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Xylene (all isomers)

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

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Keywords

xylene; neurotoxicity; maximum workplace concentration; MAK value; toxicity; peak limitation; sensitization; skin absorption

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated xylene [1330-20-7]. The critical effect of xylene is acute neurotoxicity. The former maximum concentration at the workplace (MAK value) of 100 ml/m^3 was derived on the basis of effects on the equilibrium of persons exposed at rest. It is now lowered to 50 ml/m^3 taking into account the increased respiratory volume at the workplace (see List of MAK and BAT Values, Section Ib and Ic). Since a systemic effect is critical, Peak Limitation Category II is retained. The excursion factor of 2 is confirmed on the basis of toxicokinetic studies. As skin absorption contributes significantly to systemic toxicity, the designation with "H" (for substances that can be absorbed through the skin in toxicologically relevant amounts) is retained. Xylene is not expected to be a sensitizer.

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MAK value (2019)	**50 ml/m³ (ppm) ≙ 220 mg/m³**
Peak limitation (2001)	**Category II, excursion factor 2**
Absorption through the skin (1998)	**H**
Sensitization	–
Carcinogenicity	–
Prenatal toxicity (1988)	**Pregnancy Risk Group D**
Germ cell mutagenicity	–
BAT value (1984)	**2000 mg methylhippuric acid/l urine**
Synonyms	dimethylbenzene methyltoluene *m*-xylene *o*-xylene *p*-xylene
Chemical name (IUPAC)	1,2-xylene 1,3-xylene 1,4-xylene
CAS number	xylene (all isomers): 1330-20-7 1,2-xylene: 95-47-6 1,3-xylene: 108-38-3 1,4-xylene: 106-42-3
Molar mass	106.17 g/mol
Melting point	< 25 °C (IFA 2018)
Boiling point	137–140 °C (IFA 2018)
Density at 20 °C	0.86 g/cm³ (IFA 2018)
Vapour pressure at 20 °C	8 hPa (IFA 2018)
log K_{OW}	2.98–3.15 (Eom 2011)
Solubility at 20 °C	0.2 g/l water (IFA 2018)
1 ml/m³ (ppm) ≙ 4.41 mg/m³	**1 mg/m³ ≙ 0.227 ml/m³ (ppm)**

For xylene (all isomers), there is documentation from 1983 and a supplement from 1987 (combined in one translation: Henschler 1993) as well as supplements from 1998, 2001 and 2004 (Greim 2001; Hartwig 2014 a, b).

In 2016, the Commission began using a revised approach for assessing substances with a MAK value based on systemic effects and derived from inhalation studies in animals or with volunteers at rest; this new approach takes into account that the respiratory volume at the workplace is higher than under experimental conditions. However, this does not apply to gases or vapours with a blood:air partition coefficient < 5 (DFG 2019; Section I b and I c). The blood:air partition coefficient of the xylene isomers is greater than 5 (Pierce et al. 1996). This supplement evaluates whether the MAK value for xylene needs to be changed as a result of the higher respiratory volume at the workplace.

1 Toxic Effects and Mode of Action

See also Greim (2001).

There are no clinical reports of contact allergic reactions to xylenes. The results with xylenes from experimental studies indicate a low irritant potential rather than a contact sensitizing potential.

2 Mechanism of Action

The lipophilic property of xylene is responsible for its anaesthetic and narcotic effects. The exact mechanism is unknown. As for other lipophilic organic solvents, interaction with specific targets (including ion channels and neurotransmitter receptors) in the neuronal membrane or changes in the geometry of the membrane of nerve cells is suspected (Meulenberg et al. 2016). These effects could impair the transmission of nerve impulses (ATSDR 2007). In general, a "narcosis pathway" is assumed for organic solvents, which is based on the enhancement of inhibitory processes and the reduction of excitatory processes in the nervous system and thus leads to sedative effects in humans (van Thriel 2014). Xylene can react also with other cell membranes and lead to membrane damage (Niaz et al. 2015).

Above all in vitro experiments showed that the irritant effect could be due to the dissolution of cell membranes. Cytotoxic effects are associated with the formation of hydroxyl radicals, lipid peroxidation and the release of oxidative intermediates with subsequent necrosis. Nephrotoxicity and apoptotic effects may be related to caspase activation (ATSDR 2007). In leukaemia cells, however, the induction of apoptosis independent of caspase was observed (Sarma et al. 2011; see Section 5.7).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

After physical exercise on a bicycle ergometer at 100 watts, the absorption of xylene by inhalation—and thus the concentration in the blood of test persons—was about 2.5 times as high as that when the persons were at rest. A concentration peak of 400 ml/m^3 led to a doubling of the concentration in blood compared with that after constant exposure to 200 ml/m^3 (Laine et al. 1993).

Twenty-seven male volunteers were exposed at rest to concentrations of 0 to 40 ml/m^3 of the 3 xylene isomers for 2 hours. Systemic clearance from the blood was 116 ± 34 l/hour, 129 ± 33 l/hour and 117 ± 23 l/hour for *o*-xylene, *m*-xylene and *p*-xylene, respectively. The terminal half-lives after exposure were 38.5 ± 18.2 hours for *o*-xylene, 33.0 ± 11.7 hours for *m*-xylene and 30.3 ± 10.2 hours for *p*-xylene. With the data obtained, a physiologically-based pharmacokinetic (PBPK) model was developed. The authors concluded that, because of the large interindividual variability in internal exposure, a Biological Exposure Index is more appropriate for protection at the workplace than a limit value in air (Adams et al. 2005).

The PBPK model of Tardif et al. (1997) was verified using data from subjects exposed to *m*-xylene concentrations of 12.5 or 25 ml/m^3 for 7 hours (Marchand et al. 2015).

The dermal absorption of *m*-xylene from the gaseous phase was determined after the exposure of volunteers to a xylene vapour concentration of 29.4 mg/m^3 on the forearm and hand for 20, 45, 120 and 180 minutes. The amount absorbed was calculated from the xylene concentration in the exhaled air. The systemic kinetics of xylene was calculated after inhalation exposure to 19 mg/m^3. The average flux through the skin was 0.091 µg/cm^2 and hour 20 minutes after the start of exposure and 0.061 µg/cm^2 and hour after 180 minutes. An opposite course was found for the maximum flux into the blood: after 20 minutes this was 0.034 and after 180 minutes 0.063 µg/cm^2 and hour. The average permeability coefficient at the steady state was 0.061 cm/h. With the IH SkinPerm model, a permeability coefficient of 0.18 cm/h was predicted (Kezic et al. 2004).

In a similar earlier study by the authors, a permeability constant of 0.12 cm/h was obtained for *m*-xylene. The contribution of the dermal absorption of xylene from the gaseous phase after whole-body exposure was about 1% of the total amount absorbed by inhalation and via dermal exposure (Kezic et al. 2000).

Using studies with exposed volunteers and a PBPK model, it was shown that dermal exposure to an *m*-xylene concentration of 3000 ml/m^3 over a 12-hour period is necessary to achieve a body burden similar to that following inhalation exposure to 50 ml/m^3 at rest over a 12-hour period. The contribution of the dermal route of absorption after whole-body exposure was thus 1.8%. The authors assumed an exposed body surface of 1.26 m^2. Based on the data from inhalation exposure, it was shown that the steady state of *m*-xylene in the blood is almost reached after exposure to 50 ml/m^3 for 4 hours. The concentration of *m*-xylene achieved in the blood was 0.55 mg/l after 4 hours and 0.6 mg/l after 8 hours. The elimination half-life of *m*-xylene in alveolar air was 30 minutes. For the PBPK model, a half-life of 46 minutes was used for excretion with the urine (Loizou et al. 1999).

In 72 car sprayers exposed to xylene and toluene who wore respiratory protection, the excretion of methylhippuric acid, a major metabolite of xylene and toluene, was determined (in a concentration of 0.0436 ± 0.0346 g/l urine). The amount excreted was higher in persons with eczema on the hands than in persons with healthy skin (Hino et al. 2008).

In volunteers, undiluted *m*-xylene was applied to 27 cm^2 of skin for 3 minutes. The flux through the skin was 46 nmol/cm^2 and minute, corresponding to 293 µg/cm^2 and hour (Kezic et al. 2001). Due to the short exposure time, extrapolation to longer periods may overestimate the flux through the skin. Slightly lower fluxes were determined for longer exposure periods.

When both hands were exposed to liquid *m*-xylene for 15 minutes, with the exclusion of inhalation exposure, the dermal absorption rate was about 2 µg/min/cm^2 (120 µg/cm^2 and hour). The amount of xylene in the exhaled air and the level of the main metabolite methylhippuric acid in the urine were determined. After exposure of one hand for 15 minutes, the xylene concentration in venous blood from the exposed arm was found to be about 5 mg/l and from the arm not exposed to be about 0.1 mg/l. A similar study with 13 volunteers exposed for 20 minutes yielded a dermal absorption rate of 2.45 µg/min/cm^2 (147 µg/cm^2 and hour). Marked irritation was reported after 10 minutes of exposure (Greim 2001). In the latter study, the absorption rates in the exposed persons ranged from 0.7 to 4.3 µg/min/cm^2 (42 to 258 µg/cm^2 and hour) (Henschler 1993).

The skin of rats was found to be 12 times more permeable to an aqueous solution of *o*-xylene than human skin. The permeability constants were 0.005 cm/hour for human skin and 0.058 cm/hour for rat skin. The rats were exposed under occlusive conditions to *o*-xylene at a concentration of 200 mg/l and the volunteers to 0.5 mg/l. The amount absorbed was determined on the basis of the exhalation of xylene (Thrall and Woodstock 2003).

In an in vitro study with skin from hairless rats, the flux of undiluted xylene (isomer not specified) through the skin was found to be 220 µg/cm^2 and hour (Ahaghotu et al. 2005). The value of 0.22 µg/cm^2 and hour shown in the figure of the publication is not plausible, in the text 0.22 mg/cm^2 and hour is given.

Using thermogravimetry, diffusion coefficients of 43 and 7.2×10^{-9} cm^2/s were determined when pig skin was exposed to gaseous *m*-xylene. The skin:air partition coefficients were 100 and 92. In a diffusion cell, 1 ml of *m*-xylene was applied to 0.64 cm^2 of split pig skin. The flux was 80 µg/cm^2 and hour, the permeability coefficient 0.91×10^{-4} cm/hour and the diffusion coefficient 1.5×10^{-10} cm^2/s (Rauma and Johanson 2009).

Sprague Dawley rats were exposed to an *m*-xylene concentration of 2000 ml/m^3 4 hours daily for 5 days. At the end of the exposure, the xylene concentrations in 4 different areas of the brain were determined by head-space gas chromatography. The highest concentration (976 ± 93.4 µg/g tissue) was determined in the cerebellum and the lowest (467 ± 43.6 µg/g tissue) in the cerebral cortex. The binding of [^{35}S]-tert-butylbicyclophosphorothionate, as an indicator of changes at the GABA (gamma-aminobutyric acid) receptor, or the increased release of GABA were found to a statistically significant higher degree in the cerebellum of the exposed animals than in that of the control animals (Ito et al. 2002).

3.2 Metabolism

See also Greim (2001).

In a study in rats, *m*-xylene and its metabolites *m*-tolylaldehyde and 3-methylbenzyl alcohol were shown to inhibit various cytochrome P450 (CYP) isozymes of the lung and nasal mucosa. Male Sprague Dawley rats were exposed for 6 hours to 100 or 300 ml *m*-xylene/m^3, 50 or 100 ml *m*-tolylaldehyde/m^3 or 50 or 100 ml methylbenzyl alcohol/m^3. Immediately after the exposure, microsomes were isolated from lung tissue and the nasal mucosa. *m*-Xylene inhibited CYP2B1, CYP2E1 and CYP4B1 in the lung and CYP2B1 and CYP2E1 in the nasal mucosa in a concentration-dependent manner. 3-Methylbenzyl alcohol inhibited CYP2B1 and CYP4B1 in the lung, but CYP2E1 and CYP4B1 in the nasal mucosa in a concentration-dependent manner. Tolylaldehyde inhibited CYP2B1 and CYP2E1 in the lung and CYP2B1, CYP2E1 and CYP4B1 in the nasal mucosa in a concentration-dependent manner. This suggests that, in the case of co-exposure, *m*-xylene could alter the metabolism of other xenobiotics in an organ-specific manner via changes in CYP activities (Vaidyanathan et al. 2003).

Groups of 6 Sprague Dawley rats were exposed to *m*-xylene concentrations of 100 or 300 ml/m^3 for 6 hours. The activities of CYP1A1, CYP2B1, CYP2E1, CYP1A2 and CYP4B1 in nasal mucosa, lung and liver were investigated. Immediately after exposure, all the enzymes in the nasal mucosa were inhibited in a concentration-related manner, except for CYP4B1, which was inhibited the most at 100 ml/m^3. The inhibition persisted for up to 2 days, and on day 5 the activities were increased. Concentration-related inhibition of CYP2B1, CYP2E1, and CYP4B1 in the lungs was observed, but the enzyme activities returned to the normal range after 1 day. On day 5 after the exposure, the activity of CYP2B1 was increased in the lungs. The activities of CYP1A2, CYP2E1, CYP2B1 in the liver were increased, but only after exposure to 300 ml/m^3, and were back within the range of the control values on day 5 (Foy and Schatz 2004).

4 Effects in Humans

4.1 Single exposures

In several studies, CNS effects (impaired balance) were observed in subjects after exposure to an *m*-xylene concentration of 200 ml/m^3 (Greim 2001; Henschler 1993).

From these studies and the toxicokinetic study by Loizou et al. (1999), it was concluded that CNS effects occur at blood concentrations of 2 mg xylene/l or higher. A concentration of 200 ml/m^3 corresponds approximately to a xylene concentration of 2.4 mg/l blood (MacDonald et al. 2002).

After exposure of 16 volunteers to 70 ml *p*-xylene/m^3 at rest for 4 hours, no neurotoxic effects (simple reaction time, short-term memory, choice reaction time) and no increased self-reported irritant effects were observed (Greim 2001; Olson et al. 1985).

After the exposure of 15 volunteers at rest to 100 or 300 ml/m^3 of a xylene mixture for 70 minutes, no neurotoxic effects were observed in the tests performed. Only at the level of 100 watts on a bicycle ergometer did reduced performance occur during the first 30 minutes of exposure. Due to the additional physical exercise on the bicycle ergometer during half of the exposure time, the uptake of xylene during the whole exposure period was overall 2.2 times as high as that at rest (Gamberale et al. 1978). The study by Loizou et al. (1999) shows that after 1 hour a blood concentration of 0.35 mg/l is reached after exposure to 50 ml xylene/m^3 at rest, and thus 0.7 mg/l at 100 ml/m^3 and 2.1 mg/l at 300 ml/m^3. Since no adverse effects were observed at the estimated 2.1 mg/l in the study by Gamberale et al. (1978), this would be consistent with the effect threshold of 2 mg/l derived by MacDonald et al. (2002). After 8-hour exposure to 50 ml/m^3, the concentration in the blood is 0.6 mg/l (Loizou et al. 1999). Physical exercise at 50 watts doubles absorption by inhalation, so that a concentration of 1.2 mg xylene/l blood is reached after 8 hours of increased respiratory volume and exposure to 50 ml/m^3.

In a study with volunteers, 28 healthy women and men were exposed to *m*-xylene concentrations of 0 or 50 ml/m^3 for 2 hours at rest. The subjects themselves served as controls, and so it was possible to record even small changes in the level of symptoms. A questionnaire was used to assess the severity of 10 complaints immediately before the start of exposure, during exposure (3, 60 and 118 minutes) and 140 and 350 minutes after the beginning of exposure. The complaints included discomfort in the eyes, nose, throat or airways as well as fatigue, headache, nausea, dizziness and a feeling of intoxication caused by the solvent smell. The complaints were rated on a scale from 0 to 100. The verbal gradations were "not at all", "hardly", "somewhat", "rather", "quite", "very", and "almost unbearable". In addition, colour vision, lung function and nasal swelling of the subjects were determined before exposure, at the end of exposure and 3 hours after exposure. The inflammatory markers myeloperoxidase and albumin were determined in the nasal lavage fluid before and 3 hours after exposure, and the blinking frequency was monitored throughout the exposure. After exposure to an *m*-xylene concentration of 50 ml/m^3 for 60 minutes, there was a slight increase in all symptom ratings except nausea in females. These increases were statistically significant at least at one time point ($p < 0.05$). However, the average ratings during solvent exposure did not exceed the gradation "somewhat". During exposure, the correlation between smell and other ratings of symptoms was very weak. This suggests that the perception of exposure did not in itself heavily influence the magnitude of symptom rating. Women's ratings of throat and respiratory symptoms were higher than those of men. Three hours after exposure to *m*-xylene, but not immediately afterwards, the FVC (forced vital capacity) was decreased in a slight but statistically significant manner and the FEV_1 (forced expiratory volume in one second)/FVC and FEF_{75} (forced expiratory flow at 75% of the FVC) were increased in women compared with the values before exposure. An increase in the FEF_{75} is not adverse. The other 2 parameters were only marginally changed by 1% to 3%. No statistically significant effects on lung function were observed in the men. Increases of 20% to 50% in the concentrations of myeloperoxidase and albumin were observed in the women. However, the authors consider this to be coincidental because of the large variability of these markers. Exposure to *m*-xylene did not cause any change in blinking frequency. This study provides evidence that women are somewhat more sensitive to exposure to *m*-xylene, and that slight effects can be detected in both men and women after exposure to an *m*-xylene concentration of 50 ml/m^3 (Ernstgård et al. 2002). Neuropsychological tests were not performed in this study. It is therefore not suitable for demonstrating acute neurotoxic effects or physiological evidence of sensory irritation.

4.2 Repeated exposure

A 28-year-old man, who had been drinking 60 g alcohol per day and sniffing paints since his youth, was diagnosed with renal tubular acidosis 2 weeks after sniffing paints containing xylene. Serum analysis revealed hyponatraemia, hypochloraemia, hypokalaemia, an increase in creatinine up to 30 mg/l, metabolic acidosis with an anion gap of 31 mmol/l, but no anaemia and hypoproteinaemia were detected. The urine osmolarity was 322 (Sarmiento Martinez et al. 1989).

In a study with 459 workers in the paint and lacquer industry, who had been exposed for many years to a mixture of white spirit, toluene, butyl acetate, ethyl acetate and xylene, subclinical peripheral neuropathy with lower conduction velocities and other symptoms such as cramps, numbness and weakness were observed. There is no information on the exposure level in the study and the results were not evaluated separately for the different solvents (Jovanović et al. 2004).

One case of retrocochlear hearing loss has been reported. The patient was exposed to xylene at unknown levels for 6 months (Draper and Bamiou 2009).

Fifteen men and women exposed to a mixture of xylenes (21% *p*-xylene content) in hospital laboratories in Chile exhibited poorer hearing in audiological tests compared with 30 age-matched control subjects. A statistically significant difference in hearing thresholds of about 5 decibels was measured in the exposed compared with the unexposed. This impairment increased with increasing cumulative exposure and excretion of methylhippuric acid in the urine. However, this correlation was described as only "moderate" by the authors themselves. The persons were exposed to 36.5 ± 66.6 (range 8–217) mg xylene/m^3 for an average of 11.8 (range 2–29) years and thus to lower concentrations than the limit value permissible in Chile of 347 mg/m^3 (78.8 ml/m^3). However, the study did not describe how often and with

which method the xylene concentration was determined. The exposure duration was evaluated with a questionnaire and was thus not exactly determined. The maximum urinary excretion of methylhippuric acid was 500 mg/g creatinine. Exposure to noise was 72.9 ± 4.5 (range 65.9–84.5) dBA. The exposed persons were divided into 3 groups based on their cumulative exposure. The cumulative exposure derived from the data is also uncertain, as the authors point out that the exposure to noise and to xylene might have been higher in the past, but measured data were not available. In the exposed persons, slight but statistically significant changes in the "pitch pattern sequence test", "dichotic digit test" and the "hearing in noise test" were found (Fuente et al. 2013). In summary, it can be stated that the exposure levels and duration were not precisely recorded, the time between the last exposure and the examinations was 16 hours and therefore only acute, but no chronic effects could be detected. A dose–response relationship was not obtained for effects on central hearing. Therefore, this study is not included in the derivation of a threshold limit value.

4.3 Local effects on skin and mucous membranes

The spillage of paint containing xylene into the eyes caused hyperaemic conjunctivae and photophobia, irritation and partial loss of the corneal epithelium in 2 individuals (Ansari 1997). A worker in a chemical plant whose eyes came into contact with heated xylene experienced similar damage, with epithelial damage persisting for 4 weeks after the initial injury (Narváez and Song 2003). Vacuolar keratopathy of the eyes was reported after the spraying of an insecticide containing xylene (Trujillo et al. 2003).

4.4 Allergenic effects

Apart from the reports already listed in Greim (2001) on 2 persons with urticarial reactions to xylene (Altman 1977; Palmer and Rycroft 1993) and the negative result of a maximization test with volunteers (Greim 2001), there are no further case studies or information on positive findings in patch tests and also no cases of sensitizing effects of xylenes on the airways.

4.5 Reproductive and developmental toxicity

A cross-sectional study of 1408 workers in petrochemical plants in China investigated the association between exposure to organic solvents such as benzene, styrene, toluene and xylene and menstrual disturbance. Of the workers, 440 were exposed to solvents. The average concentrations of toluene, styrene and xylene were all lower than 1 ml/m^3. None of the workers were exposed to xylene alone. After adjustment for confounders, a 53% increase in the incidence of oligomenorrhoea (odds ratio (OR) 1.53; 95% confidence interval (CI): 1.00–2.34) was found after 3 years or more of exposure to the mixture of solvents (Cho et al. 2001). As the exposure was to a mixture of substances, the results of this study cannot be included in the evaluation of the effects of xylene.

4.6 Genotoxicity

There are no new studies available.

4.7 Carcinogenicity

In a case–control study with 3730 people suffering from 15 different types of cancer and 533 control subjects in the general population, it was shown that an increased cancer risk is not to be expected after exposure to xylene. Only the OR for colon carcinomas was increased in a slight, but not statistically significant manner (OR 1.5; 95% CI: 0.8–2.8) (Gérin et al. 1998).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

The RD_{50} for *o*-xylene in mice is 1467 ml/m³ (de Ceaurriz et al. 1981) and that for *p*-xylene is 1325 ml/m³ (Kuwabara et al. 2007).

Groups of 6 Sprague Dawley rats were exposed to *m*-xylene concentrations of 100 or 300 ml/m³ for 6 hours. The protein level and the lactate dehydrogenase activity in the lavage fluids of the nose and lungs were unchanged compared with the values in the control animals. There was a statistically significant increase in the activity of gamma-glutamyl transferase in the lavage fluids of the nose and lungs, and in the activities of aspartate and alanine aminotransferase in serum (Foy and Schatz 2004).

5.1.2 Oral administration

There are no new data available.

5.1.3 Dermal application

There are no new data available.

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Groups of 20 male LOD Wistar rats were exposed to *m*-xylene concentrations of 100 or 1000 ml/m³ for 6 hours daily, on 5 days per week for 12 weeks. *m*-Xylene did not affect the parameters of vigilance status obtained by electroencephalographic recordings from different brain areas. In the exposed animals of both groups, a reduced number of SWD bursts (spike and wave discharges, usually an electroencephalographic marker in epilepsy research) and a longer, cumulative duration of SWD episodes were found after the exposure phase (day 84 of the post-exposure period). In the behavioural test, the radial maze, there were only weak effects on learning performance 70 to 83 days after exposure. Especially on the final day of the test, the two *m*-xylene groups required more time for the task and they committed more omission errors. Exposure to *m*-xylene did not lead to a statistically significant effect on the body weights of the animals. A concentration–response relationship was not observed (Gralewicz et al. 1995).

In a second study, Wistar rats were exposed to *m*-xylene concentrations of 100 or 1000 ml/m³ for 6 hours daily, on 5 days per week for 6 (100 ml/m³) or 3 months (1000 ml/m³). Only in the rats exposed to 1000 ml/m³ for 3 months was a statistically significant reduction in lymphocytes and an increase in monocytes in the blood found. Performance in the rotarod test was reduced in all exposed animals, regardless of the level and duration of exposure. In rats exposed to 1000 ml/m³, performance in the rotarod test was about 40%, compared with the values in the control animals, at 100 ml/m³ about 68%. The spontaneous activity of the animals exposed to 100 ml/m³ was reduced to about 400 movements/hour, that of the control animals was 800. The tests were performed 24 hours after the last exposure (Korsak et al. 1992). However, the presentation of results is inadequate.

Male Wistar rats were exposed to *m*-xylene concentrations of 50 or 100 ml/m³ for 6 hours daily, on 5 days per week for 3 months. A statistically significant decrease in the haemoglobin content and erythrocyte count was observed in the exposed animals. There was a statistically significant decrease in the body weights of the animals exposed to 100 ml/m³ after 1 and 2 months of exposure, but not after 3 months of exposure. A statistically significant decrease in

performance in the rotarod test was found only in the animals of the 100 ml/m³ group, whereas pain sensitivity was increased in all exposed animals but statistically significant only at 100 ml xylene/m³ (see Table 5 in Korsak et al. 1994).

Wistar rats were exposed to *m*-xylene concentrations of 100 ml/m³ for 6 hours daily, on 5 days per week for 4 weeks. Two weeks after the end of exposure, the behaviour of the animals was examined with a battery of 5 different tests. Neither in the radial maze test nor in spontaneous activity was there a statistically significant reduction in performance in the exposed animals, but 24 hours after the administration of electric shocks to the paws the animals exhibited impaired passive avoidance behaviour in the hot plate assay and longer paw licking latencies, which were statistically significant (Gralewicz and Wiaderna 2001). According to the authors, the cause of these effects could be stress from the odour of xylene. On the basis of this study and the evidence from the other subchronic studies, a concentration of 100 ml *m*-xylene/m³ can be derived as the LOAEC (lowest observed adverse effect concentration). Also at 50 ml *m*-xylene/m³ first indications of effects on pain-induced avoidance learning were observed (not statistically significant) (Korsak et al. 1994). In this study, however, the analyses were less detailed and adjustment for multiple comparisons was not made.

Female Sprague Dawley rats (12 weeks old; n = 24) were exposed to concentrations of technical xylene of 300 ml/m³ for 8 hours daily for 6 weeks. At the end of exposure, total protein, albumin, urea and creatinine in serum, the activities of superoxide dismutase, catalase and glutathione peroxidase, and glutathione and malondialdehyde in kidney tissue were determined. In the serum, the concentration of urea, and in the kidney tissue that of glutathione and malondialdehyde were increased in a statistically significant manner (Kum et al. 2007 b).

One-day-old SD rat embryos (in utero), neonatal (1-day-old), young (4-week-old) and adult (12-week-old) rats were exposed to technical xylene at a concentration of 300 ml/m³ for 8 hours daily over 6 weeks. The number of apoptotic cells in lung and bronchus-associated lymphoid tissue was higher in the young and adult rats than that in the controls (Sandikci et al. 2009). Body and liver weights were decreased in the exposed embryos and 1-day-old neonatal rats. Increased liver weights were found in the 4-week-old animals, increased catalase activity and increased malondialdehyde levels in the exposed embryos, decreased glutathione levels in the 1-day-old rats, and decreased superoxide dismutase activity in the 4-week-old rats. Such changes were not seen in the adult animals (Kum et al. 2007 a). The number of CD4-positive and CD8-positive lymphocytes was increased in the young and adult rats (Sandikci et al. 2007).

Exposure of male Wistar rats to an *m*-xylene concentration of 400 mg/m³ for 5 hours daily for 5 months did not lead to an increase in lipid peroxidation or glutathione depletion in the liver (Jajte et al. 2003).

Ototoxicity

Male SD rats were exposed to the 3 xylene isomers at concentrations of 0, 450, 900 or 1800 ml/m³ for 6 hours daily, on 6 days per week for 13 weeks. Only *p*-xylene caused ototoxicity at 900 ml/m³ and above. The NOAEC (no observed adverse effect concentration) for ototoxicity was thus 1800 ml/m³ for *o*-xylene and *m*-xylene and 450 ml/m³ for *p*-xylene (Gagnaire et al. 2001).

A 3-week exposure of rats confirmed that, of the xylene isomers, only *p*-xylene is ototoxic (Maguin et al. 2006).

Ototoxicity was determined in rats after exposure to ethylbenzene and 2 mixtures of ethylbenzene and xylene isomers. The mixtures contained ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene in ratios of 1:1:2:1 and 1:3:5:1, respectively, to simulate technical xylenes with a high and a low proportion of the ototoxic substances ethylbenzene and *p*-xylene. Male Sprague Dawley rats were exposed to the mixtures at concentrations of 0, 250, 500, 1000 or 2000 ml/m³ for 6 hours daily, on 6 days per week for 13 weeks. Xylene potentiated the ototoxicity of ethylbenzene. A NOAEC was not obtained for the mixture with the high proportion of ethylbenzene and *p*-xylene; 500 ml/m³ was derived for the mixture with the low proportion (Gagnaire et al. 2007).

5.2.2 Oral administration

After oral administration of the 3 xylene isomers at a dose level of 8.47 mmol/kg body weight and day (900 mg/kg body weight) on 5 days per week for 2 weeks, only *p*-xylene caused ototoxicity in rats. The animals were examined histopathologically 10 days after exposure. Ototoxicity was determined on the basis of lesions of the organ of Corti (Gagnaire and Langlais 2005).

5.2.3 Dermal application

There are no new data available.

5.3 Local effects on skin and mucous membranes

Xylene vapour is irritating to the eyes and airways. Liquid xylene is irritating to the skin (Greim 2001; Henschler 1993).

An amount of 230 µl *m*-xylene was applied occlusively for 1 hour to the skin of hairless rats. Erythema and transepidermal water loss were observed during the 24-hour recovery phase. In the blood, the expression of interleukin-1 alpha (IL-1 alpha) and in the skin, the expression of tumour necrosis factor-alpha (TNF-alpha) and monocyte chemoattractant protein 1 (MCP-1) were increased (Chatterjee et al. 2005).

After dermal application of 250 µl *m*-xylene to male Fischer rats (6 to 10 per group) for 1 hour, the resulting low molecular weight DNA fragments and the levels of oxidative species in the skin were analysed. Reactive oxygen species were detected by determining the oxidation of 2′,7′-dichlorofluorescein diacetate. Two hours after the start of treatment, the level of oxidative species was increased. A statistically significant increase in DNA fragments was observed 2, 4 and 6 hours after the start of application. These can serve as indicators of skin irritation or skin injury (Rogers et al. 2001). In another study, granulocyte infiltration and separation of the epidermis from the dermis were observed at the same exposure conditions. The inflammatory markers IL-1 alpha, protein levels and inducible NO synthase were elevated. The authors suggested that these proteins could serve as early indicators of skin irritation (Gunasekar et al. 2003).

An amount of 15 µl xylene (isomer not specified) was applied non-occlusively to the skin of hairless rats every 2 hours for 8 hours daily, for 4 days. Erythema formation and transepidermal water loss increased with the treatment duration. Histopathological examination revealed granulocyte penetration, swelling of the epidermis and severe disturbance and damage of the stratum corneum. IL-1 alpha expression in the blood was increased, and in the skin TNF-alpha expression (Ahaghotu et al. 2005) and MCP-1 were increased (Chatterjee et al. 2005).

Application of *m*-xylene to the abdominal skin of rats caused increased vascular permeability in the skin. This was attributed in part to the activation of the tachykinin NK1 receptor by the release of tachykinin from sensory nerve endings. Mast cells and the transient receptor potential cation channel vanilloid 1 (capsaicin receptor) were not considered to play a role (Futamura et al. 2009).

An amount of 25 µl xylene (isomer not specified) was applied in concentrations of 10%, 50% or 100% to the ear skin of BALB/c mice once a week for 5 weeks. Ear thickness increased as a result of the irritant effect in a concentration and time-dependent manner. Undiluted xylene led to the migration of inflammatory cells into the skin (Saito et al. 2011).

Exposure of human skin to concentrations of xylene (isomer not specified) of 100 to 1 000 000 ml/m^3 in the gaseous phase for 8 hours decreased tissue viability, the glutathione level, the activities of glutathione *S*-transferase, catalase, and superoxide dismutase, and increased the levels of malondialdehyde and carbonyl compounds and DNA fragmentation in the comet assay. Some of these effects occurred even at 1000 ml/m^3 and were attributed by the authors to oxidative damage (Costa et al. 2006).

5.4 Allergenic effects

There are several findings from (modified) local lymph node assays (LLNAs) with xylene, which, however, are to be regarded as false positives:

In an incompletely documented compilation of findings from LLNAs, stimulation indices of 3.0 and 3.1 were reported for the two highest xylene concentrations (probably 50% and undiluted substance) (Basketter et al. 1996). A concentration of 95.8% was given as the EC3 value, that is the concentration that leads to the tripling of lymphocyte proliferation (Estrada et al. 2003).

In an interlaboratory validation of alternative end points in the LLNA conducted in 9 laboratories, lymph node weights and the lymphocyte count in BALB/c mice were used to determine lymphocyte proliferation instead of determining ^{3}H-thymidine incorporation. Ear weights served as a marker of irritation. Xylene was used in 10% and 30% preparations in dimethylacetamide/acetone/ethanol (4:3:3) as well as undiluted and led to an increase in the lymphocyte count in 8 of the 9 laboratories as well as in one additional laboratory in each case in which ^{3}H-thymidine incorporation was investigated or in which NMRI mice were used (Ehling et al. 2005).

In a modification of the LLNA according to OECD Test Guideline 442A (LLNA:DA with determination of lymphocyte proliferation based on ATP content), a two-stage protocol was used. In the first screening step, only the highest possible concentration was examined in only 2 BALB/c mice per group. Substances that resulted in a stimulation index ≥ 1.8 in this step were tested in the second step using the original LLNA:DA with 3 concentrations and groups of 4 animals. Undiluted xylene resulted in a stimulation index of 2.4 in the first step. In the second step, stimulation indices of 1.3, 1.8 and 2.7 were determined for 10% and 50% preparations in acetone/olive oil (4:1) and for undiluted xylene, respectively, and an EC1.8 of 46.9% was obtained (Zhang et al. 2017).

Undiluted xylene (no information on isomer) applied to the ears of BALB/c mice once a week for 5 weeks evoked mild ear swelling and marginal inflammatory cell invasion from the second application onwards. The maximum ear thickness was reached about 1 hour after application in each case. The expression of interleukin-4 and interferon-γ was not increased (Saito et al. 2011).

In several compilations of in vitro findings, only negative results were found with xylene, for example in a modified (quantitative) direct peptide reactivity assay (DPRA) (Wareing et al. 2017) and in the DPRA according to OECD Test Guideline 442C (Bauch et al. 2012), with the test methods according to OECD Test Guideline 442D, the KeratinoSens assay (Bauch et al. 2012; Natsch et al. 2015) and the LuSens assay (Bauch et al. 2012; Ramirez et al. 2014), with the test methods according to OECD Test Guideline 442E, the h-Clat assay (Bauch et al. 2012; Urbisch et al. 2015), the U-Sens assay (Bauch et al. 2012; EURL ECVAM 2016) and the IL-8 Luc assay (OECD 2017), as well as with test methods not (yet) included in an OECD test guideline, the GARD assay (Forreryd et al. 2018; Zeller et al. 2017) and the SENS-IS assay using a reconstructed human skin model (Episkin^{R}) (Cottrez et al. 2016).

5.5 Genotoxicity

In human lymphocytes, 50, 100 or 200 µM *m*-xylene, *p*-xylene and *o*-xylene induced a concentration-dependent increase in DNA damage as measured by the alkaline and neutral comet assays (Chen et al. 2008). In the comet assay with promyelocytic leukaemia HL-60 cells, *o*-xylene caused a statistically significant increase of the tail moment (Sarma et al. 2011).

Xylenes were not found to be genotoxic in vitro and in vivo (Greim 2001), and the results of the new indicator tests have not led to a change in this assessment.

5.6 Carcinogenicity

Xylenes are not carcinogenic in rats and mice after oral administration (Greim 2001).

5.7 Other effects

In an in vitro study, *m*-xylene induced the release of MCP-1 in A549 lung epithelial cells at concentrations of 1 to 10 000 mg/m^3. At a concentration of 100 000 mg/m^3, the release of IL-6 and MCP-1 was inhibited and that of IL-8 was increased (Fischäder et al. 2008).

In LLC-PK1 cells (porcine proximal tubular cells), *p*-xylene was cytotoxic at a concentration of 106 mg/l (the concentration unit "mg/ml" given in the publication is wrong) and increased caspase-3 activity. These effects were reversed by a caspase-3 inhibitor. Caspase-3 may play a role in the observed apoptosis of proximal tubular cells by xylene and tubular renal toxicity in exposed persons after chronic exposure to solvents (Al-Ghamdi et al. 2003). In another study in LLC-PK1 cells, it was demonstrated that 1 mM *p*-xylene (106 mg/l) can induce caspase-9 activity and cause increased expression of Bax protein. Xylene-induced DNA fragmentation was prevented by a caspase-9 inhibitor (Al-Ghamdi et al. 2004).

In dermal fibroblasts in a collagen matrix, *m*-xylene induced cytotoxicity and decreased the cellular thiol level and catalase activity (Coleman et al. 2003).

After exposure of rat liver mitochondria to 0.25 to 1 mM xylene (isomer not specified), mitochondrial uncoupling occurred. At 0.1 mM reactive oxygen species were released and at 1 mM the mitochondrial ATP was depleted (Revilla et al. 2007).

In human promyelocytic leukaemia HL-60 cells, the gene expression pattern was investigated after exposure to cytotoxic *o*-xylene concentrations. There was an increased expression of genes involved in immune response, apoptosis, transcriptional regulation, cell cycle regulation and transport (Sarma et al. 2010).

In HL-60 cells, *o*-xylene caused apoptosis. The expression of haemoxygenase-1 and Noxa proteins (involved in apoptosis) was increased. The release of reactive oxygen species was increased and, in the comet assay, the tail moment was increased, a sign of apoptosis. These effects could be reduced by administration of the antioxidant *N*-acetylcysteine. This haemoxygenase-1 and Noxa-induced apoptosis is caspase-independent (Sarma et al. 2011).

6 Manifesto (MAK value/classification)

The critical effects of xylene are the acute pre-narcotic effects in humans and in animal studies.

MAK value. The previous MAK value of 100 ml/m^3 was based on the effects on the equilibrium function after exposure to xylene concentrations of 200 ml/m^3 and more. As shown in Greim (2001), if the MAK value of 100 ml xylene/m^3 is observed, xylene concentrations in the blood are already attained during mild physical activity, as they occurred under resting conditions in experimental studies in the range of 200 and 300 ml xylene/m^3. In one study (Laine et al. 1993), however, no clear increase in effects due to increased breathing was found. However, only 9 subjects were examined in this study (Greim 2001).

A threshold concentration of 2 mg xylene/l blood has been derived for pre-narcotic effects (MacDonald et al. 2002). At 50 ml xylene/m^3, a concentration of about 0.6 mg xylene/l is reached after 8 hours at rest and about 1.2 mg/l with exercise at 50 watts, which corresponds to the increased respiratory volume at the workplace. Therefore, the MAK value has been set at 50 ml xylene/m^3 to avoid pre-narcotic effects.

Peak limitation. The MAK value for xylenes was established on the basis of systemic effects and they remain assigned to Peak Limitation Category II. In the toxicokinetic study by Loizou et al. (1999), an elimination half-life of 30 minutes was given for *m*-xylene in alveolar air. This corresponds to the elimination half-life from the blood. This half-life would necessitate an excursion factor of 1. For substances with a half-life of 30-minutes, a 15-minute exposure at the end of the shift with an excursion factor of 2 would be expected to increase the concentration in the blood by 50% (Hartwig and MAK Commission 2017). Since exposure at the level of the MAK value corresponds to a concentration of

1.2 mg xylene/l blood, with an excursion factor of 2 a concentration of 1.8 mg/l can be expected. Because effects on the CNS occurred only at more than 2 mg/l, an excursion factor of 2 has been set in deviation from the usual procedure.

Absorption through the skin. Several in vitro and in vivo studies in animals and humans are available for absorption of xylene through the skin. For a quantitative estimation of the transdermal absorption of the substance, the in vivo experiments in humans are particularly suitable. In these experiments, the dermal flux of xylene was determined to be 293 µg/cm^2 and hour for an exposure duration of 3 minutes; for exposures lasting 15 and 20 minutes, the fluxes were lower at 120 and 147 (42–258) µg/cm^2 and hour, respectively. The highest flux thus probably represents an overestimation resulting from extrapolation to longer exposure times. For exposure of both hands and forearms (2000 cm^2) for 1 hour, the dermal absorption of about 300 mg xylene on average can be assumed, but also a maximum of 516 mg xylene. This compares with an inhaled amount of 1320 mg of xylene at 60% inhalation retention (Greim 2001) and a respiratory volume of 10 m^3 for an 8-hour exposure at the level of the MAK value. The estimated mean percutaneous absorption is about 23% of this value. It may be higher in individual persons, so that observance of the established MAK value alone does not provide sufficient protection with certainty. The designation of xylene with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts) has therefore been retained (Greim 2001).

Sensitization. There are no reliable clinical reports of contact allergic reactions to xylenes. The results with xylenes from experimental studies indicate a low irritant potential rather than a contact sensitizing potential. There are no findings of sensitizing effects on the airways. Xylenes have therefore not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

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

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