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Xylidine isomers (2,3-xylidine, 2,5-xylidine, 3,4-xylidine, 3,5-xylidine)

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

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Keywords

2,3-xylidine; 2,5-xylidine; 3,4-xylidine; 3,5-xylidine; carcinogenicity; germ cell mutagenicity; skin absorption; methaemoglobin; toxicity

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the xylidine isomers 2,3-, 2,5-, 3,4- and 3,5-xylidine [87-59-2, 95-78-3, 95-64-7, 108-69-0] considering all toxicological end points. The critical effects are methaemoglobinaemia, anaemia and effects on the spleen, liver and kidneys. A carcinogenicity study was performed with only 2,5-xylidine. Although the study is of limited validity, the angiosarcomas observed in male mice and fibromas and fibrosarcomas in rats are tumours typical for aromatic amines. Therefore, a carcinogenic potential is assumed and 2,3-, 2,5-, 3,4- and 3,5-xylidine are classified in Carcinogen Category 3 B for suspected carcinogens. All 4 isomers are genotoxic both in vitro with metabolic activation and in somatic cells in vivo. However, these findings are not quite consistent, this is typical for aromatic amines. Therefore, 2,3-, 2,5-, 3,4- and 3,5-xylidine are classified in Category 3 B for germ cell mutagenicity. A maximum concentration at the workplace (MAK value) is not derived because of the suspected mutagenicity. There are only limited data for percutaneous absorption, but as the systemic toxicity of these 4 isomers is high and they are suspected mutagens and carcinogens, the designation with "H" is retained. Studies of the sensitization potential are not available.

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MAK value	–
Peak limitation	–
Absorption through the skin (1966)	**H**
Sensitization	–
Carcinogenicity (2019)	**Category 3 B**
Prenatal toxicity	–
Germ cell mutagenicity (2019)	**Category 3 B**
BAT value	–
Melting point	2,3-xylidine: 2 °C (NCBI 2023 a) 2,5-xylidine: 15.5 °C (NCBI 2023 b) 3,4-xylidine: 51 °C (NCBI 2023 c) 3,5-xylidine: 9.8 °C (NCBI 2023 d)
Boiling point	2,3-xylidine: 221.5 °C (NCBI 2023 a) 2,5-xylidine: 214 °C (NCBI 2023 b) 3,4-xylidine: 228 °C (NCBI 2023 c) 3,5-xylidine: 220.5 °C (NCBI 2023 d)
Density at 20 °C	2,3-xylidine: 0.99 g/cm3 (IFA 2019 a) 2,5-xylidine: 0.98 g/cm3 (IFA 2019 b) 3,4-xylidine: 1.07 g/cm3 (IFA 2019 c) 3,5-xylidine: 0.97 g/cm3 (IFA 2019 d)
Vapour pressure	2,3-xylidine: 0.1 hPa at 25 °C (NCBI 2023 a) 2,5-xylidine: 0.2 hPa at 20 °C (NCBI 2023 b) 3,4-xylidine: 0.04 hPa at 25 °C (NCBI 2023 c) 3,5-xylidine: 0.2 hPa at 25 °C (calculated; NCBI 2023 d)
Solubility in water	2,3-xylidine: no data (ECHA 2018 a) 2,5-xylidine: < 0.1 mg/l (ECHA 2019) 3,4-xylidine: < 0.1 mg/l (ECHA 2018 b) 3,5-xylidine: 4.8 g/l (ECHA 2018 c)

Note: The substances can occur simultaneously as vapour and aerosol.

This supplement re-evaluates the carcinogenicity classification of the xylidine isomers 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine. In 1998, 2,4-xylidine and 2,6-xylidine were classified in Carcinogen Category 2. These 2 xylidine isomers are not the subject of the present supplement.

Since the documentation from 1998 and the supplement from 2000 (published in one combined translation: Greim 2003) new studies concerning methaemoglobin formation, the mechanism of action, subacute toxicity and genotoxicity of the isomers have been published, as well as a case-control study in patients with bladder cancer.

1 Toxic Effects and Mode of Action

The target organs of 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine are the blood, spleen, liver and kidneys. All 4 isomers form methaemoglobin; 3,5-xylidine was shown to be the strongest methaemoglobin former of the 4 isomers (Greim 2003). There are no consistent data for genotoxicity in vitro and in vivo: mutagenicity tests with Salmonella typhimurium strains yielded positive as well as negative results. Mutagenicity in mammalian cells has been demonstrated only in the case of 3,5-xylidine. Two of the 4 isomers were found to have clastogenic effects in vitro. In vivo, the test results with all 4 isomers were positive in the comet assay; the micronucleus test yielded negative results. DNA binding was observed with 3,5-xylidine. In the MutaMouse®, only 2,5-xylidine caused mutations in nasal tissue, but not 3,5-xylidine. Overall, a genotoxic effect is suspected. Human carcinogenicity data suitable for inclusion in the evaluation are not available. The studies of carcinogenicity in mice and rats are older but indicate that 2,5-xylidine causes tumours typical of this substance class, namely blood vessel tumours, fibromas and fibrosarcomas. This, together with the unclear genotoxicity as well as the mechanism of action and structure, suggests the 4 isomers possess carcinogenic potential.

3,5-Xylidine is not irritating to the skin and eyes. Occlusive application of 2,3-xylidine for 24 hours led to severe skin irritation, although studies in accordance with OECD test guidelines are not available for this isomer. Studies of the sensitizing effects of xylidines are not available. There are also no reproductive toxicity studies, but a study of the incorporation of thymidine into testicular DNA demonstrated that 2,5-xylidine and 3,5-xylidine reach the testes of male mice.

2 Mechanism of Action

All 4 isomers produce methaemoglobin, but to varying degrees. The haemoglobin binding index (adduct concentration [mmol/mol Hb] per dose [mmol/kg body weight]) is 0.7 for 3,4-xylidine, 7.3 for 2,5-xylidine and 14.0 for 3,5-xylidine (Greim 2005). The formation of methaemoglobin is the result of the co-oxidation of the *N*-hydroxy derivatives and oxyhaemoglobin produced during metabolism (Greim 2003).

In 2 publications, possible reaction pathways and metabolites of 3,5-xylidine are postulated; these can be assumed to apply to all the isomers (Chao et al. 2012; Skipper et al. 2010). According to these publications, there are different metabolic pathways that may contribute to the genotoxicity of xylidines:

***N*-Hydroxylation:** Xylidines are oxidized by cytochrome P450 (CYP) to *N*-hydroxylamine, which, catalyzed by acyltransferases or sulfotransferases, forms an unstable *N*-*O*-ester. The resulting highly reactive nitrenium ion can form DNA adducts.

After intraperitoneal administration of radiolabelled 3,5-xylidine or 2,6-xylidine at a dose of 100 µg/kg body weight, DNA binding was detected in the liver and bladder of C57Bl/6 mice. DNA binding was higher after exposure to 3,5-xylidine than after exposure to 2,6-xylidine: the covalent binding indices in the bladder and liver were 21 and 84 for 3,5-xylidine and 5 and 14 for 2,6-xylidine, respectively. However, DNA adducts were not identified in this study (Skipper et al. 2006).

Four different DNA adducts could be identified in vitro. Calf thymus DNA was incubated with *N*-AcO-3,5-xylidine, a metabolite formed from *N*-hydroxylamine after phase II conjugation which also forms a nitrenium ion as an intermediate. Two adducts from the reaction with deoxyadenosine (4-(deoxyadenosin-*N*6-yl)-3,5-dimethylaniline and *N*-(deoxyadenosin-8-yl)-3,5-dimethylaniline) were identified and adducts from the reaction with deoxycytidine (*N*-(deoxycytidin-5-yl)-3,5-dimethylaniline) and deoxyguanosine (*N*-(deoxyguanosin-5-yl)-3,5-dimethylaniline) (Cui et al. 2007). In vitro, also deoxyguanosine C8 adducts could be formed with the *N*-(acyloxy) derivatives of all xylidine isomers (Marques et al. 1996, 1997). In addition, a correlation was found between the substitution pattern and the stability of the nitrenium ion (Sabbioni 1992) or mutagenicity (Marques et al. 1997). The enthalpy of formation for nitrenium ions is lower for para-substituted alkylanilines than for meta-substituted alkylanilines. It can therefore be concluded that para-substituted isomers have a greater ability to form adducts and accordingly have stronger mutagenic effects.

Tests with *N*-hydroxyarylamines and Salmonella typhimurium TA100 without metabolic activation yielded a weak mutagenic response with the double meta-substituted isomer (*N*-hydroxy-3,5-dimethylaniline). An alkyl group in the para position increased the mutagenic effect. It is believed that the para-substituted alkylanilines can form adducts more effectively due to the greater stability and resulting longer half-life of the nitrenium ion. Substitution in the ortho position also enhances the mutagenic response, even to the extent that *N*-hydroxy-2,6-dimethylaniline has been shown to be the *N*-hydroxyalkylaniline with the strongest mutagenicity. However, since this is not associated with increased DNA adduct formation, the authors concluded that adducts of ortho-substituted alkylanilines have a higher intrinsic mutagenicity compared with those of para-substituted or meta-substituted alkylanilines (Marques et al. 1997).

Ring hydroxylation: Another possibility is metabolism to an aminophenol. The aminophenol can be formed directly by oxidation, likewise with the participation of CYP. But also the nitrenium ion and the *N*-hydroxylamine can rearrange themselves to form an aminophenol ("Bamberger rearrangement"). From the aminophenol, a quinone imine is formed by spontaneous or peroxidase-catalyzed oxidation. Due to its electrophilic properties, this is highly reactive and capable of redox cycling and thus of forming reactive oxygen species (ROS). The quinone imine of 2,6-xylidine induces sister chromatid exchange (SCE). Protein adducts with quinone imines could be detected; the formation of DNA adducts with quinone imines has not been reported to date (Skipper et al. 2010). The products formed by the reaction of quinone imines with proteins can be indirectly mutagenic as a result of the generation of ROS.

The formation of ROS and of DNA strand breaks in AS52 cells was demonstrated for the ring and *N*-hydroxy metabolites of 3,5-xylidine and 2,6-xylidine. This suggests that quinone imines play a crucial role also in the mechanism of action of xylidines (Chao et al. 2012).

When S9 mix was added or metabolically competent cells were used, 3,5-xylidine hydroxylated on the ring was found to be more cytotoxic and mutagenic in CHO (Chinese hamster ovary) cells at lower concentrations than the corresponding *N*-hydroxylamine. Both hydroxy derivatives were more cytotoxic than unmetabolized xylidine. The authors concluded that ROS are more important for the mutagenicity of xylidines than DNA adducts (Chao et al. 2012).

2,6-Xylidine and 3,5-xylidine and the corresponding *N*-hydroxy derivatives and aminophenols were studied in cell lines that are either nucleotide excision repair-deficient (NER-deficient, AA8 CHO cells) or NER-proficient (UV5 cells). None of the cell lines expresses phase I or phase II enzymes. The cells were treated with 50 µM to 1 mM 2,6-xylidine and 3,5-xylidine or with the corresponding *N*-hydroxy and aminophenol metabolites (5 to 500 µM). After 24 hours, cytotoxicity (trypan blue) and mutagenicity (8-azaadenine selection) were examined. 2,6-Xylidine and 3,5-xylidine and the corresponding *N*-hydroxy and aminophenol metabolites were cytotoxic without metabolic activation by S9 mix, but not mutagenic. Cytotoxicity was higher in the NER-deficient cells than in the NER-proficient cells. This suggests that also other products that are not mutagenic in this test contribute to the toxicity by causing DNA damage. 2,6-Xylidine and 3,5-xylidine were cytotoxic and mutagenic in CHO cells expressing mouse CYP1A2 genes and human aryl sulfotransferase or *N*-acetyltransferase (NAT) genes. NER deficiency increased the cytotoxicity and mutagenicity. The authors concluded that alkylanilines must be activated by phase I and phase II enzymes for mutagenic activity (Skipper et al. 2010).

3 Toxicokinetics and Metabolism

There are no data available for inhalation exposure. The xylidines are absorbed well from the gastrointestinal tract after oral absorption (Greim 2003).

There are no studies available for absorption through the skin. The dermal LD_{50} of 2,3-xylidine in guinea pigs is between 500 and 1000 mg/kg body weight (Eastman Kodak Co 1994); the substance can therefore be assumed to be absorbed through skin contact. However, severe skin irritation occurred, which may have led to an increase in absorption.

Methaemoglobin formation with the structural analogue 2,4-xylidine was observed in cats and dogs after dermal application (Greim 2003).

For a saturated aqueous solution of 3,5-xylidine (log K_{OW} about 2; Greim 2003), fluxes of 706 and 54 µg/cm² and hour were calculated using the models of Fiserova-Bergerova (1990) and the algorithm of the IH SkinPerm model (Tibaldi et al. 2014), respectively. Assuming the exposure of 2000 cm² of skin (area of hands and forearms) for 1 hour, this would correspond to absorbed amounts of 1412 and 108 mg, respectively. There are no data for the solubility in water of 2,3-xylidine, so that dermal absorption of the substance cannot be calculated. As the water solubility of the other xylidine isomers is less than 0.1 mg/l, their absorption through the skin is negligible according to these models.

2,5-Xylidine: Male Osborne Mendel rats were given daily doses of 200 mg 2,5-xylidine/kg body weight by gavage for 8 days. The substance was excreted either unchanged or as metabolites with the urine. The main metabolite was 4-hydroxy-2,5-xylidine; 2-amino-4-methylbenzoic acid and 3-amino-4-methylbenzoic acid were found in lower concentrations (Greim 2003; OECD 2012).

3,4-Xylidine: The conjugates identified in the rat were 4-acetamido-2-methylbenzoic acid, 2-amino-4,5-dimethylphenyl sulfate and 4-amino-2-methylbenzoic acid as glucuronide (Greim 2003).

3,5-Xylidine: Male C57BL/6 mice were given intraperitoneal injections of 100 µg [^{14}C]-3,5-xylidine/kg body weight. About 45% of the radioactivity was found in the 25-hour urine collected. However, the authors assumed that the amount may have been underestimated because it was difficult to collect such small amounts of urine. The clearance of 3,5-xylidine from plasma was biphasic, with slower clearance at later times, indicating that binding of metabolites to tissue takes place. As the earliest plasma sample was collected after 2 hours, the data do not represent a complete absorption phase. DNA binding was found in both the bladder and the liver. The half-life for the clearance of DNA adducts from the bladder was 15 hours, that from the liver 21 hours (Skipper et al. 2006).

4 Effects in Humans

No or no new data are available for single and repeated exposures, reproductive toxicity, genotoxicity and allergenicity.

In a case-control study, 298 patients with bladder cancer were examined. The control group consisted of 308 people, who were matched for age, sex, ethnicity and neighbourhood to the patients. Personal interviews were used to gather information about smoking habits and other risk factors for bladder cancer. Blood samples were taken at the end of the interviews and the arylamine-haemoglobin adduct levels were determined. Determinations included haemoglobin adducts of 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine. Covariance and regression analyses were used to investigate the relationship between the arylamine-haemoglobin adducts and bladder cancer. Arylamine-haemoglobin adduct concentrations were higher in smokers than in non-smokers (except 2,6-xylidine), and the haemoglobin adduct levels of all arylamines were higher in patients with bladder cancer than in control subjects. Taking confounders such as smoking behaviour at the time of sampling into account, a statistically significant association between the haemoglobin adducts of 3,5-xylidine and the risk of bladder cancer was found. The association between 3,5-xylidine and bladder cancer also remained statistically significant when only non-smokers (at the time of sampling) were included (relative risk 2.7; 95% confidence interval 1.2–6.0) (Gan et al. 2004). Since exposure concentrations were not recorded in the study, it cannot be included in the evaluation of the carcinogenicity of xylidines.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

There are no data available.

5.1.2 Oral administration

The oral LD_{50} values for the 4 xylidine isomers in male SD rats and CF-1 mice are shown in Table 1. The clinical signs are not described.

Tab. 1 Oral LD_{50} values for xylidines in rats and mice (mg/kg body weight (95% confidence interval))

Species	2,3-Xylidine	2,5-Xylidine	3,4-Xylidine	3,5-Xylidine
rat	930 (630–1380)	1300 (940–2140)	810 (590–1120)	710 (470–1070)
mouse	1070 (730–1590)	840 (470–1490)	710 (520–960)	420 (280–640)

The methaemoglobin concentration was determined in groups of 3 SD rats before and 1, 2, 4 and 6 hours after the administration of 4.8 mmol 2,3-xylidine, 2,5-xylidine, 3,4-xylidine or 3,5-xylidine/kg body weight. At all time-points, a statistically significant difference between the value determined and the control value (methaemoglobin concentration before exposure) was found only for 3,5-xylidine. The highest methaemoglobin concentration of 31.3% ± 1.5% was reached 6 hours after administration. The methaemoglobin concentration produced by the other 3 isomers remained below 3% at all times. The authors assumed 4.8 mmol/kg body weight (580 mg/kg body weight) to be the NOAEL (no observed adverse effect level) for 2,3-xylidine, 3,4-xylidine and 2,5-xylidine (Cauchon and Krishnan 1997).

Methaemoglobin formation after single oral doses of 0.24, 0.48, 0.72, 0.96, 1.2, 1.8, 2.4 or 4.8 mmol 3,5-xylidine/kg body weight in corn oil was investigated also in groups of 3 rats. Blood samples from the caudal vein were taken before and 0.25 to 56 hours after administration and the methaemoglobin levels were determined. A NOAEL of 0.96 mmol/kg body weight (116 mg/kg) and a LOAEL (lowest observed adverse effect level) of 1.2 mmol/kg body weight (145 mg/kg) were obtained (Greim 2003; Shardonofsky and Krishnan 1997).

5.1.3 Dermal application

The LD_{50} in guinea pigs after occlusive application of 2,3-xylidine for 24 hours was found to be between 500 and 1000 mg/kg body weight (Eastman Kodak Co 1994).

5.1.4 Intravenous injection

After groups of 5 adult cats were given intravenous injections of a single dose of 2,3-xylidine, 2,5-xylidine, 3,4-xylidine or 3,5-xylidine of 30 mg/kg body weight, the methaemoglobin concentration was determined in the blood during the first 5 hours after treatment. The highest methaemoglobin concentration (46.5% ± 3.9%) was found in the animals treated with 3,5-xylidine. The maximum methaemoglobin concentrations in the first 5 hours after the exposure to the remaining 3 isomers were 36.3% ± 7.1% (2,5-xylidine), 20.2% ± 5.4% (2,3-xylidine) and 18.0% ± 3.4% (3,4-xylidine) (BUA 1995).

Groups of 3 Sprague Dawley rats were given single intravenous injections of 0.06, 0.12, 0.24, 0.48 or 0.60 mmol 3,5-xylidine/kg body weight (purity 99%, dissolved in Emulphor, methanol and saline (1:1:4)). Blood samples from the caudal vein were taken before and 0.25 to 6 hours after the exposure and the methaemoglobin levels were determined. The results showed a dose-dependent induction of methaemoglobinaemia, with the highest level of 28.9% methaemoglobin at the highest dose tested of 0.60 mmol/kg body weight. The NOAEL was 0.06 mmol 3,5-xylidine/kg body weight (7.3 mg/kg body weight), the LOAEL was 0.12 mmol/kg body weight (14.5 mg/kg body weight) (Greim 2003; Shardonofsky and Krishnan 1997).

5.1.5 In vitro

In in vitro studies in rat erythrocytes with a 2-compartment dialysis system, all 4 xylidine isomers caused the formation of methaemoglobin at a concentration of 1 mM with metabolic activation by rat liver S9 mix (incubation time: 0.5, 1, 2, 3 and 4 hours). Incubation for 1 hour with 3,5-xylidine caused the highest methaemoglobin concentration of 24.84% ± 2.56% (2,3-xylidine: 10.95% ± 1.43%, 2,5-xylidine: 21.36% ± 7.44%, 3,4-xylidine: 17.05% ± 2.61%, no data for control value). Likewise, with regard to the formation of methaemoglobin over time, 3,5-xylidine caused the most pronounced

effects. The AUC_{metHb} (area under the methaemoglobin concentration–time curve) for 3,5-xylidine was 60.75% methaemoglobin × h, while that of the other isomers was 45.33 (2,3-xylidine), 49.43 (2,5-xylidine) and 56.83% methaemoglobin × h (3,4-xylidine). Without metabolic activation, the methaemoglobin concentration of all the isomers remained below 3% after incubation for 1 hour. The results show that xylidine isomers can form methaemoglobin only after metabolic activation. At a lower xylidine concentration of 0.3 mM, methaemoglobin formation for 3,5-xylidine was 19.56% ± 2.22% after incubation for 2 hours, while that of the other isomers was significantly lower (2,3-xylidine: 4.83% ± 0.72%, 2,5-xylidine: 5.94% ± 0.61%, 3,4-xylidine: 7.15% ± 0.19%, control value: 3.90% ± 0.10%). After incubation with 0.06 mM xylidines for 1 hour, the methaemoglobin formation caused by 3,5-xylidine was still 9.64% ± 0.51% (2,3-xylidine: 3.59% ± 0.42%, 2,5-xylidine: 3.10% ± 0.27%; 3,4-xylidine: 5.84% ± 0.37%, control value: 2.90% ± 0.27%). After incubation for 2 hours only a low level of methaemoglobin formation was observed with all the xylidines (3,5-xylidine: 4.53% ± 0.47%, 2,3-xylidine: 4.11% ± 1.20%, 2,5-xylidine: 2.90% ± 0.32%, 3,4-xylidine: 3.34% ± 0.23%, control value: 2.56% ± 0.13%) (Cauchon and Krishnan 1997).

5.2 Subacute, subchronic and chronic toxicity

The subacute, subchronic and chronic toxicity of the isomers were already described in detail in the documentation from 1998 and in the supplement from 2000 (Greim 2003). Therefore, this supplement presents only new data.

Since the last supplement from 2000 (Greim 2003), 28-day oral studies have been conducted in CRJ:CD (SD) rats with 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine. The study reports are available in Japanese, with English tables and, with the exception of 2,5-xylidine, with an English summary (MHLW 2018 a, b, c, d). All 4 studies were carried out according to OECD Test Guideline 407 in different test facilities and included additional animals for a 14-day recovery period. 2,3-Xylidine, 2,5-xylidine and 3,5-xylidine led to an increase in the level of methaemoglobin in the blood of male and female rats; the methaemoglobin level in the study with 3,4-xylidine was not determined. An increase in reticulocytes, a decrease in erythrocytes and the haemoglobin level and changes in other blood parameters were observed at and above 60 mg/kg body weight and day with 2,3-xylidine and 3,5-xylidine, at and above 250 mg/kg body weight and day with 3,4-xylidine and at 300 mg/kg body weight and day with 2,5-xylidine. Increased haemosiderin deposits in the liver and spleen as a secondary effect of haemolysis were observed at and above 60 mg/kg body weight and day with 3,5-xylidine and with 3,4-xylidine and 2,5-xylidine at the highest doses tested of 250 and 300 mg/kg body weight and day, respectively. Haemosiderin deposits in the kidneys were reported also for 3,5-xylidine. Female rats given 2,3-xylidine were found to have increased haemosiderin deposits in the spleen at 12 mg/kg body weight and day; in male rats, this effect occurred only at 60 mg/kg body weight and day and above. In each case, the highest tested doses of all 4 isomers led to an increase in specific urine gravity, an increase in urine volume and a change in the pH and protein composition of the urine. Mainly in the male animals, hyaline droplets or casts were found in the kidneys: for 2,3-xylidine and 3,4-xylidine at 60 mg/kg body weight and day and for 3,5-xylidine at 360 mg/kg body weight and day. In the highest dose groups, all isomers except 3,4-xylidine led to increased kidney weights and necrosis. With 2,3-xylidine and 3,5-xylidine at 60 and 50 mg/kg body weight and day, respectively, slight increases in weight or enlargement of the liver were found. At the highest doses tested of 250 or 360 mg/kg body weight, the absolute or relative liver weights were increased with all 4 isomers and hypertrophy of hepatocytes was observed. Effects on the spleen, such as increased relative and absolute weights and congestion, were observed at the highest doses (250 to 360 mg/kg body weight). For 2,3-xylidine, the authors derived a NOAEL of 12 mg/kg body weight and day for male rats due to haematological effects and findings in the liver, kidneys, bone marrow and spleen at 60 mg/kg body weight and above. In the female rats, haemosiderin deposits in the red pulp of the spleen were increased at 12 mg/kg body weight and day, so that 12 mg/kg body weight was considered to be the LOAEL. The NOAEL for female and male rats for 2,5-xylidine was 12 mg/kg body weight and day, based on haematological effects and effects on the kidneys and the forestomach at 60 mg/kg body weight and day. A NOAEL of 10 mg/kg body weight and day was derived for 3,4-xylidine and 3,5-xylidine. At the next-higher 3,4-xylidine dose of 50 mg/kg body weight and day, hyaline droplets in the kidneys of the male animals and enlargement of the liver in the female animals were observed. At 60 mg 3,5-xylidine/kg body weight, haematological effects on the bone marrow, liver and spleen and their sequelae were observed (Table 2; MHLW 2018 a, b, c, d; OECD 2012).

Tab. 2 28-Day oral studies in CRJ:CD (SD) rats with 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine

Substance exposure number per group	Findings	References
2,3-xylidine 0, 12, 60, 300 mg/kg body weight and day in olive oil, by gavage, 7 days/week 6 ♂ and 6 ♀, 14-day recovery period: additional 6 ♂ and 6 ♀ at 0, 60, 300 mg/kg body weight and day	**12 mg/kg body weight**: ♂: **NOAEL**; **12 mg/kg body weight and above**: spleen: ♀: haemosiderin deposits (5/6 severity level 3, controls: 6/6 severity level 2); **60 mg/kg body weight and above**: blood: ♂ and ♀: Hb ↓; ♀: erythrocytes, haematocrit ↓, MetHb ↑, liver: ♂: relative weights 10% ↑, spleen: ♂: haemosiderin deposits, kidneys: ♂: hyaline casts, bone marrow: ♂ and ♀: haematopoiesis ↑; **300 mg/kg body weight**: 1 ♂ died on day 27 (heart: adhesion to lung; lung: adhesion to heart and other lung lobes, reddened lung, spleen enlargement); ♂ and ♀: water intake ↑; ♀: body weight gains ↓, biochemistry: ♂ and ♀: total bilirubin, BUN ↑; ♂: γ-GTP, indirect bilirubin, potassium ↑; ♀: ALT, total cholesterol, phospholipids ↑, blood: ♂ and ♀: MCV, MCH, reticulocytes, leukocytes ↑; ♂: haematocrit, erythrocytes, MCHC ↓, MetHb ↑, urine: ♂ and ♀: volume ↑, specific gravity ↓, spleen: ♂ and ♀: absolute and relative weights ↑, extramedullary haematopoiesis ↑; ♂: congestion, kidneys: ♂ and ♀: absolute and relative weights ↑, papillary necrosis, dilation of the renal tubules, cellular infiltration; ♂: basophilia of the renal tubules, mineralization of the inner medulla, high occurrence of eosinophilic bodies in the tubular epithelia; ♀: hyaline casts, liver: ♀: relative weights ↑; ♂ and ♀: hypertrophy of the centrilobular hepatocytes, biliary duct proliferation, haemosiderin deposits in Kupffer cells, extramedullary haematopoiesis, recovery period: reversal of findings, except in the kidneys	MHLW 2018 a
2,5-xylidine 0, 12, 60, 300 mg/kg body weight and day in corn oil, by gavage, 7 days/week 6 ♂ and 6 ♀, 14-day recovery period: additional 6 ♂ and 6 ♀ at 0 and 300 mg/kg body weight and day	**12 mg/kg body weight**: **NOAEL**; **60 mg/kg body weight and above**: blood: ♀: prothrombin time ↑, biochemistry: ♂: protein fraction changed, glucose ↓, forestomach: ♂ and ♀: squamous epithelial hyperplasia, hyperkeratosis; ♂: thickening of the focal mucosa, kidneys: ♂: eosinophilic bodies in the epithelium of the proximal tubules; **300 mg/kg body weight**: ♂ and ♀: staggering gait, lacrimation, water intake ↑, ♂: body weight gains ↓, food intake ↓ (in ♀ only up to days 5 and 7), urine: ♂ and ♀: volume ↑, specific gravity ↓, protein content ↓; ♂: pH ↓, blood: ♂ and ♀: erythrocytes, haematocrit, Hb ↓, MetHb, reticulocytes ↑; ♂: MCHC ↓, segmented neutrophils ↑, lymphocytes ↓, biochemistry: ♂ and ♀: total bilirubin ↑; ♂: inorganic phosphorous ↑; ♀: protein fraction changed, forestomach: ♀: focal thickening of mucosa, kidneys: ♂ and ♀: discoloration of papilla, relative weights ↑, oedema, papillary necrosis, cellular infiltration, regeneration, mineralization; ♂: dilation, liver: ♂ and ♀: relative weights ↑, centrilobular hypertrophy, spleen: ♂ and ♀: relative weights ↑, congestion, haemosiderin deposits, heart: ♂: absolute weights ↓, brain: ♂: absolute and relative weights ↑, pituitary: ♂: absolute weights ↓, testes: relative weights ↑, prostate: absolute weights ↓, seminal vesicles: absolute weights ↓, recovery period: effects partly reversible	MHLW 2018 b

Tab. 2 *(continued)*

Substance exposure number per group	Findings	References
3,4-xylidine 0, 10, 50, 250 mg/kg body weight and day in corn oil, by gavage, 7 days/week 5 ♂ and 5 ♀, 14-day recovery period: additional 5 ♂ and 5 ♀ at 0 and 250 mg/kg body weight and day	**10 mg/kg body weight**: **NOAEL**; **10 mg/kg body weight and above**: blood: ♂: neutrophilic leukocytes ↑, lymphocytes ↓ (only at 10 und 50 mg/kg body weight, no dose-dependency), MetHb not investigated; **50 mg/kg body weight and above**: blood: ♂: prothrombin time ↓ (only at 50 mg/kg body weight), ♀: total cholesterol ↑, liver: ♀: enlarged (1/5), kidneys: ♂: hyaline droplets (4/5); **250 mg/kg body weight**: ♂ and ♀: salivation; ♂: body weights ↓, body weight gains ↓, blood: ♂ and ♀: haematocrit, Hb, erythrocytes ↓, reticulocytes, ALT, total bilirubin, PLT ↑; ♀: neutrophilic leukocytes ↓; lymphocytes ↑; ♂: leukocytes, total cholesterol, albumin, albumin/globulin, potassium ↑, γ-GTP ↓; ♀: glucose, calcium ↑, chloride ↓, urine: ♂ and ♀: volume ↑, pH, sediment in urine ↓; ♀: specific gravity ↓, liver: ♂ and ♀: absolute and relative weights ↑, enlarged (♂ 2/5; ♀ 5/5), hypertrophy, pigment deposit (presumably haemosiderin); ♂: single cell necrosis; ♂: black discoloration (3/5), spleen: ♂ and ♀: absolute and relative weights ↑; black discoloration (♂ 5/5; ♀ 4/5), enlarged (♂ 2/5; ♀ 3/5); congestion, pigment deposits (presumably haemosiderin), haematopoiesis ↑, adrenal glands: ♀: relative weights ↓, testes: ♂: relative weights ↑, bone marrow: haematopoiesis ↑, kidneys: ♂: hyaline droplets; recovery period: changes not completely reversible with the exception of pigment deposits	MHLW 2018 c
3,5-xylidine 0, 10, 60, 360 mg/kg body weight and day in corn oil, by gavage, 7 days/week 6 ♂ and 6 ♀, 14-day recovery period: additional 6 ♂ and 6 ♀ at 0 and 360 mg/kg body weight and day	**10 mg/kg body weight**: **NOAEL**; **60 mg/kg body weight and above**: blood: ♂ and ♀: Hb ↓, reticulocytes ↑; ♀: erythrocytes, MCV, MCHC ↓, ALT ↑; ♂: haematocrit ↓, spleen: ♂ and ♀: absolute weights ↑, extramedullary haematopoiesis, haemosiderin deposits in red pulp, liver: ♂ and ♀: haemosiderin deposits, bone marrow: ♂: sternum and femur haematopoiesis ↑; **360 mg/kg body weight**: ♂ and ♀: cyanosis, paleness, salivation, staggering gait, absolute body weights, food consumption ↓ (not every day); ♀: ophthalmopathy, urine: ♂ and ♀: volume, sodium, chloride ↑, osmotic pressure, specific gravity, pH ↓; ♀: potassium ↑, blood: ♂ and ♀: MCH, MetHb ↑; ♂: erythrocytes, MCHC ↓, MCV, ratio of neutrophilic leukocytes ↑; ♀: total leukocytes ↑, haematocrit, aPTT ↓, biochemistry: ♂ and ♀: bilirubin, AST, phospholipids, inorganic phosphorous ↑; ♂: ALT ↑, glucose, potassium, chloride ↓; ♀: total cholesterol, calcium ↑, spleen: ♂ and ♀: enlarged (in all animals examined), relative weights ↑, thyroid gland: ♂ and ♀: absolute and relative weights ↑, hypertrophy, liver: ♂ and ♀: relative weights ↑ (♂: only relative weights), hypertrophy, extramedullary haematopoiesis, kidneys: ♂ and ♀: relative weights ↑ (absolute only in ♀), blackish-brown discoloration, haemosiderin deposits; ♂: hyaline droplets; ♀: papillary necrosis, testes: absolute and relative weights ↑, heart: ♂: relative weights ↑, brain: ♂: relative weights ↑; recovery period: papillary necrosis und haemosiderin deposits were not reversible	MHLW 2018 d

ALT: alanine aminotransferase; aPTT: activated partial prothrombin time; AST: aspartate aminotransferase; BUN: blood urea nitrogen; GTP: glutamyl transpeptidase; Hb: haemoglobin; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular Hb concentration; MCV: mean corpuscular volume; MetHb: methaemoglobin; PLT: platelets

In 1971, the effects of 2,5-xylidine on the liver and kidneys were studied in 5 male and 5 female rats and 1 male and 1 female beagle dog per dose group. The rats were given gavage doses of 0, 20, 100 or 500 mg/kg body weight daily for 4 weeks. After 2 weeks, the high dose was increased to 700 mg/kg body weight and day. In the high dose group, body weight gains were reduced in the male animals. In the female rats of the high dose group, decreased haematocrit values and haemoglobin concentrations were observed, as well as an "enlarged liver" (no other data) and increased ornithine carbamyltransferase activity. One animal died on study day 25. The NOAEL was 100 mg/kg body weight and day (BUA 1995; OECD 2012). The dogs were given oral doses of 0, 2, 10 or 50 mg/kg body weight daily for 4 weeks. At 10 mg/kg body weight and day and above, vomiting and moderate steatosis occurred in the male dog. At 50 mg/kg body weight and day, both animals lost weight and displayed a poor general condition. Severe steatosis, hyperproteinaemia, hyperbilirubinemia and generalized jaundice were found in the male dog. Minor steatosis was observed in the female. The NOAEL was 2 mg/kg body weight. However, the study was not conducted according to current test guidelines and was considered to have limited validity due to the lack of statistics (rats) and the small number of animals (dogs) (BUA 1995).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

2,3-Xylidine: In a study to determine the dermal LD_{50}, severe skin irritation was observed in guinea pigs after occlusive exposure to 500 to 1000 mg 2,3-xylidine/kg body weight for 24 hours (no other data; Eastman Kodak Co 1994). The 24-hour occlusive application does not comply with the 4-hour semi-occlusive application required by OECD Test Guideline 404.

2,5-Xylidine and **3,4-xylidine**: There are no data available.

3,5-Xylidine: The semi-occlusive application of 0.5 ml for 4 hours according to OECD Test Guideline 404 did not cause skin irritation in New Zealand White rabbits (no other data) (BUA 1995; ECHA 2018 c).

5.3.2 Eyes

2,3-Xylidine, 2,5-xylidine and **3,4-xylidine**: There are no data available.

3,5-Xylidine: In New Zealand White rabbits (no other data) both slight corneal opacity and slight conjunctival erythema were found 1 hour to 72 hours after the instillation of 0.1 ml undiluted 3,5-xylidine (purity 98%; OECD Test Guideline 405), which subsided after 7 days (BUA 1995; ECHA 2018 c). According to the CLP regulation criteria, the substance is therefore considered to be not irritating to the eyes.

5.4 Allergenic effects

There are no findings available.

5.5 Reproductive and developmental toxicity

There are no data available.

5.6 Genotoxicity

5.6.1 In vitro

For all xylidine isomers there are numerous bacterial mutagenicity tests with the Salmonella typhimurium strains TA97, TA98, TA100 and TA1537. These were already described in detail in the documentation from 1998, in the supplement from 2000 (Greim 2003) and in the BUA report (BUA 1995). Despite not entirely consistent results, it can be concluded that all xylidine isomers are mutagenic after metabolic activation in the above-mentioned Salmonella

typhimurium strains. Since the last documentation, a bacterial mutagenicity test with **2,3-xylidine** (purity 99.7%) with the strains TA98, TA100, TA1535 and TA1537, and the bacterial strain Escherichia coli WP2 uvrA according to OECD Test Guidelines 471 and 472 has been published (MHLW 2018 a). A mutagenic effect was found only in strain TA100 with metabolic activation (S9, rat liver) at 625 µg/plate and above with a doubling of the number of revertants. Cytotoxicity occurred at 1250 µg/plate and above. The negative and positive controls yielded the expected results (ECHA 2018 a; MHLW 2018 a; OECD 2012). The results of this study are therefore in line with the studies mentioned above. The reverse mutations induced primarily in the Salmonella strain TA100 indicate the occurrence of base pair substitutions.

2,5-Xylidine induced DNA repair in rat hepatocytes (Greim 2003).

In chromosomal aberration tests, 2,3-xylidine, 3,4-xylidine and 3,5-xylidine (purity 99.7%–99.9%) were examined in Chinese hamster lung (CHL/IU) cells according to OECD Test Guideline 473 (MHLW 2018 a, c, d; OECD 2012). At a concentration of 0.6 mg **2,3-xylidine**/ml with metabolic activation, there was no statistically significant difference between the findings and those in the control; in 18.8% of the cells, aberrations (without gaps) were detected compared with no aberrations in the solvent control. The cytotoxicity at 0.6 mg/ml reached a level of 70% and was thus very high (ECHA 2018 a; MHLW 2018 a). In another study, **2,3-xylidine** induced chromosomal aberrations in CHL/IU cells after 6-hour treatment with metabolic activation at the highest concentration tested of 0.6 mg/ml, again with a very high level of cytotoxicity of more than 50% (Kusakabe et al. 2002). **3,4-Xylidine** did not induce either clastogenicity or polyploidy. Concentrations of 0, 0.24, 0.47, or 0.94 mg/ml (short-term treatment, with and without metabolic activation) and of 0, 0.11, 0.23, or 0.45 mg/ml (continuous treatment, without metabolic activation) were tested. Cytotoxicity occurred at and above 0.3 mg/ml (continuous treatment without metabolic activation and short-term treatment with metabolic activation) and at and above 0.6 mg/ml (short-term treatment without metabolic activation) (MHLW 2018 c; OECD 2012). In the above-mentioned study by Kusakabe et al. (2002), chromosomal aberrations were not detected after exposure to **3,4-xylidine**. The highest concentration tested was chosen to induce cytotoxicity of 50% or higher. **3,5-Xylidine** induced clastogenicity (chromatid breaks, chromatid exchange and chromosomal breaks; without gaps) in 12% of the cells without metabolic activation at the highest concentration tested of 900 µg/ml; in the solvent control such findings were observed in 0.5% of the cells. With metabolic activation, chromosomal aberrations (chromatid breaks, chromatid exchange and chromosomal breaks; without gaps) were found in 22.5% of the cells compared with in 1% of the cells in the solvent control (ECHA 2018 c; MHLW 2018 d; OECD 2012). The reports are written in Japanese, only the summary and some tables and figures are available in English.

3,5-Xylidine caused base pair substitutions with metabolic activation in CHO-AS52 cells with the transfected bacterial *gpt* gene (54%; control 48%; difference not statistically significant). Most mutations occurred as A:T to G:C transitions (20% of the total number of mutations; statistically significant compared with the findings for the control). At 100 µmol/plate and above, cell growth was impaired (100 µmol/plate by 20%, 300 µmol/plate by 35%) (Chao et al. 2012).

5.6.2 In vivo

Male C57BL/6 mice (n = 13), were given a single intraperitoneal dose of about 100 µg radiolabelled **3,5-xylidine**/kg body weight. After 2, 4, 8, 16 and 24 hours, the DNA was isolated from the bladder, liver, kidneys, colon, lungs and pancreas, and the radioactivity was determined. From the results, the authors concluded that 3,5-xylidine is metabolized in vivo to electrophilic intermediates that bind to DNA and form adducts in the DNA of the bladder and liver (Skipper et al. 2006). The structures of the adducts formed or the metabolites from which the adducts were formed were not determined.

Three male ddY mice per group were given gavage doses of 0 or 200 mg **2,3-xylidine**, **2,5-xylidine**, **3,4-xylidine** or **3,5-xylidine**/kg body weight. The animals were killed 3 or 24 hours after administration; the bone marrow, liver, kidneys and lungs were removed and the tissues prepared for the comet assay. For evaluation, 500 randomly selected cells per sample were examined. Three hours after exposure, the number of damaged cells with tail formation and evident migration and the number of cells with increased fragmentation in the kidneys and lungs was increased for all the xylidine isomers tested. In the liver, increased numbers of cells with incipient tail formation or tail formation and evident migration were found, likewise for all the xylidines administered. Only after exposure to 3,4-xylidine

and 3,5-xylidine was the number of cells with small tail formation and cells with tail formation and evident migration increased in the bone marrow. The strongest effects in the kidneys were caused by 2,3-xylidine and 2,5-xylidine and in the liver by 2,5-xylidine. The effects were less pronounced in the lungs after treatment with 3,4-xylidine and 3,5-xylidine. DNA damage was not found in the organs removed after 24 hours (Kohara et al. 2018).

In a gene mutation test, groups of 5 male MutaMice® were given gavage doses of 100 mg **2,5-xylidine** or **3,5-xylidine**/kg body weight once a week for 4 weeks. Seven days after the last dose, bone marrow, liver and the entire nasal cavity were collected. In the nasal tissue of the mice treated with 2,5-xylidine, a statistically significant increase in the mutation frequency of the *lacZ* and *cII* genes was observed. Statistically significant effects in the nasal cavity were not observed in the mice treated with 3,5-xylidine. In the liver, no effects on the mutation frequency were found with either 2,5-xylidine or 3,5-xylidine. In the bone marrow of mice treated with 2,5-xylidine the mutation frequency was not significantly increased for the *cII* gene. It was not possible to determine whether the findings were statistically significant for *lacZ* because 4 animals of the control group could not be evaluated (total number of plaques less than 100 000). A sequence analysis of the mutations on the *cII* gene caused by 2,5-xylidine revealed a statistically significant increase in the number of transitions (GC to AT; AT to GC) and transversions (GC to TA) (Kohara et al. 2018).

For a micronucleus test, cells from the bone marrow of the same ddY mice used in the comet assay described above were collected and examined. The frequency of polychromatic erythrocytes with micronuclei was not increased with any of the xylidine isomers tested. The ratios of polychromatic to normochromatic erythrocytes were 1.1 ± 0.0 (control), 0.8 ± 0.2 (2,3-xylidine), 1.1 ± 0.4 (2,5-xylidine), 1.1 ± 0.1 (3,4-xylidine) and 0.9 ± 0.2 (3,5-xylidine) and were therefore unaffected (Kohara et al. 2018).

In another study, groups of 5 male MutaMice® were given single gavage doses of 100 mg of 2,5-xylidine or 3,5-xylidine/kg body weight, and blood samples were taken from the tail vein 48 hours after the treatment. The animals were the same animals exposed in the gene mutation test described above. The blood samples were taken after the first of a total of 4 doses and tested for micronucleated reticulocytes. In each case 1000 reticulocytes per animal were tested. The frequency of micronucleated reticulocytes was not increased in the peripheral blood of the exposed animals (Kohara et al. 2018).

Accessibility of the germ cells: To study the effects of 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine on the incorporation of thymidine into the testicular DNA, male mice (strain not specified) were given either a single oral dose of 200 mg of 2,3-xylidine or 2,5-xylidine/kg body weight or a single intraperitoneal dose of 100 mg 3,4-xylidine or 3,5-xylidine/kg body weight. 2,5-Xylidine and 3,5-xylidine led to a statistically significant reduction in thymidine incorporation, whereas no effects were observed with 2,3-xylidine and 3,4-xylidine (BUA 1995; Seiler 1977). It can therefore be assumed that 2,5-xylidine and 3,5-xylidine reach the testes.

5.7 Carcinogenicity

A carcinogenicity study in male Charles River SD rats and male and female CD1 HaM/ICR mice was already described in the documentation of 1998 (Greim 2003). The rats were fed 0, 300 or 600 mg 2,5-xylidine/kg body weight and day for 5 months (6000 or 12 000 mg/kg diet × conversion factor 0.05 (for chronic exposure) according to EFSA 2012). Thereafter, the dose was reduced by half for a further 13 months due to toxicity. In the rats, increased incidences of subcutaneous fibromas and fibrosarcomas occurred in both the high dose group (9/17; 53%) and the low dose group (7/17; 41%), which were statistically significant only compared with a combined control of 5 time-staggered control groups (18/111; 16%), but not compared with the concurrent control (8/17; 47%). The male and female mice were given 0, 900 or 1800 mg 2,5-xylidine/kg body weight (6000 or 12 000 mg/kg diet × conversion factor 0.15 (for chronic exposure) according to EFSA 2012) with the diet for 18 months. In the male mice, increased incidences of vascular tumours occurred in the high (7/19; 37%) and low (5/18; 28%) dose group, which were statistically significant only in comparison with the time-staggered control groups (5/99; 5%) (concurrent control 2/16; 13%). In the female mice, a statistically significant increase in the number of hepatomas was found only in the low dose group compared with both control groups (5/16; 31%; high dose group: 2/20; 10%; concurrent control: 0/13; time-staggered controls: 1/102; 1%; Weisburger et al. 1978). The results of

this study are, however, only of limited validity, since, among other things, the small number of animals used in the treated and control groups does not meet the requirements of the test protocols according to the OECD test guideline.

Studies of the carcinogenicity of 2,3-xylidine, 3,4-xylidine und 3,5-xylidine are not available.

6 Manifesto (MAK value/classification)

All 4 xylidine isomers cause the formation of methaemoglobin, reduce the haemoglobin content and are toxic to the liver, kidneys and spleen (see also Greim 2003). These effects are confirmed by the new 28-day studies in rats published since the last documentation.

Carcinogenicity. In vitro and in vivo, all 4 xylidine isomers display genotoxic properties after metabolic activation. The results are not entirely consistent, but genotoxicity is typical of this substance class. Studies of the mechanism of action suggest that the metabolism of xylidines can lead, among other things, to the formation of highly reactive nitrenium ions and quinone imines, which are capable of forming DNA adducts or, by redox cycling, ROS.

Valid studies of the carcinogenicity of the xylidine isomers in humans are not available. A comparison of the monocyclic aromatic amino compounds shows that a common basic pattern can be identified for the organotropy of the developing tumours. The main effects are tumours in the blood vessels of rats and mice. Fibromas and fibrosarcomas are also frequently observed (Greim 2005). Data for carcinogenicity from animal experiments are available only for 2,5-xylidine, which induced hepatomas in female CD1 HaM/ICR mice in the low dose group, but not in the high dose group. A dose-response relationship is not evident. The increase in the incidences of vascular tumours in male mice and of fibromas and fibrosarcomas in rats was statistically significant compared with the time-staggered control, but not compared with the concurrent control in the experiment. Thus, a statistically significant increase in tumour incidences occurred in only one species and in one sex. The study is, however, only of limited validity, since, among other things, the small number of animals in the dose and control groups does not meet the requirements of the test protocols according to the OECD test guideline.

However, the tumours that occurred correspond to the above-mentioned common basic pattern for monocyclic aromatic amino compounds, so that a substance-related effect cannot be excluded. An association between the development of these tumours and the mechanisms that contribute to the formation of methaemoglobin can be assumed. It is highly probable that the very high doses used in these studies also contributed to the development of tumours as a result of their toxicity.

Overall, the animal data, together with the known mechanism of action and the fact that the xylidines belong to the substance class of aromatic amines, indicate that the isomers are suspected to have a carcinogenic effect. Therefore, 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine are classified in Carcinogen Category 3B.

Germ cell mutagenicity. In vitro, 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine are mutagenic in bacteria with metabolic activation. 3,5-Xylidine is mutagenic also in mammalian cells. Mutagenicity tests with mammalian cells are not available for the other xylidines. 2,3-Xylidine and 3,5-xylidine have been shown to be clastogenic. Clastogenicity has not been tested with 2,5-xylidine; 3,4-xylidine was not clastogenic.

In vivo, DNA binding has been demonstrated in the bladder and liver of C57BL/6 mice, but the structures of the DNA adducts are unknown. In the comet assay in mice, all 4 xylidine isomers caused DNA damage to the lungs, kidneys and liver, and 3,4-xylidine and 3,5-xylidine also to the bone marrow. The micronucleus test in the bone marrow yielded negative results for all 4 xylidines. 2,5-Xylidine and 3,5-xylidine did not induce micronuclei in peripheral blood, either; there is no corresponding test for the other 2 isomers. 2,5-Xylidine, but not 3,5-xylidine, led to mutations in nasal tissue in the MutaMaus®.

Inhibition of testicular DNA synthesis in mice has been demonstrated for 2,5-xylidine and 3,5-xylidine. Accessibility of the germ cells can therefore be assumed.

Studies in germ cells are not available.

Overall, based on the available results, mutagenicity in germ cells is suspected, and 2,3-xylidine, 2,5-xylidine, 3,4-xylidine and 3,5-xylidine have therefore been classified in Category 3 B for germ cell mutagens.

MAK value and peak limitation. The NOAELs obtained in the 28-day studies in rats are approximately the same: 2,3-xylidine: 12 mg/kg body weight and day for males (LOAEL for females); 2,5-xylidine: 12 mg/kg body weight and day for males and females; 3,4-xylidine and 3,5-xylidine: 10 mg/kg body weight and day for males and females. As genotoxic effects of the xylidine isomers are suspected both in vitro and in vivo, a MAK value cannot be derived. Therefore, peak limitation does not apply.

Absorption through the skin. There are no studies available for absorption of the substances through the skin. The mathematical models yield a high level of dermal absorption of up to 1412 mg for 3,5-xylidine; for the other isomers it is negligible or cannot be calculated due to the lack of data. For 2,3-xylidine the dermal LD_{50} in guinea pigs is between 500 and 1000 mg/kg body weight. Methaemoglobin formation with the structural analogue 2,4-xylidine was observed in cats and dogs after dermal application (Greim 2003). Systemic toxicity is high with a LOAEL/NOAEL for the xylidine isomers in the range of 10 mg/kg body weight and day, and carcinogenicity and mutagenicity are suspected. For this reason, the xylidine isomers remain designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts) despite the insufficient data available for absorption through the skin.

Sensitization. Findings of sensitizing effects of xylidines in humans and results from experimental animal or in vitro studies are not available. Although such effects might be expected based on the structural similarity with some amine-substituted monocyclic aromatics with a low contact sensitization potential, such as aniline or *p*-toluidine, the xylidine isomers have not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways) due to lack of data.

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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