons without exposure to these substances (Casale et al. 2014).

A cohort study investigated liver and kidney toxicity in 52 persons (anaesthetists, surgeons, nurses) working in a hospital in Iran. The control group was composed of 52 hospital administrators who were not exposed but matched the exposed persons in age, weight, size, body mass index (BMI) and length of employment. The group of 52 exposed persons included 51 non-smokers and the control group 50 non-smokers. The mean duration of exposure was 10.79 ± 5.63 years (exposed persons) and the length of employment 8.69 ± 6.57 years (control persons). The following serum parameters were determined: ALT, AST, alkaline phosphatase (ALP), γ-GT, albumin, total protein, bilirubin, urea, creatinine, calcium, phosphorus, potassium, α-glutathione-*S*-transferase (α-GST) and KIM-1 (type 1 transmembrane glycoprotein: biomarker for nephrotoxicity). Urine samples were analysed after the 3-hour morning shift. The concentrations in the urine were: isoflurane 4.95 ± 3.43 µg/l (range: 0.78–14.9) (the unit ppm (mg/l) used in the original publication was corrected by the authors to µg/l in an erratum, Neghab et al. 2021), N_2O 175.8 ± 77.52 µg/l (range: 7.98–319.91) and sevoflurane 15.03 ± 16.06 µg/l (range: 0.76–46.40). After adjusting for age, sex and BMI, the levels of AST, ALT, γ-GT, α-GST, creatinine, KIM-1 and calcium in the serum of exposed persons were increased with statistical significance in comparison with the levels found in the control persons (Neghab et al. 2020 a). The study is not suitable for the evaluation of the liver enzymes and kidney parameters that were determined in serum because the operating theatre personnel were exposed to several inhalation anaesthetics and a reliable adjustment for alcohol consumption cannot be made.

A cross-sectional study was carried out with 53 anaesthetists and surgeons who were exposed to inhalation anaesthetics for 40 hours a week for an average period of 10 years; 90% of the time to a mean isoflurane concentration of 6 ml/m³ and a mean sevoflurane concentration of 6 ml/m³, and about 10% of the time to a desflurane concentration of

15 ml/m³ and a N_2O concentration of 100 ml/m³. The following parameters were analysed in the blood: ALT, AST, γ-GT, ALP, C-reactive protein (hs-CRP), adrenocorticotropin (ACTH), cortisol and prolactin. In comparison with the levels determined in a control group without exposure to anaesthetics, none of the parameters were increased with statistical significance. If men and women were evaluated separately, the prolactin and cortisol concentrations were increased in the men with statistical significance in comparison with the values determined in the control group (Aun et al. 2021).

4.2.2 Neurotoxicity

Studies of neurotoxicity induced by anaesthesia with isoflurane are not included in the evaluation and thus not discussed in this addendum.

In 112 hospital workers who were exposed to N_2O and isoflurane in the operating theatre, no differences in behaviour (complex reaction time test, subjective stress levels, basic intellectual abilities) were observed in comparison with the findings for 135 test persons in the control group. The concentrations of halogenated gases in the ambient air of the operating theatres were expressed as a geometric mean of 0.4 ml/m³ (95th percentile 3.8, range 0.1–6.2) for the 1st day of the working week and of 0.3 ml/m³ (95th percentile 2.7, range 0.1–3.9) for the last day; the nitrous oxide concentrations were 23.2 ml/m³ (95th percentile 127, range 3–183) and 20.6 ml/m³ (95th percentile 114, range 4–154.5), respectively. The determinations were performed with stationary monitors. The geometric mean of urinary isoflurane at the end of the shift was 0.7 µg/l (95th percentile 2.6, range 0–4.7) on the first day and 0.8 µg/l (95th percentile 2.0, range 0–5.6) on the last day of the working week (Lucchini et al. 1997).

In 51 members of surgical teams who were exposed to N_2O (mean 36 ml/m³, range 5–360 ml/m³), halothane (mean 0.5 ml/m³, range 0.1–6.2 ml/m³) or isoflurane (median 0.5 ml/m³, range 0.2–24.1 ml/m³), no neurotoxic effects (determination of reaction time, Wechsler Adult Intelligence Scale) or effects on behaviour were observed. The control group was composed of 31 persons without exposure (Marraccini et al. 1992).

The studies available for evaluation do not provide evidence that isoflurane induces neurotoxic effects at low concentrations of on average 0.5 ml/m³ up to a maximum of 24.1 ml/m³.

A balance control analysis was carried out with 53 female and male nurses who were exposed to N_2O, isoflurane, desflurane and sevoflurane for more than 5 years. The mean 8-hour time average concentration of N_2O was 59 ml/m³ (median 45 ml/m³, range 10–100 ml/m³). Without providing specific data, the concentrations of isoflurane, desflurane and sevoflurane were given to be lower by a factor of 10. Another group of 53 female and male nurses working at the same hospital who were not exposed to anaesthetic gases were used as controls. The study found that the exposed nurses had slight postural instability (Vouriot et al. 2005). Only one end point was determined. It is improbable that the findings of peripheral neurotoxicity are attributable to isoflurane because of its effects on the central nervous system. In addition, there was exposure to a mixture of substances.

4.3 Local effects on skin and mucous membranes

The supplement from 2007 (Hartwig and MAK Commission 2016) included a study with 11 male volunteers who were exposed for 15 seconds to the MACs of enflurane (1.68%), halothane (0.77%), isoflurane (1.15%) or sevoflurane (1.71%) and, after a 15-minute break, to the test substances at 2-fold MAC. After exposure to isoflurane, 3 of the 11 volunteers reported an urge to cough and subjective irritation that was more pronounced than that experienced after exposure to the other anaesthetic gases. Changes to certain respiratory parameters (decrease in volumetric capacity, increase in respiratory frequency and changes to the expiratory reserve volume) were observed most frequently after inhalation of isoflurane.

4.4 Allergenic effects

The mechanism of idiosyncratic hepatitis is discussed in Section 2.

4.4.1 Sensitizing effects on the skin

The supplement from 2007 includes 2 case studies of anaesthetists. However, these case studies do not provide clear evidence of contact sensitizing effects (Hartwig and MAK Commission 2016). New studies have not been published since that time.

4.4.2 Sensitizing effects on the airways

After handling isoflurane and sevoflurane for 4 years, a person who worked in an operating theatre developed workplace-related respiratory symptoms (dyspnoea, rhonchus, angina pectoris, rhinitis) that decreased in severity on days off work. A delayed asthmatic reaction was observed in the bronchial provocation test (decrease in the forced expiratory volume in 1 second (FEV_1) by up to 32% 4–5 hours after exposure), while the $PD_{20(methacholine)}$ value decreased from above 4800 µg to 2127 µg in the methacholine provocation test. Although this does not fulfil the standard positivity criterion requiring a decrease by a factor of 3 (Chan-Yeung et al. 2003; Ochmann and Nowak 2016), this finding suggests an increase in non-specific respiratory sensitivity. Although this person had tolerated general anaesthesia without symptoms on four previous occasions, renewed exposure to sevoflurane caused an anaphylactic reaction. Another person responded for the first time with an asthmatic reaction after having handled isoflurane and sevoflurane for 17 years. However, the authors regarded the results of the bronchial provocation test that showed a 15% decline in the FEV_1 after 8 hours as questionable. After working in the operating theatre for only 3 years, a third person developed generalized redness of the skin and swelling of the face that frequently was accompanied by the feeling of a tightness in the chest. Provocation tests with isoflurane or sevoflurane did not affect the FEV_1; however, a pruritic skin rash developed after exposure to isoflurane. Inhalation of isoflurane led to a decrease in the $PD_{20(methacholine)}$ value from above 4800 µg to 1745 µg in the methacholine provocation test (Vellore et al. 2006). The Commission regards these findings as suggestive of an immunological reaction after occupational exposure to isoflurane.

Anaphylactoid reaction to isoflurane

In a 13-year-old patient, severe haemodynamic instability occurred during general anaesthesia that was induced with midazolam, fentanyl, propofol and atracurium and maintained with isoflurane (1% in 100% O_2). Five minutes after starting isoflurane, severe hypotension and tachycardia developed. As a result of the clear temporal relationship and the administration of intravenous anaesthesia without complications 2 weeks later, the authors suspect an anaphylactoid reaction to isoflurane. Allergological tests were not carried out (de Souza Hobaika et al. 2007). A 7-year-old female patient was premedicated for anaesthesia with a mixture of atropine, codeine and diazepam. Anaesthesia was induced with N_2O-O_2 (60% : 40%) and halothane, which was switched to isoflurane during the operation. About a year later, a second operation was carried out using the same anaesthetic regimen (switching from halothane to isoflurane after 10 minutes). Within 5 minutes of starting isoflurane, a generalized erythematous rash appeared, accompanied by bronchospasm and tachycardia. There were no signs of shock or hypotension. After immediate discontinuation of isoflurane, intubation and treatment with antihistamines, the skin reaction and bronchospasm disappeared. Surgery was continued with halothane without further complications. Again, no allergological tests were carried out (Slegers-Karsmakers and Stricker 1988).

Symptoms of acute coronary syndrome developed in a 2-year-old patient during a surgical procedure for atrial septal defect repair and a subsequent surgical operation. The authors postulated that Kounis syndrome may have been responsible for the symptoms. As the patient had been given rocuronium and isoflurane during anaesthesia in both cases, the authors suggested that 1 of the 2 drugs may have been the cause. However, allergological tests were not carried out (Parent et al. 2011).

As the complications occurred after the administration of different anaesthetics in the described cases and no allergological tests were carried out, the reported cases cannot be used to evaluate the sensitizing effects on the respiratory tract.

4.5 Reproductive and developmental toxicity

There are no epidemiological studies that investigated the effects on the male reproductive organs or on male fertility.

Sperm samples collected from healthy men were exposed to isoflurane (0, 1.4, 2.8, 4.2 or 5.6 vol%) in vitro for 0.5 to 4 hours. In each concentration group, sperm motility and sperm vitality were increased with statistical significance in comparison with the values determined in the control samples without exposure. Sperm motility and sperm vitality decreased with the increasing length of the incubation period (Wang et al. 2008).

Since the publication of the supplement in 2007, several reviews have become available that studied the association between reduced fertility, premature births, cognitive effects and miscarriages and exposure to anaesthetic gases at the workplace (Boivin 1997; Nilsson et al. 2005; Oliveira et al. 2021 (incidence of spontaneous abortions, 18 studies); Quansah and Jaakkola 2010). According to Oliveira et al. (2021), about half of the studies carried out prior to 2000 found an association between exposure to anaesthetic gases in the ambient air and a higher incidence of spontaneous abortions. This association was not found by studies carried out after 2000. All of the authors stated that a conclusive evaluation of the risk of toxic effects on development cannot be made due to the heterogeneity of the studies. None of the studies listed provide data for the level of exposure to the various inhalation anaesthetics. In addition, in most cases it is unclear to which inhalation anaesthetics the staff members had been exposed. For this reason, no conclusions can be drawn with respect to the reproductive toxicity of isoflurane. Therefore, there is evidence that suggests that exposure to inhalation anaesthetics may lead to toxic effects on reproduction, but these findings must be studied further with respect to isoflurane.

4.6 Genotoxicity

The studies published up to 2005 were discussed in detail in the 2007 supplement (Hartwig and MAK Commission 2016). All studies with exposure data that were published from 2005 onwards are shown in Table 3 and described in detail. Studies that do not include exposure data (Chandrasekhar et al. 2006; El-Ebiary et al. 2012; Izdes et al. 2010; Reitz et al. 1994; Rozgaj et al. 2009; Szyfter et al. 2016) have not been included in the evaluation.

4.6.1 Workplace studies

The lymphocytes of 153 female staff members who worked in the operating theatre were examined for chromosomal aberrations, sister chromatid exchange (SCE) and micronuclei. The control groups were composed of 197 female radiologists without exposure to inhalation anaesthetics and 153 persons from the general population. The group of exposed personnel included 99 smokers and 54 non-smokers. The controls were not divided into groups of smokers and non-smokers. Two readings were taken to determine the concentrations of isoflurane. The first reading was taken in a ventilated operating theatre; the isoflurane concentration was < 0.1 ml/m^3 and the N_2O concentration was 0.1 to 0.27 ml/m^3. The operating theatre was not ventilated when the second reading was taken and the concentrations were found to be 3.9-fold and 4.7-fold as high as the permissible exposure concentration. However, this concentration was not specified. The halothane concentration was about 0.5 ml/m^3. The incidences of chromosomal aberrations, sister chromatid exchange and micronuclei were increased with statistical significance in the exposed personnel in comparison with the levels determined in the 2 control groups. The incidence of micronuclei correlated also with age and the incidence of sister chromatid exchange with the duration of employment (Bilban et al. 2005). The study is not suitable for the evaluation of genotoxicity induced by isoflurane because of the unclear exposure data, the additional exposure to N_2O and halothane, the additional positive correlation with age and the inadequate biostatistics.

In 55 female nurses and 29 male anaesthetists, no correlation was found between increased DNA damage (comet assay) in the blood leukocytes and isoflurane concentrations in the range of 0.07 to 1.9 ml/m^3. The concentrations were obtained by stationary determinations. However, a correlation ($r = 0.56$) was found between the N_2O concentration (range: 6–1502 ml/m^3) and DNA damage. The control group was composed of 52 female nurses and 31 physicians who were not exposed to inhalation anaesthetics. Smokers made up 40% to 54% of the control group. The groups were

adjusted for age, sex and smoking status. Excluded from the study were smokers who smoked less than 10 cigarettes and persons with exposure to X-rays (Wrońska-Nofer et al. 2009).

The incidences of DNA strand breaks in the lymphocytes and of micronuclei in the epithelial cells of the oral mucosa were examined in 20 male and 10 female anaesthetists who had been exposed to anaesthetic gases in the operating theatre for on average 16 years (2–44 years). Fasting samples were taken in the morning. A control group was composed of persons without exposure who were matched for age, sex and lifestyle (including diet, physical activity, alcohol and tobacco consumption). Stationary determinations in the breathing zone of the anaesthetists during surgery found concentrations of isoflurane of 5.5 ± 4.4 ml/m^3 (0.4–16.5), of sevoflurane of 7.7 ± 8.7 ml/m^3 (0.2–34.4), of desflurane of 16.4 ± 6.0 ml/m^3 (8.2–23.2) and of N_2O of 150.3 ± 135.7 ml/m^3 (61–350, time-weighted average (TWA): 178 ± 152). In the exposed group, the incidence of micronuclei in the epithelial cells of the oral mucosa was increased with statistical significance and the number of basal cells was decreased. Likewise, the incidences of karyorrhexis (nuclear fragmentation) and pyknosis were increased in the exposed group with statistical significance in comparison with the levels determined in the control group. The incidence of DNA strand breaks in the lymphocytes was not increased (Souza et al. 2016).

The blood lymphocytes of 10 surgeons (7 men, 3 women), 18 male nurses, 16 female nurses and 16 technicians (5 men, 11 women) working in operating theatres were examined for the increased formation of micronuclei and chromosomal aberrations. The control group was composed of 60 male and female nurses who were not exposed to inhalation anaesthetics. Concentrations of 2.4 ± 0.9 ml/m^3 (0.5–4.2) for isoflurane and 0.2 ± 0.1 ml/m^3 (0.01–0.59) for sevoflurane were determined by personal air monitoring and concentrations of 851 ± 920 ml/m^3 (10–2895) were obtained for N_2O by stationary determinations. Glutathione *S*-transferase genotypes (GSTM1, GSTT1 and GSTP1) were determined. An evaluation at the group level found that the number of chromosomal aberrations and micronuclei were increased in the exposed persons with statistical significance in comparison with the levels determined in persons without exposure. When the same results were evaluated by sub-group, the effects were found in male and female nurses and the technical staff, but not in the surgical staff. In the exposed persons, the increase in micronuclei formation correlated with the combined occurrence of polymorphisms in all three glutathione *S*-transferases. Cytotoxicity determined by CBPI (cytokinesis-block proliferation index) did not differ with statistical significance between persons with and without exposure. As exposure of the personnel to other chemicals and to X-rays had been ruled out, the authors proposed that the observed genotoxic effects can be attributed to exposure to inhalation anaesthetics and that the effects are modulated by polymorphisms in the glutathione *S*-transferases. These effects require further investigation (Kargar Shouroki et al. 2019). A correlation between oxidative stress and the increased formation of micronuclei and chromosomal aberrations was investigated in what is assumed to be the same collective. Malondialdehyde levels, the activity of superoxide dismutase (SOD) and the levels of total antioxidant capacity (TAC) were determined in the blood as markers for oxidative stress. TAC (exposed persons: 1.76 ± 0.59 mM; controls: 2.13 ± 0.64 mM) and SOD activity levels (exposed persons: 11.22 ± 5.11 U/ml, controls: 13.36 ± 4.12 U/ml) were decreased with statistical significance in the persons with exposure in comparison with the levels determined in the persons without exposure. The malondialdehyde concentrations were increased with statistical significance in the exposed group (exposed persons: 2.46 ± 0.66 µM; controls: 2.19 ± 0.68 µM). Statistically significant associations were found between the incidence of micronuclei and inhalation anaesthetics, age, SOD and TAC activity as well as between chromosomal aberrations and exposure to inhalation anaesthetics and SOD (Neghab et al. 2020 b).

DNA damage in the lymphocytes and micronuclei in the cells of the oral mucosa were analysed in 12 female and 20 male physicians at the end of their 3-year residency in surgery or anaesthesiology. During their residency, they had been exposed to various anaesthetic gases for 37 hours a week. The control group was composed of physicians without exposure who matched in terms of age, sex and BMI. Exclusion criteria included smoking and high alcohol consumption. The concentrations determined in the breathing zone of the physicians during surgery in operating theatres without ventilation were 9.2 ml/m^3 (3.0–17.8) for isoflurane, 16.4 ml/m^3 (5.3–34.1) for sevoflurane and 235 ml/m^3 (120–350) for N_2O. In operating theatres with ventilation, concentrations of 1.3 ml/m^3 (0.3–3.2) were determined for isoflurane, 2.9 ml/m^3 (1.0–7.2) for sevoflurane and 66 ml/m^3 (61–70) for N_2O. The exposed persons worked in each of the operating theatres. A statistically significant increase (1.6-fold) in DNA damage was determined by comet assay

(tail intensity) in the exposed group. However, micronuclei did not form in the cells of the oral mucosa and oxidative base damage was not found in the blood. Markers for oxidative stress, such as malondialdehyde, were not increased in the blood, but the pro-inflammatory marker IL-17A was induced (Braz et al. 2020).

Tab. 3 Studies of genotoxic effects in operating theatre personnel and veterinarians

Collective	Exposure	Results[a)]	References
exposed: 27 anaesthetists (non-smokers), controls: 27 internists (non-smokers)	N_2O: 11.8 ml/m³ (8-hour TWA), isoflurane: 0.5 ml/m³ (8-hour TWA), 100% < 2 ml/m³, 3 months, readings: end of shift, end of week	sister chromatid exchange: positive, exposed: 9.0 ± 1.3 SCE/cell, controls: 8.0 ± 1.4 SCE/cell	Hoerauf et al. 1999 c
exposed: 10 veterinary surgeons (non-smokers), controls: 10 veterinarians (non-smokers)	N_2O: 12.3 ml/m³ (8-hour TWA), isoflurane: 5.3 ml/m³ (8-hour TWA), 86% < 2 ml/m³, 94% < 10 ml/m³	sister chromatid exchange: positive, exposed: 10.2 ± 1.9 SCE/cell, controls: 7.4 ± 2.4 SCE/cell, micronuclei: negative, exposed: 8.7 ± 2.95 MN/500 BNC, controls: 6.80 ± 2.47 MN/500 BNC	Hoerauf et al. 1999 a
exposed: 25 hospital employees (anaesthesia personnel), controls: 25 employees of an Eastern European university	N_2O: 170 ml/m³, halothane: 4 ml/m³, isoflurane: 4 ml/m³	sister chromatid exchange: positive, estimated from diagram: exposed: about 12.6 SCE/cell, controls: about 9.8 SCE/cell, micronuclei: positive, exposed: 14 MN/1000 BNC (9–26.7), controls: 11.3 MN/1000 BNC (3.2–19.4)	Wiesner et al. 2001, 2002
exposed: 25 persons, controls: 25 employees of a German university	N_2O: 12 ml/m³, isoflurane, sevoflurane, desflurane < 0.5 ml/m³	micronuclei: negative, median: exposed: 9.8 MN/1000 BNC (4.2–20), controls: 10.5 MN/1000 BNC (5.0–20.5)	Wiesner et al. 2001
exposed: 153 female hospital employees (99 smokers, 54 non-smokers), controls: 197 female radiologists, 153 women of the general population, not divided into smokers and non-smokers	1st reading: room ventilated: isoflurane: < 0.1 ml/m³, N_2O: 0.1–0.27 ml/m³, 2nd reading: room not ventilated: isoflurane: 4.7 and 3.9 × PEL (no other data), N_2O: no data, 3rd reading: room not ventilated: halothane: 0.5 ml/m³ (= 10% of PEL), stationary	lymphocytes: chromosomal aberrations ↑, sister chromatid exchange ↑, micronuclei ↑ in comparison with values of general population and female radiologists, chromosomal aberrations differed between smokers and non-smokers, micronuclei correlated with age, sister chromatid exchange correlated with length of employment	Bilban et al. 2005
exposed: 55 female nurses, 29 male anaesthetists, controls: 52 female nurses, 31 physicians, percentage of smokers 40%–54%	stationary: isoflurane: 0.68 ml/m³ (0.07–1.83), sevoflurane: 0.56 ml/m³ (0.05–1.8), N_2O: 242 ml/m³ (3.3–826)	no correlation between DNA damage (comet assay) and isoflurane, but with N_2O	Wrońska-Nofer et al. 2009
exposed: 30 anaesthetists (20 men, 10 women), controls: 27 physicians (18 men, 9 women)	isoflurane: 5.5 ± 4.4 ml/m³ (0.4–16.5), sevoflurane: 7.7 ± 8.7 ml/m³ (0.2–34.4), desflurane: 16.4 ± 6.0 ml/m³ (8.2–23.2), N_2O: 150.3 ± 135.7 ml/m³ (61–350), 16 years on average, stationary in the breathing zone, blood and samples taken prior to start of shift, fasting	epithelial cells of the oral mucosa: micronuclei ↑, nuclear fragmentation, pyknosis, number of basal cells ↓, lymphocytes: no effects, comet assay: negative results	Souza et al. 2016
exposed: 10 surgeons (7 men, 3 women), 34 nurses (18 men, 16 women), 16 technicians (5 men, 11 women), controls: 60 nurses (35 men, 25 women)	isoflurane: 2.4 ± 0.86 ml/m³ (0.49–4.15), N_2O: 851 ± 920 ml/m³ (10–2895), sevoflurane: 0.18 ± 0.14 ml/m³ (0.01–0.59)	lymphocytes: chromosomal aberrations and micronuclei: ↑, in female and male nurses and technical staff, but not in surgical staff; CBPI did not differ with statistical significance	Kargar Shouroki et al. 2019

Tab. 3 *(continued)*

Collective	Exposure	Results[a)]	References
exposed: 31 physicians (19 men, 12 women), controls: 32 physicians (20 men, 12 women)	operating theatre without ventilation: isoflurane: 9.2 ml/m³ (3.0–17.8), sevoflurane: 16.4 ml/m³ (5.3–34.1), N_2O: 235 ml/m³ (120–350), operating theatre with ventilation: isoflurane: 1.3 ml/m³ (0.3–3.2), sevoflurane: 2.9 ml/m³ (1.0–7.2), N_2O: 66 ml/m³ (61–70); 3 years, 37 hours/week	lymphocytes: comet assay (tail intensity) ↑ (1.6-fold), epithelial cells of the oral mucosa: micronuclei no effects; no data for cytotoxicity	Braz et al. 2020

a) unless specified otherwise, the changes described were statistically significant
BNC: binucleated cells; CBPI: cytokinesis-block proliferation index; MN: micronuclei; PEL: permissible exposure limit; SCE: sister chromatid exchange; TWA: time-weighted average

4.6.2 Studies in persons under and after narcosis

The lymphocytes of 12 men and 8 women (aged 18 to 45 years) were examined for DNA damage, oxidative base damage as well as the altered expression of DNA repair genes (*hOGG1, XRCC1*, *BCL2*: anti-apoptotic protein) during anaesthesia (2 hours after induction of anaesthesia) and on the first day following anaesthesia with 1.2% isoflurane (12 000 ml/m³). The anaesthesia lasted at least 2 hours and was induced with 3 mg midazolam (intravenous), fentanyl (5 µg/kg body weight, intravenous) and propofol (2 mg/kg body weight, intravenous). Anaesthesia was maintained with 1.2% isoflurane, the neuromuscular blocking agent rocuronium bromide (0.6 mg/kg body weight, intravenous) and 40% oxygen. Excluded from the study were persons with pre-existing conditions who regularly took medication, persons who took antioxidant supplements, smokers, alcoholics and persons who had undergone radiation therapy. In comparison with the values obtained prior to anaesthesia, no increase in DNA damage or in oxidized purines and pyrimidines and no increased cytotoxicity in the lymphocytes (determination of CD4+ helper cells and CD8+ T cells) were found in the comet assay at any time point. Apoptosis was not induced in the T cells (annexin V/7-amino-actinomycin staining). The genes *hOGG1, XRCC1* and *BCL2* were down-regulated on the first day following anaesthesia (Braz et al. 2011 b).

In another study carried out by the same research group, the lymphocytes of 8 men and 7 women (aged 18 to 40 years) were examined by comet assay during anaesthesia (2 hours after induction of anaesthesia) and on the first day following anaesthesia with 1.2% isoflurane (12 000 ml/m³) (minimally invasive surgery: ear operation or straightening of the nasal septum; for the anaesthetic regimen, see Braz et al. 2011 b). In comparison with the levels determined prior to surgery, no increase in DNA damage was detected (Braz et al. 2011 a).

The lymphocytes of 9 women and 3 men ranging in age from 20 to 66 years who had been anaesthetized with 1% to 1.5% isoflurane for abdominal surgery were examined by comet assay prior to anaesthesia, 1 and 2 hours following the induction of anaesthesia and on days 1, 3 and 5 following surgery. A comparison group of controls was composed of 12 persons who were not anaesthetized. The lymphocytes of the control persons were examined only once. The patients were described as otherwise healthy: this was their first anaesthesia, they were not undergoing radiation therapy, did not have diabetes and were non-smokers. The anaesthesia lasted on average about 133 minutes and was induced with thiopenthone (5–7 mg/kg body weight, intravenous) and 0.1 mg fentanyl citrate (intravenous). Anaesthesia was maintained with 1% to 1.5% isoflurane in an oxygen-air mixture; the neuromuscular blocking agent was vecuronium bromide (0.1 mg/kg body weight). Prior to anaesthesia, the mean number of comets detected in the treatment groups and in the control group did not differ with statistical significance. After 1 and 2 hours and on the first day following surgery, the number of lymphocytes with comets was increased with statistical significance in comparison with the values obtained prior to anaesthesia and in the control group, reaching the highest level after 2 hours. On days 3 and 5 following anaesthesia, the values no longer differed with statistical significance from those prior to anaesthesia and those of the control persons. According to the authors, there is no evidence to date that the other substances that were used for anaesthesia induce genotoxic effects (Karabıyık et al. 2001; Şardaş et al. 1998). It is unclear whether the findings can be attributed solely to effects induced by isoflurane. Only 100 cells per patient were analysed.

4.6.3 Summary

Cross-sectional studies investigating DNA damage in operating theatre personnel found only suggestive evidence that isoflurane has genotoxic potential in this occupational group. However, as the personnel was exposed to a number of different inhalation anaesthetics in the operating theatres, data are not available for past exposure to other inhalation anaesthetics and the biostatistics are in part unclear, it is not possible to attribute specific genotoxic effects to isoflurane from the findings of the available studies. Negative findings were obtained by studies investigating the development of DNA damage during or following anaesthesia induced with isoflurane in patients without pre-existing conditions undergoing minimally invasive surgery.

4.7 Carcinogenicity

There are no data available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

As the neurotoxic effects in humans are the most sensitive end point, the studies investigating neurotoxicity in rats and mice after acute inhalation exposure are shown in detail in Table 4. The available studies all investigated isoflurane only at narcotic concentrations. Reduced cognitive performance was observed in the Morris water maze test and in the Barnes maze test. In most cases, the memory parameters tested by these assays remained unchanged. Decisive for the effects on neurotoxicity were the age of the animals, the concentration and the duration of narcosis as well as the time point of examination (see also Section 5.5.2.2).

Tab. 4 Studies of neurotoxicity after acute exposure to isoflurane

Species, strain, number per group	Concentration, duration	Time of examination	Cognitive effects	Effects on the hippocampus[a)]	References
SD rats, 6–12 ♂, age: 20 months	0, 1.5%, 4 hours	directly following exposure	cognitive deficits	HIR-1α ↑, VEGF ↑, MMP-2 ↑, type IV collagen ↓ and occludin ↓, accumulation: HIR-1α, VEGF	Cao et al. 2018
F344 rats, 12, no other data, age: 18 months	group 1: 100% O_2, group 2: 1.2% isoflurane in 100% O_2, group 3: 2 hours 1.2% isoflurane, then UTI, group 4: first UTI then 2 hours 1.2% isoflurane	16 hours, 14 days	14 days: Barnes maze test: reduced cognitive performance, effects reduced by UTI	16 hours: caspase-3 ↑, β-amyloid peptide ↑, IL-1β ↑, TNF-α ↑, effects reduced by UTI, 13 minutes after Barnes maze test: apoptosis ↑, apoptosis reduced by UTI	Guo et al. 2019
Wistar rats, 8 ♂, age: not specified	0, 1.2%, 3 hours; 1.2%, 3 hours and 2 hours recovery	directly following exposure, 72 hours	not tested	protein mapping: altered expression of many proteins	Kalenka et al. 2010
SD rats, 76 ♂ in total, age: 20 months	0 (100% O_2), 1.5%, 1, 4 hours	3, 6, 12, 24 hours	Morris water maze test: 4 hours: escape latency increased from day 4 onwards	1 hour: unchanged: Beclin 1, LC3B, p62, 4 hours: LC3B ↓, after 3 hours/6 hours: Beclin 1 ↑, after 12 hours/24 hours: Beclin 1 ↓	Li et al. 2015

Tab. 4 *(continued)*

Species, strain, number per group	Concentration, duration	Time of examination	Cognitive effects	Effects on the hippocampus[a)]	References
F344 rats, no other data, ♂, age: 4 months	0 (100% O_2), 1.2%, 2 hours	6 hours, 16 hours, 14 days, 29 days	Barnes maze test: 14 days: fear conditioning test: reduced freezing time, reduced spatial learning	hippocampus: 6 hours: IL-1β ↑, TNF-α unchanged, 16 hours: caspase-3 protein ↑, 14/29 days: no effects, 29 days: neural density in the CA1 region ↓, in the CA3 region ↓ (not statistically significant)	Lin and Zuo 2011
rats, no other data, 4, no other data, age: 18 months	0, 1.4%, 2 hours; VB12 administered 2 hours before isoflurane: 10 µg or 20 µg	6 hours, 2 weeks	Morris water maze test and Barnes maze test: 2 weeks: reduced cognitive performance, not as marked after addition of VB12, no effects on memory	6 hours: IL-1β ↑, caspase-3 protein ↑, unchanged: amyloid β, in the cerebral cortex: TNF-α, IL-1β	Sha et al. 2017
Wistar rats, 12 ♂, age: 4 months (24 months) data unclear	controls: 30% O_2, 2 hours, narcosis: 3%, 2 hours, then 1.5% in 30% O_2, 2 hours	0, 0.5, 1, 3, 7 days	Morris water maze test: unchanged: swimming speed, all examination time points: escape latency ↑, number of times crossing the original platform ↓	no effects: expression of BACE-1, BACE-1 protein, from day 1 onwards: expression APP ↑, from day 0.5 onwards: amyloid-β42 protein ↑, APP protein ↓ (not statistically significant), from day 3 onwards: Aβ-peptide plaques ↑	Zhang et al. 2017
C57BL/6 mice, 8 ♂, age: 8–10 weeks	0, 1.2% in 100% O_2, 6 hours	1 day, 3 days, 7 days	new object recognition test: on days 1, 3, 7: explore time ↓, differentiation index ↓, fear conditioning test: reduced freezing time, cued fear memory extinction: reduced freezing time, impaired memory consolidation	protein expression: p-GSK-3β ↓, t-GSK-3β ↓, D1R ↓, COMT ↑	Du et al. 2020
C57BL/6 mice, 12 ♂, age: 8 weeks	0.5 hours: 0.7%; 2 hours: 0.7%; 2 hours: 1.4%; 4 hours: 1.4%, controls: 0%	24 hours, 2 weeks	10 days: Morris water maze test: no changes in swimming speed, 0.7%, 0.5 and 2 hours: time to reach platform (escape latency) ↓, number of platform crossings ↑, 1.4%, 4 hours: time to reach platform (escape latency) ↑, number of platform crossings ↓	24 hours: 0.7%, 0.5 hours and 2 hours: NDMA receptor protein ↑, ERK1/2 ↑, 1.4%, 2 hours and 4 hours: caspase-3 protein ↑, ERK1/2 ↓, 2 weeks: 0.7%, 0.5 hours and 2 hours: NDMA receptor protein ↑, 1.4%, 4 hours: caspase-3 protein ↑, 1.4%, 2 hours and 4 hours: NDMA receptor protein ↓, ERK1/2 ↓	Liu et al. 2014
C57BL/6 mice, 10–12 ♂, 8–10 ♀, age: 7 days	0, 1.5% in 30% O_2 and 70% N_2, 6 hours	2 hours, 32 days	no effects in the Morris water maze test	2 hours: S100β in the plasma ↑, caspase-3 protein ↑, no effects: TNF-α, IL-1β, IL-6	Yang et al. 2014

[a)] unless specified otherwise, the changes described were statistically significant

APP: amyloid precursor protein; BACE-1: β-site APP cleavage enzyme-1; COMT: anti-catechol *O*-methyltransferase; D1R: dopamine receptor; ERK: extracellular signal-regulated kinase; p-GSK-3β: anti-phospho-glycogen synthase kinase-3β; HIR-1α: hypoxia-inducible factor; HO-1: haem oxygenase-1; HSP: heat shock protein; IL: interleukin; LC3B: microtubule-associated protein 1 light chain-3B; NDMA receptor: *N*-methyl-D-aspartate receptor (glutamate receptor); p62: marker for autophagy; SD: Sprague Dawley; TNF: tumour necrosis factor; UTI: ulinastatin (anti-inflammatory drug); VB12: vitamin B12; VEGF: vascular endothelial growth factor

5.1.2 Oral administration

Groups of 7 rabbits were given liquid isoflurane at doses of 0 (0.9% NaCl), 5 or 10 ml/kg body weight via a nasogastric tube. The animals were observed at 5-minute intervals. The examined organs were: liver, kidneys and lungs (time point not specified). In the liver, lymphocyte infiltration, enlarged portal areas and nuclear polymorphisms in the hepatocytes were observed at 5 ml/kg body weight and above. Necrosis was not detected. Lymphocyte and neutrophil infiltration were likewise observed in the kidneys in addition to "substance accumulation" in the interstitium. Alveolar damage was found in the focal areas of the lungs and, in 1 rabbit, thickness was detected in one area. Oedema and congestion were observed (Arici et al. 2013). This study has not been included in the evaluation because its route of administration is not relevant for the workplace.

5.1.3 Dermal application

There are no data available.

5.2 Subacute, subchronic and chronic toxicity

The only studies with inhalation exposure that are available are those that were reviewed in detail in the documentation published in 1993 and in the supplement from 2007. Table 5 below provides an overview of these studies.

After exposure for 9 weeks, no histopathological effects on the liver, kidneys, brain, testes and ovaries were observed in mice up to an isoflurane concentration of 5000 ml/m^3. AST, CYP, cytochrome b5 and haematocrit remained unchanged up to this concentration (Rice et al. 1986). A NOAEC of 4000 ml/m^3 was derived for mice based on the findings of a chronic toxicity study (see Table 5) (Baden et al. 1988).

After continuous exposure of male rats to 20 ml/m^3 for 30 weeks, no histopathological effects were observed in the liver and kidneys. Also the CYP levels in the liver and the ALT activity in the serum were unchanged. A NOAEC of 20 ml/m^3 was derived for liver and kidney toxicity in male rats (Plummer et al. 1986).

There are no studies available of neurotoxicity after chronic exposure; as a result, this end point cannot be evaluated.

Tab. 5 Studies of repeated inhalation exposure to isoflurane

Species, strain, number per group	Exposure	Findings	References
mice, Swiss Webster, 82–92 ♀, ♂	0, 1000, 4000 ml/m^3, 4 hours/day, 5 days/week, 78 weeks	**4000 ml/m^3**: NOAEC, no histopathological findings in all 39 organs and tissues examined	Baden et al. 1988
mice, Swiss Webster, 23–27 ♀	0, 60, 600, 6000 ml/m^3, 4 hours/day, gestation days 6–15	**up to 600 ml/m^3**: no toxicity, no abnormal behaviour, **6000 ml/m^3**: body weights ↓ (by 9.7%) (statistically significant), ataxia caused by mild anaesthesia	Mazze et al. 1985
mice, Swiss Webster, 15 ♀, 15 ♂	0, 200, 1000, 5000 ml/m^3, 4 hours/day, 5 days/week, 9 weeks	**1000 ml/m^3**: NOAEC, **5000 ml/m^3**: body weights ↓ (not statistically significant), no effects on organ weights (liver, spleen, kidneys, testes, uterus), haematocrit, aspartate aminotransferase, CYP, cytochrome b5, no effects found by histopathological examination: brain, liver, kidneys, testes, ovaries	Rice et al. 1986
rats, F344, 12 ♂	0, 20 ml/m^3, 24 hours/day, 7 days/week, 30 weeks, groups of 4 animals examined after 43, 133 and 210 days	**20 ml/m^3**: NOAEC for body weights, liver, kidneys, CYP level, alanine aminotransferase, 2-fold to 3-fold increase in excretion of fluoride with the urine	Plummer et al. 1986

5.3 Local effects on skin and mucous membranes

There are no studies available.

5.4 Allergenic effects

There are no studies available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

New studies that have become available since the publication of the 2007 supplement (Hartwig and MAK Commission 2016) are described in detail below.

Groups of 8 male Sprague Dawley rats were exposed whole-body in a closed exposure chamber to isoflurane concentrations of 0, 50, 300, 1800 or 10 800 ml/m^3 for 2 hours a day for a total of 15 days. Weights of the testes, epididymides and seminal vesicles and body weights were unchanged. At isoflurane concentrations of 300 ml/m^3 and above, the number of sperm and the daily sperm production were decreased with statistical significance. At this concentration and above, the histopathological examination revealed damage to the seminiferous tubules and a decrease in the number of germ cells. The spermatozoa were either present in reduced numbers or were completely absent in the seminiferous tubules of several animals. The epithelium in the seminiferous tubules of several animals was disorganized with disrupted germ cell layers. The findings observed in the spermatocytes by electron microscope were nucleus agglutination, large lipid drops (at 50 ml/m^3 and above), swollen mitochondria, enlarged endoplasmic reticulum and autophagosomes in the cytoplasm. In the spermatozoa, vacuoles in the acrosome and altered mitochondria in the tail were observed. Additional findings were necrosis of spermatogonia, of the spermatocytes and of the Sertoli cells. In the serum, lower testosterone levels were found at concentrations of 300 ml/m^3 and above and decreased FSH levels at concentrations of 1800 ml/m^3 and above; both changes were statistically significant. The LH value remained unchanged (Table 6; Xu et al. 2012). A concentration–effect relationship was not evident for the sperm parameters; this is probably due to the fact that the maximum effect size was already reached at 300 ml/m^3. A concentration-dependent decrease in LH, FSH and testosterone is plausible, although the hormone variability was relatively high. Rats are hyperfertile; this means that they remain fertile even if only about 10% of sperm are present. Humans are generally considered subfertile, and the effects observed at concentrations of 300 ml/m^3 and above are therefore evaluated as adverse. As histopathological effects were not observed in the testes at 50 ml/m^3, and the LH, FSH and testosterone concentrations as well as the sperm count and daily sperm production did not differ with statistical significance from the values determined in the control group, this concentration is considered the NOAEC for effects on the male reproductive organs of rats.

Tab. 6 Effects on sperm, FSH, LH and testosterone induced by isoflurane in Sprague Dawley rats (n = 8) (Xu et al. 2012)

Isoflurane (ml/m^3)	Sperm count	Sperm production (10^6/day/g)	FSH (IU/l)	LH (IU/l)	Testosterone (ng/ml)
0	265.1 ± 22.7	43.5 ± 3.7	2.39 ± 0.49	1.09 ± 0.69	11.14 ± 4.34
50	236.6 ± 42.5	38.8 ± 7.0	2.00 ± 0.57	0.88 ± 0.46	9.06 ± 2.64
300	179.0 ± 75.1*	29.3 ± 12.3*	1.92 ± 1.02	0.91 ± 0.56	4.82 ± 2.34*
1800	158.8 ± 27.8*	26.0 ± 4.5*	1.29 ± 0.77*	0.89 ± 0.71	2.64 ± 1.59*
10 800	171.3 ± 32.3*	28.1 ± 5.3*	0.41 ± 0.3*	0.88 ± 0.56	1.74 ± 0.96*

*$p < 0.05$

This study and other studies investigating fertility and the effects on the reproductive organs are shown in Table 7.

A group of 50 male Sprague Dawley rats were exposed for 1 hour a day, for 25 days, to an isoflurane concentration of 2 ml/m^3 (Ding et al. 2015). As according to the authors an anaesthesia group is compared with a control group, it is

assumed that instead a concentration of 2% (20 000 ml/m³) was used because general anaesthesia cannot be induced at 2 ml/m³. This assumption is supported by the finding that the histopathological changes in the testes and the effects on hormone status and sperm parameters observed in this study (see Table 7) occurred with the same strength and order of magnitude as those found in the study of Xu et al. (2012) after exposure to the highest concentration of 10 800 ml/m³. This suggests that the concentration used in this study must have been considerably higher (Ding et al. 2015). The study has not been included in the evaluation because the concentration used is not clear.

Sperm motility and sperm counts were markedly lower in Sprague Dawley rats after euthanasia by isoflurane anaesthesia followed by exsanguination than after euthanasia by CO_2. The authors suggested that isoflurane may inhibit the contraction of the smooth muscle of the vas deferens (Campion et al. 2012).

Groups of 20 female C57 mice were exposed to isoflurane concentrations of 0, 2500, 5000, 10 000 or 20 000 ml/m³ for 1.5 hours a day, for 15 days. The length of the oestrus cycle was examined in 6 animals of each group. The blood, ovaries and hormone status were analysed in all animals. At concentrations of 5000 ml/m³ and above, FSH and LH levels were increased in the serum with statistical significance and the anti-Mullerian hormone (AMH) levels were decreased. At concentrations of 10 000 ml/m³ and above, the length of the oestrus cycle and the oestradiol concentrations in the blood were increased with statistical significance. In addition, at this concentration and above, the follicles had irregular nuclei, distorted zona pellucida and a disorganized granular environment. The number of atretic follicles was not increased with statistical significance. The length of the oestrus cycle was prolonged with statistical significance after exposure to 20 000 ml/m³ (Tang et al. 2020). On the basis of this study, a NOAEC of 2500 ml/m³ has been derived for the effects induced by isoflurane on the reproductive organs of female mice.

Tab. 7 Studies investigating reproductive organs and hormone status after exposure to isoflurane

Species, strain, number per group	Exposure	Findings[a)]	References
rats, SD, no data	single, 50 000 ml/m³ (5%), comparison of euthanasia after isoflurane anaesthesia and CO_2 euthanasia, 5, 10 minutes	sperm motility ↓, sperm count ↓ in comparison with values in CO_2 group	Campion et al. 2012
rats, SD, 50 ♂, control group 10 ♂	0, 2 ml/m³ (probably 2%, see text), 1 hour/day, 25 days	days 0–14: GnRH ↓ (no longer from day 14 onwards); LH ↓, FSH ↓, testosterone ↓ (near-complete return to initial values after 28 days), sperm count ↓ (up to day 7), sperm motility ↓ (up to day 7), abnormal sperm ↑ (up to day 14), marked narrowing of the seminiferous tubules, disruption of spermatogenesis, changes in the interstitial tissue, no Leydig cells, expression of genes relevant for reproduction: hypothalamus: AR, Kiss-1, GPR54, GnRH ↓, pituitary gland: LH-β ↓, FSH-β ↓, GnRH-R ↓, testes: LH-R ↓, FSH-R ↓ examined: hypothalamus, pituitary gland, testes, sperm, on the last day of exposure, after 7, 14, 21 and 28 days, determination of LH, FSH, testosterone, GnRH	Ding et al. 2015
rats, Charles River albino, 10 ♂, 20 ♀	0, about 16 000 ml/m³, 1 hour/day, for 5 days, 1–5, 6–10 or 11–15 days before mating	**NOAEC**: **16 000 ml/m³**: fertility: no effects on mating, foetal development, surviving foetuses	Kennedy et al. 1977
rats, Sprague Dawley, 8 ♂	0, 50, 300, 1800, 10 800 ml/m³, whole-body exposure, 2 hours/day, 15 days	see also Table 6, **50 ml/m³: NOAEC** ♂ reproductive organs, electron microscope: spermatocytes: nuclear agglutination, large lipid droplets, **300 ml/m³ and above**: damage to the seminiferous tubules, number of germ cells ↓, spermatozoa ↓: vacuoles in the acrosome, altered mitochondria in the tail, seminiferous tubule sections: disorganized epithelium, spermatocytes: nuclear agglutination, large lipid droplets, swollen mitochondria, enlarged endoplasmic reticulum, autophagosomes, necrosis of spermatogonia, spermatocytes and Sertoli cells	Xu et al. 2012

Tab. 7 *(continued)*

Species, strain, number per group	Exposure	Findings[a)]	References
mice, C57, 5 ♂	0, 1000, 10 000 ml/m³, 4 hours/day, 5 days/week, 28 days	no statistically significant effects on the percentage of abnormal spermatozoa	Land et al. 1981
mice, Swiss Webster, 32–41 ♀, no data for ♂	0, 1000, 4000 ml/m³, 4 hours/day, 14 days, followed by mating within the treatment group (not specified if ♂ also exposed), exposure during mating and gestation	**4000 ml/m³**: mild anaesthesia, no effects on body weights, copulation, gestation, litters, implantations, resorptions	Mazze 1985
mice, Swiss Webster, 15–24 ♂	0, 1000, 4000 ml/m³, 4 hours/day, 6 weeks, followed by mating with 2 untreated ♀ each		
mice, C57, 20 ♀	0, 2500, 5000, 10 000, 20 000 ml/m³, 1.5 hours/day, 15 days, oestrus cycle examined in 6 animals per group	**2500 ml/m³**: **NOAEC** ♀ reproductive organs, **5000 ml/m³ and above**: in serum: FSH ↑, LH ↑, AMH ↓, **10 000 ml/m³ and above**: prolonged oestrus cycle, serum E2 ↑, follicles: irregular nuclei, distorted zona pellucida, disorganized granular environment, atretic follicles ↑ (not statistically significant), developing follicles ↓ (not statistically significant), **20 000 ml/m³**: prolonged oestrus cycle, examination of the oestrus cycle for 15 days, examined: blood, oestrus cycle, ovaries, hormone status	Tang et al. 2020

[a)] unless specified otherwise, the changes described were statistically significant
AMH: anti-Mullerian hormone; AR: androgen receptor; E2: oestradiol; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; GPR54: Kiss-1-derived peptide receptor; Kiss-1: kisspeptin encoding gene; LH: luteinizing hormone; SD: Sprague Dawley

Summary: Studies investigating repeated, 2-hour exposure of rats derived a NOAEC for effects on the male reproductive organs (testes, sperm, hormone status) of 50 ml/m³ (Xu et al. 2012). In rats, no effects on fertility were observed at anaesthetic concentrations (16 000 ml/m³) (Kennedy et al. 1977). In mice, a NOAEC for effects on the female reproductive organs (oestrus cycle, hormone status and ovaries) of 2500 ml/m³ was derived after exposure for 1.5 hours (Tang et al. 2020). In a long-term study with mice, no histopathological effects on the testes or ovaries were observed up to a concentration of 4000 ml/m³ (Baden et al. 1988; Rice et al. 1986). No effects on fertility were observed in mice up to a concentration of 4000 ml/m³ (Mazze 1985).

5.5.2 Developmental toxicity

All studies that found prenatal and postnatal effects after prenatal exposure are shown in Table 8.

5.5.2.1 Prenatal effects

No new studies have become available since the most recent supplement was published in 2007 (Hartwig and MAK Commission 2016). Overall, the findings of the animal studies reviewed in this addendum showed that isoflurane induces embryotoxic effects in mice and rats at anaesthetic concentrations. Evidence of an increased incidence of cleft palates was found in mice at 6000 ml/m³, but not in rats and rabbits. The exposure of mice to an isoflurane concentration of 600 ml/m³ yielded neither embryotoxic nor maternally toxic effects. However, prenatal exposure led also to postnatal toxicity. These effects were observed in mice at isoflurane concentrations of 4000 ml/m³. A NOAEC was not established for these effects.

5.5.2.2 Postnatal effects

Exposure of Swiss Webster mice to concentrations of up to 4000 ml/m³ did not induce effects on the body weights of the offspring up to postnatal day 28 (Mazze 1985). Postnatal effects such as delayed latency of the righting reflex and delayed ability to swim were observed in juvenile Swiss Webster mice born of dams exposed to an isoflurane concentration of 4000 ml/m³ from gestation days 6 to 15 (Rice 1986). However, these findings cannot be evaluated because

the data were not reported in the study report in sufficient detail. The number of offspring per litter was reduced on postnatal days 1 and 4 in CR albino rats born of dams exposed to a concentration of 17 400 ml/m³ for 1 hour a day from gestation days 15 to 20. The dams were under mild anaesthesia and had decreased body weight gains (see Table 8) (Kennedy et al. 1977).

The publications that have become available since 2007 are presented in detail below.

Inhalation exposure of rats to an isoflurane concentration of 30 000 ml/m³ for 1 hour on gestation day 14 caused effects in behavioural tests for spatial learning and memory. The number and density of caspase-3-positive cells in the hippocampus were increased. These effects were not observed at 13 000 ml/m³ (Kong et al. 2012 a). After inhalation exposure of rats to isoflurane at a concentration of 13 000 ml/m³, either as a single exposure for 4 hours on gestation day 14 or as repeated exposure for 2 hours a day on gestation days 14 to 21, the latency period for acquiring spatial information was increased and fewer crossings of the original platform were observed in the Morris water maze test (Kong et al. 2011, 2012 b). After the single inhalation exposure, the number and density of the C/EBP homologous transcription factor protein and caspase-12 protein in the hippocampus were increased with statistical significance, suggesting apoptosis (Kong et al. 2011). After multiple inhalation exposures, the levels of growth-associated protein-43 and neuropeptide Y as well as their mRNA were decreased in the hippocampus (Kong et al. 2012 b).

After a single exposure of Sprague Dawley rats to an isoflurane concentration of 14 000 ml/m³ for 4 hours on gestation day 14, behavioural tests carried out with the offspring on postnatal day 28 revealed an increased latency period for acquiring spatial information and decreased anxiety (Palanisamy et al. 2011). In the offspring of Sprague Dawley rats exposed once by inhalation to a concentration of 13 000 ml/m³ for 6 hours on gestation day 21, a decrease in caspase-3 and TUNEL-positive cells (**T**erminal Deoxynucleotidyl Transferase Biotin-d**U**TP **N**ick **E**nd **L**abeling) in the hippocampus was observed 2 hours after exposure, but no changes in behavioural tests carried out at the age of 28 and 115 days (Li et al. 2007).

After a single exposure of Hartley guinea pigs by inhalation to a concentration of 5500 ml/m³ for 4 hours at some point between gestation days 35 to 40, neuroapoptosis was increased in various regions of the foetal brain and the neural density was decreased (Rizzi et al. 2008).

Overall, a NOAEC for postnatal effects on behaviour after prenatal exposure cannot be derived on the basis of these studies.

Tab. 8 Prenatal and postnatal developmental toxicity after inhalation of isoflurane before and during gestation

Species, strain, number per group	Exposure	Findings	References
prenatal effects			
mice, Swiss Webster, 32–41 ♀, ♂ not specified	0, 1000, 4000 ml/m³, 4 hours/day, 14 days, followed by mating within the treatment group (not specified if also ♂ exposed), exposure during mating and gestation, examination: 2/3 of the dams on GD 18, 1/3 produced offspring (see below)	**4000 ml/m³**: dams: mild anaesthesia; no effects on copulation, gestation, litter parameters	Mazze 1985
mice, Swiss Webster, 23–31 ♀	0, 60, 600, 6000 ml/m³, 4 hours/day, GD 4–15, examination GD 18	**600 ml/m³**: **NOAEC for developmental toxicity and maternal toxicity**; **6000 ml/m³**: dams: body weight gains ↓, ataxia, mild anaesthesia; foetuses: body weights ↓, late resorptions ↑, ossification ↓, changes to the kidneys ↑, incidence of cleft palates ↑	Mazze et al. 1985
rats, SD, 30 ♀, controls: 40 ♀	0, 3500 ml/m³, 24 hours, GD 8, examination GD 20	**3500 ml/m³**: dams: body weight gains ↓, mild anaesthesia; no developmental toxicity	Fujinaga et al. 1987

Tab. 8 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rats, SD, 21–25 ♀, controls: 39–50 ♀	0, 10 500 ml/m³, 6 hours/day, GD 8–10, GD 11–13, GD 14–16, screening for skeletal and soft tissue anomalies, examination GD 20	**10 500 ml/m³**: **GD 8–10:** dams: mild anaesthesia; foetuses: body weights ↓; **GD 11–13:** dams: mild anaesthesia; **GD 14–16**: dams: mild anaesthesia, body weight gains ↓; foetuses: body weights ↓; no developmental toxicity	Mazze et al. 1986
rats, Charles River albino, 11–20 ♀	0, about 16 000 ml/m³, 1 hour/day, GD 1–5, GD 6–10, GD 11–15, screening for skeletal and soft tissue anomalies, examination GD 14 and 20	**16 000 ml/m³**: no developmental toxicity	Kennedy et al. 1977
rabbits, New Zealand White, 15 ♀	0, 23 000 ml/m³, 1 hour/day, GD 6–9, GD 10–14, GD 15–18, screening for skeletal and soft tissue anomalies, examination GD 29	**23 000 ml/m³**: no developmental toxicity	
postnatal effects			
mice, Swiss Webster, 32–41 ♀, ♂ not specified	0, 1000, 4000 ml/m³, 4 hours/day, 14 days, followed by mating within the treatment group (not specified if also ♂ exposed), exposure during mating and gestation, examination: offspring from 1/3 of the dams PND 1–28	**4000 ml/m³**: dams: mild anaesthesia; no effects on copulation, gestation, litter parameters, survival and lactation index, body weights of the offspring on PND 1–28	Mazze 1985
mice, Swiss Webster, ♀ not specified	0, 4000 ml/m³, 4 hours/day, GD 6–15, examination PND 2–11 and PND 6–20	**4000 ml/m³**: offspring: righting reflex ↓, swimming ability ↓	Rice 1986
rats, not specified, 5 ♀	0, 13 000 ml/m³, 1 × 4 hours, GD 14, examination from PND 28 onwards: behavioural tests: Morris water maze test: spatial learning and memory	**13 000 ml/m³**: offspring: latency period for acquiring spatial information ↑, number of crossings of the original platform ↓ (memory impairment), number and optical density of CHOP and caspase-12 proteins in the hippocampus ↑, swimming speed unchanged (no sensorimotor effects)	Kong et al. 2011
rats, not specified, 8 ♀	0, 13 000, 30 000 ml/m³, 1 × 1 hour, GD 14, examination from PND 28 onwards: behavioural tests: Morris water maze test: spatial learning and memory	**13 000 ml/m³: NOAEC for postnatal effects on behaviour,** **30 000 ml/m³**: offspring: latency period for acquiring spatial information ↑, number of crossings of the original platform ↓ (memory impairment), number and optical density of caspase-3-positive neurons in the hippocampus ↑, swimming speed unchanged (no sensorimotor effects)	Kong et al. 2012 a

Tab. 8 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rats, SD, 5 ♀	0, 13 000 ml/m³, 2 hours/day, GD 14–21, examination from PND 28 onwards: behavioural tests: Morris water maze test: spatial learning and memory	**13 000 ml/m³**: offspring: latency period for acquiring spatial information ↑, number of crossings of the original platform ↓ (memory impairment), number and optical density of GAP-43, NPY and mRNA level in the hippocampus ↓, swimming speed unchanged (no sensorimotor effects)	Kong et al. 2012 b
rats, SD, 3 ♀	0, 14 000 ml/m³, 1 × 4 hours, GD 14, examination from PND 35 onwards: behavioural tests: spatial learning and memory	**14 000 ml/m³**: offspring: latency period for acquiring spatial information ↑, anxiety ↓, no effects on locomotor activity, spontaneous changes or object recognition memory	Palanisamy et al. 2011
rats, Charles River albino, 11–20 ♀	0, 17 400 ml/m³, 1 hour/day, GD 15–20, examination up to PND 21	**17 400 ml/m³**: dams: body weight gains ↓, ataxia, mild anaesthesia; offspring: decrease in the number of offspring/litter on PND 1 and 4	Kennedy et al. 1977
rats, SD, 3 ♀	0, 13 000 ml/m³, 1 × 6 hours, GD 21, examination of the brain after 2 hours, 18 hours, 5 days and in behavioural tests with juveniles (from PND 28) and adults (from PND 115): spatial learning and memory	**13 000 ml/m³**: 2 hours: spontaneous apoptosis reduced in the hippocampus (caspase-3, TUNEL), no effects after 18 hours and 5 days as well as in behavioural tests (spatial learning and memory)	Li et al. 2007
guinea pigs, Hartley, 2 ♀	0, 5500 ml/m³, 1 × 4 hours, GD 20–25, GD 35–40, GD > 50, examination 2 hours and 35 to 40 days after the end of exposure	**5500 ml/m³**: **GD 35–40:** 3-fold to 6-fold increase in neuroapoptosis in the cortex, 3-fold to 11-fold increase in neuroapoptosis in the amygdala, subiculum and hippocampus (caspase-3 and caspase-9-positive cells) and decrease in neuron density, no evaluation of behaviour	Rizzi et al. 2008

CHOP: C/EBP homologous transcription factor protein; GAP-43: growth-associated protein-43; GD: gestation day; PND: postnatal day; NPY: neuropeptide Y; SD: Sprague Dawley; TUNEL: **T**erminal Deoxynucleotidyl Transferase Biotin-d**U**TP **N**ick **E**nd **L**abeling

5.5.2.3 Neurotoxic effects on development

Numerous studies reviewed by Colon et al. (2017), Maloney et al. (2019) and McCann and Soriano (2019) provide evidence that isoflurane induces neurotoxic effects in foetal/neonatal and juvenile rodents in anaesthetic concentrations. Several studies investigating the effects of a single inhalation exposure lasting several hours on 7-day-old mice and rats found that isoflurane increased neuroapoptosis at concentrations of 7500 ml/m³ and above. This concentration proved to be the lowest effect concentration for neurotoxicity. Exposure of rats and mice at this age caused impairments in learning and memory in adulthood (Colon et al. 2017). In 7-day-old mice that were exposed to anaesthetic concentrations of isoflurane, a very strong effect on cell death (20-fold to 68-fold increase in neural degeneration) was observed (Deng and Loepke 2014).

Neonatal and juvenile animals are more susceptible to neurotoxic effects than adults. The studies that investigated this end point are described in the following. Anaesthesia induced with isoflurane (17 000 ml/m³) daily for 35 minutes on 4 consecutive days impaired object recognition and reversal learning in 14-day-old male Wistar rats and male C57BL/6J mice in comparison with the effects found in 60-day-old animals; these effects grew stronger with age. These findings were not observed in the animals treated at the age of 60 days. The memory deficit was paralleled by a decrease in the hippocampal stem cell pool and by persistently reduced neurogenesis (Zhu et al. 2010). In male and female neonatal C57BL6/J mice (postnatal day 7) anaesthetized once for 6 hours with isoflurane (15 000 ml/m³), increased apoptosis (determined using the marker activated cleaved caspase-3) was found in the neocortex (11-fold increase), caudoputamen

(10-fold), in the cornu ammonis region 1 of the hippocampus (3-fold) and in the cerebellum (4-fold). This effect was not observed in 21 and 49-day-old mice. Neonatal mice did not exhibit increased sensitivity in the dentate gyrus and olfactory bulb regions of the brain, but a 15-fold increase in the marker for apoptosis was observed in the dentate gyrus of juvenile animals (Deng et al. 2014).

In mice, isoflurane induces stronger neurotoxic effects on development (neurodegeneration, impaired learning and memory) than sevoflurane and desflurane (Liang et al. 2010; Liu et al. 2017; Seubert et al. 2013; Tao et al. 2016).

In foetal/neonatal rhesus monkeys, increased apoptosis of the oligodendrocytes was observed after undergoing anaesthesia (concentration not specified) for up to 8 hours (Brambrink et al. 2012; Creeley et al. 2014; Noguchi et al. 2017). After undergoing anaesthesia for 8 hours with an isoflurane concentration of 10 000 ml/m^3, no significant neurotoxic effects such as the activation of caspase-3 or neural damage (silver-positive cells) were observed (Zou et al. 2011).

5.5.3 Summary

In animal studies, exposure to isoflurane in anaesthetic concentrations led to reduced foetal weights, delays in ossification and an increased incidence of late resorptions in mice at 6000 ml/m^3 (Mazze et al. 1985) and to reduced foetal weights in rats at 10 500 ml/m^3 (Mazze et al. 1986). An increased incidence of cleft palates was observed in mice, but not in rats and rabbits, at 6000 ml/m^3. Neither embryotoxic nor maternally toxic effects were detected in mice after exposure to an isoflurane concentration of 600 ml/m^3 (NOAEC for prenatal developmental toxicity) (Mazze et al. 1985). However, prenatal exposure caused also postnatal effects on behaviour in mice and rats. These effects were observed in mice after daily exposure for 4 hours to an isoflurane concentration of 4000 ml/m^3 (Rice 1986) and in rats after daily exposure for 2 or 4 hours to a concentration of 13 000 ml/m^3 (Kong et al. 2011, 2012 b). However, the study did not investigate lower concentrations that would be suitable for the derivation of a NOAEC. On the basis of the findings of studies with juvenile rodents and monkeys, the developing brain appears to be vulnerable to exposure to isoflurane, responding with neurodegeneration at concentrations of 7500 ml/m^3 and above (Colon et al. 2017). There are no studies available with exposure of neonatal or juvenile animals to non-anaesthetic concentrations that could be used to derive a NOAEC for neurodevelopmental effects induced by isoflurane.

5.6 Genotoxicity

5.6.1 In vitro

Isoflurane concentrations in the range of 100 to 300 000 ml/m^3 (0.01%–30%) did not cause mutagenic effects in the Salmonella typhimurium strains TA98, TA100 and TA1535 either with or without the addition of a metabolic activation system. Exposure to isoflurane occurred either in a desiccator with the room air or dissolved in medium (Baden et al. 1977; Greim 1996; Waskell 1978). Urine samples from patients who had been anaesthetized with isoflurane likewise did not induce mutagenic effects in the strains TA100 or TA1535 (Baden et al. 1977; Greim 1996).

In tests for sister chromatid exchange, negative results were obtained in CHO cells (a cell line derived from Chinese hamster ovary) with isoflurane concentrations of up to 11 500 ml/m^3 both with and without the addition of a metabolic activation system (Greim 1996; White et al. 1979) as well as in V79 cells with isoflurane concentrations of up to 40 000 ml/m^3 (Hartwig and MAK Commission 2016; Trudnowski et al. 1987). An incubation period of 1 hour was used in each case.

In vitro treatment of the blood lymphocytes of 8 male test persons with isoflurane (pure isoflurane in liquid form) for 72 hours at concentrations of 0.3, 0.6 or 1.2 mM (55.4, 110.7, 221.4 mg/l) induced a dose-dependent increase in sister chromatid exchange per cell (9.15 ± 1.0, 9.55 ± 1.4, 9.95 ± 1.8) at the low concentration and above that was statistically significant in comparison with the levels determined in the controls (7.65 ± 1.5). The mitotic index (MI) and proliferation index were decreased with statistical significance in comparison with the values determined in the controls only at the high concentration. Lymphocyte cultures incubated with 50% N_2O in air for 1 hour under agitation were likewise

found to have increased levels of sister chromatid exchange that were statistically significant at a significantly lower mitotic index. No data for a positive control were given (Hoerauf et al. 1999 b).

In the alkaline comet assay, isoflurane caused a concentration-dependent and statistically significant increase in DNA damage (total comet length) in human blood lymphocytes at concentrations of 1 mM and above. The lymphocytes were incubated at 4 °C with 0, 0.1, 1 and 10 mM (0, 18.45, 184.5, 1845 mg/l) isoflurane in 1% DMSO for 10 or 30 minutes. After subsequent incubation of the treated cells in a substance-free medium at 37 °C, the DNA damage in the lymphocytes that had initially been exposed to 1 mM isoflurane was reduced to a statistically significant degree after 20 minutes; after 60 minutes the damage was at the same level as the control values. The authors regarded this as evidence that the damage is to be attributed to the genotoxic effects of the substance and not to apoptotic mechanisms because it would not be possible to repair the latter (Jaloszyński et al. 1999).

In another alkaline comet assay carried out according to the same procedure, human blood lymphocytes were incubated for 10 or 30 minutes at 4 °C or 37 °C with isoflurane (liquid, dissolved in DMSO) in concentrations of 0, 1 or 10 mM (0, 184.5, 1845 mg/l). The authors found that the DNA damage induced by isoflurane was greater to a statistically significant degree than that determined in the controls. However, it is not possible to determine from the diagram at which concentrations the damage was induced. The DNA damage was not dependent on time and was less marked at 37 °C than at 4 °C; this may be attributable to DNA repair (Szyfter et al. 2004).

Human blood lymphocytes and the sperm from a donor were incubated for 5 minutes with (liquid) isoflurane concentrations of 0.1, 1, 10 or 100 mM (0, 18.45, 184.5, 1845, 18 450 mg/l) in 1% DMSO and analysed for DNA damage by alkaline comet assay. The incubation was carried out on ice in order to suppress any DNA repair. Isoflurane caused a concentration-dependent and statistically significant increase in DNA damage (% tail intensity) at the low concentration and above. The other anaesthetics tested, halothane, desflurane and sevoflurane, likewise caused a statistically significant increase in DNA damage (Kaymak et al. 2012).

The studies of Kaymak et al. (2012), Jaloszyński et al. (1999) and Szyfter et al. (2004) did not use standardized cytotoxicity tests or include a positive control. Therefore, the studies cannot be used to evaluate the genotoxic effects.

Incubation with 2% isoflurane (gaseous) increased the incidence of DNA damage (γH2AX expression), cytotoxicity (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test (MTT), lactate dehydrogenase assay) and apoptosis (caspase-3 activation) in human neuroglioma cells transfected to express human amyloid precursor protein (H4-APP) with statistical significance after 6 hours, but not after 3 hours (Li et al. 2017; Ni et al. 2017). Pre-incubation for 1 hour with a reactive oxygen species scavenger (*N*-acetyl-L-cysteine) or caspase inhibitor weakened isoflurane-induced γH2AX expression with statistical significance (Ni et al. 2017). If the cells were incubated under mild hypothermia (35 °C), the DNA damage and cytotoxicity caused by isoflurane were lower with statistical significance than the effects induced under standard conditions (37 °C) (Li et al. 2017).

Incubation of MLE-12 cells (murine lung epithelial cell line) with 2% isoflurane (in the ambient air), 60% oxygen (hyperoxia) or a combination of 2% isoflurane and 60% oxygen led to a statistically significant increase in 8-oxo-dG damage, both in the nucleus and in the mitochondria, after 24, but not after 2 hours. This was determined by immunofluorescence. The highest level of damage was induced by 2% isoflurane; the damage was reduced with statistical significance by co-incubation with oxygen (Kundumani-Sridharan et al. 2019).

5.6.2 In vivo

Male wild-type Drosophila melanogaster were exposed to an isoflurane concentration of 10 000 or 20 000 ml/m^3 for 1 hour and then mated with untreated females. In the sex-linked recessive lethal test, no mutagenic effects were observed in the F2 generation (Greim 1996; Kundomal and Baden 1985; Saber and Hougaard 2009).

In another test for X-chromosomal recessive lethal mutations in Drosophila melanogaster, mutagenic effects were not induced after exposure for 1 hour to isoflurane (20 000 ml/m^3) in combination with N_2O (75%) (Baden and Kundomal 1987; Hartwig and MAK Commission 2016; Saber and Hougaard 2009).

The studies reviewed in the documentation published in 1993 (Greim 1996) and the supplement from 2007 (Hartwig and MAK Commission 2016) reported negative findings in Drosophila melanogaster. The studies below have since become available:

Isoflurane induced a 2.95-fold increase in the frequency of micronuclei in the kidneys of male Sprague Dawley rats that was statistically significant. The rats were first treated with folic acid 24 hours after removing the left kidney in order to strengthen the proliferation stimulus initiated by nephrectomy. Two days later, the rats were administered an anaesthetic in a single oral dose of 4 mmol/kg body weight (about 800 mg/kg body weight). The induction of micronuclei in the kidneys was examined 2 days later. The control group was composed of 15 animals and each treatment group was made up of 6 animals. Micronuclei were induced not only by isoflurane, but also by halothane (3.48-fold), chloroform (3.32-fold), trichloroethylene (3.24-fold) and sevoflurane (2.98-fold). Enflurane was not found to have genotoxic properties using the same procedure. According to the authors, the approximately 3-fold increase in micronuclei cannot be evaluated as a clearly positive result, but only as suggestive of a genotoxic effect (Robbiano et al. 1998; Saber and Hougaard 2009). The validity of the study is limited because the method applied was not validated and only 1 dose was tested. The values determined for 5 of the 6 tested anaesthetics are of the same order of magnitude, which seems contradictory because of the varying degrees of renal toxicity.

After inhalation exposure of groups of 10 male Sprague Dawley rats to isoflurane at 10 000 ml/m^3 for 30 to 60 minutes, DNA damage in the lymphocytes, spleen, bone marrow, brain, liver and lungs was increased with statistical significance in the comet assay in comparison with the levels found in the controls. The olive tail moment values for the lymphocytes were 1.36 ± 0.02 and 1.51 ± 0.08, respectively, after 30 and 60 minutes of exposure (controls: 1.24 ± 0.01). The corresponding values for the bone marrow were 1.28 ± 0.03 and 1.88 ± 0.02, respectively, after 30 and 60 minutes compared with 1.13 ± 0.05 in the control group; for the spleen 1.26 ± 0.01 and 1.96 ± 0.02, respectively, compared with 1.02 ± 0.06, for the liver 1.74 ± 0.04 and 2.12 ± 0.05, respectively, compared with 1.48 ± 0.05, for the brain 1.44 ± 0.10 and 1.83 ± 0.05, respectively, compared with 1.36 ± 0.02, and for the lungs 2.20 ± 0.06 and 2.13 ± 0.08, respectively, compared with 1.37 ± 0.02. In all organs except for the lungs, the DNA damage increased over time. Lipid peroxidation (malondialdehyde concentration) was determined; slight increases were found in all tissues, and marked increases were found in the bone marrow. Protein oxidation was assessed by determining carbonyl groups in amino acids after derivatization with 2,4-dinitrophenylhydrazine. The highest levels were again determined in the bone marrow. No statistically significant increase was found in the spleen. On the basis of these findings, the authors did not establish an association between lipid peroxidation and protein oxidation and the DNA damage (Kim et al. 2006).

Groups of 6 Wistar rats were exposed once by inhalation to an isoflurane concentration of 20 000 ml/m^3 or a sevoflurane concentration of 40 000 ml/m^3 in oxygen for 2 hours. The whole blood cells were then examined for DNA damage by comet assay. Sevoflurane caused an increase in DNA strand breaks that was statistically significant in comparison with the control value ($p = 0.02$). Whereas isoflurane increased the number of DNA strand breaks, but not with statistical significance ($p > 0.05$). Cytotoxicity, determined by trypan blue staining, was higher than 98%. In the MTT test, exposure to isoflurane increased cell vitality with statistical significance. The authors postulate that this shows a higher antioxidant capacity of the cells, which can be seen as the cause of the lower DNA damage compared with sevoflurane (Rocha et al. 2015).

After repeated exposure by inhalation to isoflurane, sevoflurane and halothane, DNA strand breaks were induced in Ehrlich ascites tumour cells (EAT) in male Swiss mice. Groups of 4 animals were given 2×10^6 EAT cells by intraperitoneal injection. Treatment began 3 days later. For the next 3 consecutive days, the animals were exposed for 2 hours to an isoflurane concentration of 17 000 ml/m^3, a sevoflurane concentration of 24 000 ml/m^3 or a halothane concentration of 15 000 ml/m^3 in oxygen. The control group remained untreated. The DNA damage in the EAT cells was then examined by comet assay and apoptosis by flow cytometry. All 3 inhalation anaesthetics led to an increase in DNA strand breaks that was statistically significant ($p < 0.001$) compared with the control level. The mean tail length was 1.51 ± 0.61 in the group exposed to isoflurane, 1.41 ± 0.67 in the sevoflurane group, 1.89 ± 0.68 in the halothane group and 0.38 ± 0.55 in the control group. Sevoflurane and halothane did not cause significant effects on cellular apoptosis, but did significantly reduce the number of living EAT cells in the peritoneal lavage. By contrast, repeated anaesthesia with isoflurane had stimulating effects on EAT cell proliferation, shown by an increase in living cells by 26.22%, and

concurrently inhibited the apoptosis of tumour cells (6.11%) in comparison with the control values (10.26%) (Brozovic et al. 2009).

In another study carried out by the same research group, DNA damage in the peripheral leukocytes (blood), brain, liver and kidneys was examined by comet assay in groups of 5 mice after exposure to isoflurane or halothane in oxygen (the same protocol was used as by Brozovic et al. 2009). Both substances induced a statistically significant increase in DNA strand breaks ($p < 0.05$ in comparison with control group values) in all examined tissues. In a comparison of halothane and isoflurane, isoflurane caused a more marked increase in the peripheral lymphocytes and kidney cells while halothane induced stronger effects in the liver and brain (Brozovic et al. 2011).

The same research group carried out another study investigating DNA damage and repair in the kidney cells of male Swiss mice after exposure for 2 hours on 3 consecutive days to an isoflurane concentration of 17 000 ml/m^3 or a sevoflurane concentration of 24 000 ml/m^3 in oxygen. Tail length, tail moment and tail intensity were determined by comet assay 0, 2, 6 and 24 hours after the end of exposure and compared with the findings in the control group. The values of all three parameters were increased with statistical significance in the groups treated with isoflurane and sevoflurane. The number of DNA strand breaks induced by sevoflurane increased continuously for up to 24 hours. After exposure to isoflurane, the incidence of strand breaks was increased with statistical significance directly after exposure and after 2, 6 and 24 hours, with the greatest increase occurring after 2 hours. This was followed by a decline in incidence (Brozović et al. 2017).

Under anaesthetic conditions, there is concurrent exposure to high concentrations of oxygen. To investigate whether hyperoxia induces DNA damage in the lungs, mice were exposed by inhalation for 2 hours to room air with and without 2% isoflurane (20 000 ml/m^3) or to 60% oxygen (hyperoxia) with and without 2% isoflurane. The expression of genes that are markers for oxidative stress or DNA damage, and the expression of DNA repair genes and cell cycle regulatory proteins were analysed by Western blot and PCR assay. Additionally, apoptosis was determined by TUNEL assay and by assessing the levels of 8-hydroxy-2′-deoxyguanosine (8-Oxo-dG). Hyperoxia induced mitochondrial and nuclear DNA damage; these effects and apoptosis were decreased in the groups exposed also to isoflurane. Exposure to only isoflurane in a concentration of 20 000 ml/m^3 in room air, however, caused a statistically significant increase in 8-Oxo-dG in the mitochondria and the cell nucleus. Furthermore, NADPH oxidase, a source of superoxide anion generation, was inhibited by isoflurane only in combination with an increased oxygen concentration. The authors concluded that DNA damage caused by hyperoxia during anaesthesia is reduced by isoflurane, while isoflurane in combination with room air induces genotoxic effects in the lungs (Kundumani-Sridharan et al. 2019).

Groups of 3 mice (aged 18 months) were anaesthetized for 2 hours with isoflurane in 100% oxygen. The isoflurane concentration was 14 000 ml/m^3. The control animals were exposed to pure oxygen (100%). After exposure to isoflurane, the γH2A.X value in the prefrontal cortex of the brain increased to a value that was about 2.4-fold higher ($p = 0.0005$) than the control value (Ni et al. 2017).

5.6.3 Summary

In vitro: Isoflurane was not mutagenic in bacteria. DNA damage occurred only concurrently with apoptosis or cytotoxicity. Tests for sister chromatid exchange yielded inconsistent results. Sister chromatid exchange was not induced in CHO and V79 cells. Only one test for sister chromatid exchange in human blood lymphocytes yielded positive findings; however, the findings suggest that isoflurane may have clastogenic potential in vitro.

In vivo: The studies available for the evaluation of genotoxic effects in animals after inhalation exposure investigated only narcotic concentrations. The studies found evidence of DNA damage (comet assay), but are not suitable for evaluating genotoxic effects under workplace conditions. As each of the studies tested only one concentration, it is not possible to draw conclusions about a concentration–effect relationship. A micronucleus test that obtained positive findings after exposure to a single oral dose is only of limited relevance for the evaluation because the study method was not validated, only one dose was administered and the findings were of the same order of magnitude as those for 5 other anaesthetics that produced positive results in spite of inducing varying levels of renal toxicity. Overall, the genotoxic potential of isoflurane requires further study, particularly in the concentration ranges found at the workplace.

5.7 Carcinogenicity

No new data have become available for this end point since the publication of the last documentation in 2007 (Hartwig and MAK Commission 2016). After exposure of Swiss Webster mice to isoflurane concentrations of 0, 1000 and 4000 ml/m³ for 4 hours a day, on 5 days a week, for 78 weeks, the tumour incidence was not increased and no histopathological changes were observed (Baden et al. 1988).

6 Manifesto (MAK value/classification)

The most sensitive end points are the effects on the central nervous system in humans in addition to liver toxicity and the effects on the reproductive organs in animals.

MAK value. There are no data for the induction of liver toxicity in humans at non-anaesthetic concentrations. On the basis of the findings of a long-term study (24 hours a day; 30 weeks) with male F344 rats, a NOAEC of 20 ml/m³ was established for the liver, CYP levels and for ALT activity in serum. Taking the exposure scenario at the workplace into account with exposure for 8 hours a day on 5 days a week and extrapolating the findings from animals to humans (1:2), this would result in a MAK value of 42 ml/m³ (20 ml/m³ × 24/8 × 7/5 × 1/2). However, higher concentrations were not investigated. Using a read-across approach from halothane, a NOAEC of 92 ml/m³ was established for neurotoxicity induced in humans by isoflurane. As exposure lasted for 3 to 4 hours, the steady state for isoflurane is reached after about 100 minutes, and prenarcotic effects are dependent only on the concentration, the acute effects on the central nervous system are not expected to increase over time. On this basis, a MAK value of 92 ml/m³ has been derived for these effects on the central nervous system. In a long-term study in mice, no histopathological effects on the testes or ovaries were observed up to a concentration of 4000 ml/m³. A NOAEC of 2500 ml/m³ was derived for the effects on the reproductive organs of female mice (Tang et al. 2020) and a NOAEC of 50 ml/m³ for the effects on the reproductive organs of male rats, the most sensitive species (Xu et al. 2012). The increased respiratory volume does not need to be taken into account because the blood:air partition coefficient is below 5 (1.4). Using the lowest NOAEC of 50 ml/m³ as the basis for calculation and assuming that the effects are dependent on C × T, 2 hours are extrapolated to an 8-hour working day (1:4). As the length of exposure was only 15 days, but the spermatogenesis cycle takes about 56 days, the data have been extrapolated from a subacute to a subchronic duration of exposure (1:2). Additionally, the extrapolation of findings from animal studies to humans has been taken into consideration (1:2). A concentration in the air of 3.1 ml/m³ is calculated from these values. After applying the preferred value approach, this results in a MAK value of 2 ml/m³.

Peak limitation. The critical effect of the substance is systemic and it has therefore been classified in Peak Limitation Category II. However, as the derivation of the MAK value was based on the assumption that the effects on the testes are dependent on C × T, leading to the extrapolation from 2 to 8 hours, peak concentrations do not play a decisive role. For this reason, an excursion factor of 8 has been assigned.

Prenatal toxicity. In animal studies, exposure to isoflurane in anaesthetic concentrations led to a reduction in foetal weights and delays in ossification in addition to an increased incidence of late resorptions in mice at 6000 ml/m³ (Mazze et al. 1985) and a reduction in foetal weights in rats at 10 500 ml/m³ (Mazze et al. 1986). Evidence of an increased incidence of cleft palates was found in mice at 6000 ml/m³, but not in rats and rabbits. The exposure of mice to an isoflurane concentration of 600 ml/m³ (NOAEC for prenatal developmental toxicity) did not lead to either embryotoxic or maternally toxic effects (Mazze et al. 1985). In rats, the NOAEC for prenatal developmental toxicity was 4000 ml/m³ after inhalation exposure to isoflurane from gestation days 8 to 10 or 14 to 16. Without taking the increased respiratory volume into consideration (see Section "MAK value"), this results in a 300-fold to 2000-fold margin between these values and the MAK value of 2 ml/m³. However, prenatal exposure causes also postnatal behavioural effects in mice and rats. These were observed in mice at an isoflurane concentration of 4000 ml/m³ (Rice 1986) and in rats at 13 000 ml/m³ (Kong et al. 2011, 2012 b). As lower concentrations were not tested, a NOAEC for postnatal behavioural effects after prenatal exposure cannot be established.

The findings of mechanistic studies suggest that the developing brain is vulnerable to the effects of isoflurane, responding with neurodegeneration. After 7-day-old mice were exposed once for a period of 4 hours, the lowest neurotoxic effect concentration was 7500 ml/m^3 (Colon et al. 2017).

An important consideration for the evaluation of toxic effects on neurodevelopment is that expectant mothers may be exposed during pregnancy for 8 hours a day on 5 days a week until the beginning of their maternity leave 6 weeks before the scheduled date of birth. Studies with pregnant or neonatal animals that investigated a single exposure to anaesthetic concentrations are not suitable for drawing conclusions about the special conditions found at the workplace and the longer periods of exposure this entails.

While, in the human foetus, a considerable amount of brain development occurs in utero, much of this takes place after birth in rodents (Deng and Loepke 2014). By postnatal day 10, the rodent brain has reached a stage of development that is equivalent to that of the human brain at the end of pregnancy (Semple et al. 2013). As a result, toxic effects on neurodevelopment observed in rodents during the early postnatal period are critical for the in-utero development in humans and the effects observed in rats up to postnatal day 10 are relevant for the evaluation of prenatal developmental toxicity in humans.

Although the 300-fold and 2000-fold margins between the NOAEC for prenatal developmental toxicity and the MAK value would be sufficient to classify isoflurane in Pregnancy Risk Group C, a NOAEC for postnatal effects on behaviour after only prenatal exposure has not been established. In addition, evidence suggestive of toxic effects on neurodevelopment was found in neonatal and juvenile rodents and macaques. Animals are more vulnerable to the neurotoxic effects of isoflurane in these age groups than they are after reaching adulthood. However, there are no studies available with exposure of neonatal or juvenile animals to non-anaesthetic concentrations that can be used to derive a NOAEC for toxic effects on neurodevelopment induced by isoflurane.

As a result, isoflurane has been assigned to Pregnancy Risk Group D.

Carcinogenicity. New studies of the carcinogenic effects of isoflurane have not become available. A long-term study in mice did not observe an increase in tumour incidences up to a concentration of 4000 ml/m^3. Therefore, the substance has not been classified in any of the carcinogen categories.

Germ cell mutagenicity. The DNA damage observed in operating theatre personnel in cross-sectional studies is, at most, a sign that isoflurane may have genotoxic potential. However, as the personnel in the operating theatres were exposed to a mixture of various inhalation anaesthetics, information relating to earlier exposure to other inhalation anaesthetics was not given and the biostatistics are unclear in some cases, the genotoxic effects cannot be attributed specifically to isoflurane based on these findings. Isoflurane did not cause mutagenic effects in the Salmonella typhimurium strains TA98, TA100 and TA1535 or in Drosophila. There are no animal studies that carried out mammalian germ cell assays. In vitro, genotoxic effects were found only concurrently with apoptosis or cytotoxicity. Tests for sister chromatid exchange yielded inconsistent results. Sister chromatid exchange was not induced in CHO and V79 cells. However, a single positive result in a test for sister chromatid exchange using blood lymphocytes suggests that isoflurane induces clastogenic effects in vitro. The studies that are available for the evaluation of the genotoxic effects induced in animals after inhalation exposure investigated only narcotic concentrations; the findings suggest that DNA damage (comet assay) is induced. However, the findings are not suitable for use in evaluating the genotoxic potential under workplace conditions. As these studies tested only one concentration, it is not possible to draw conclusions on a concentration–effect relationship. Therefore, the substance has not been assigned to a category for germ cell mutagens. Overall, more data are needed to evaluate the genotoxic potential of isoflurane, particularly in the concentration range found at the workplace.

Absorption through the skin. Isoflurane is used only as an inhalation anaesthetic. Regular contact of the skin with liquid isoflurane is unlikely to occur, also due to its high vapour pressure. Absorption of gaseous isoflurane through the skin is not relevant (McDougal et al. 1990). Therefore, isoflurane remains not designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Only 2 case reports are available for the evaluation of skin sensitizing effects. These were already reviewed in the supplement from 2007 (Hartwig and MAK Commission 2016). There are no findings from animal studies or in vitro data. For this reason, the substance has not been designated with "Sh" (for substances which cause sensitization of the skin). Only one centre reported several findings relating to effects on the respiratory tract observed in operating theatre personnel after occupational exposure to isoflurane that are regarded as suggestive of an immunological response. However, as the substance is frequently used as an inhalation anaesthetic, the reported findings do not provide sufficient justification for assigning the "Sa" designation (for substances which cause sensitization of the airways). Therefore, isoflurane remains also not designated with "Sa".

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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