Recommendation from the Scientific Committee on Occupational Exposure Limits for 2,6-dimethylaniline (o-xylidine)

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Occupational Exposure Limits for

for 2,6-dimethylaniline (o-xylidine)

<table>
<thead>
<tr>
<th>Substance identification</th>
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<tr>
<td>2,6-Dimethylaniline:</td>
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\[
\begin{align*}
\text{Synonyms:} & \quad 2,6-xylidine; \text{o-xylidine;} 1-\text{amino-2,6-dimethylbenzene}; \text{benzenamine, 2,6-dimethyl-2-amino-1,3-xylene} \\
\text{EC No.:} & \quad 201-758-7 \\
\text{Annex 1 Index No.:} & \quad 612-161-00-X \\
\text{Classification:} & \quad \text{Carc. Cat. 3; R40 - Xn; R20/21/22 - Xi; R37/38 - N; R51-53} \\
\text{CAS No.:} & \quad 87-62-7 \\
\text{MWI:} & \quad 121.18 \\
\text{Conversion factor (20 °C, 101 kPa):} & \quad 1 \text{ ppm} = 5.03 \text{ mg/m}^3; 1 \text{ mg/m}^3 = 0.200 \text{ ppm} \\
\text{This evaluation is based on Greim (1998, 2000), ACGIH (2002), ECB (2000), NLM (2005), the references cited in these reviews and additional references from a database search.} \\
\end{align*}
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\[
\begin{align*}
\text{Physico-chemical properties} \\
2,6-Dimethyl aniline (2,6-DMA) is a colourless to reddish-yellow, clear liquid. It has a melting point of 11.2 °C and a boiling point of 216 °C. The vapour pressure at 25 °C is 0.167 hPa. 2,6-DMA is soluble in water (7.5 g/l at 20 °C) and very soluble in alcohol and ether. A log Pow of 1.96 is reported. The density is 0.979 g/cm3 at 20 °C The substance has a flash point of 91 °C (ECB, 2000; NLM, 2005).
\end{align*}
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1. Occurrence/use and occupational exposure

2,6-DMA is used as a chemical intermediate for the manufacture of pesticides, dyestuffs, antioxidants, pharmaceuticals, synthetic resins, fragrances, and other products. It is also a component of tobacco smoke, a degradation product of aniline-based pesticides, and a metabolite of certain drugs, particularly the xylide group of local anaesthetics, e.g. lidocaine (NLMP, 2005; NTP, 1990).

2. Health significance

2.1. Toxicokinetics

2.1.1. Human data

In vivo data on the toxicokinetics of 2,6-DMA are not available. The local anaesthetic lidocaine is metabolised by N-deethylation and a subsequent hydrolytic cleavage reaction yielding 2,6-DMA. 2,6-DMA is further oxidised to 4-hydroxy-2,6-DMA (4-amino-3,5-dimethylphenol) which is the main metabolite excreted in urine (Catteral and Mackie, 2001).

In vitro data indicate that oxidation of 2,6-DMA at micromolar concentrations by recombinant human P450 monooxygenases and human liver microsomes leads to 4-amino-3,5-dimethylphenol but at lower (nanomolar) concentrations, N-oxidation with the formation of N-(2,6-dimethylphenyl)hydroxylamine is substantial. Cytochrome P450 2A6 was identified as the major P450 for N-hydroxylation. Isomerisation of the N-hydroxylamine to 4-amino-3,5-dimethylphenol is catalysed by P450 2E1 (Gan et al., 2001). The product of N-oxidation may be further oxidised in vivo to the nitroso compound which may be detected as haemoglobin adduct (see below in section “biological monitoring”). Since the activity of P450 2E1 depends on environmental factors and is highly variable and 2A6 shows wide interindividual variability, it is suggested that large individual differences in N-(2,6-dimethylphenyl)hydroxylamine production may exist and that such differences may be related to the interindividual variability of 2,6-DMA-haemoglobin adduct levels (Gan et al., 2001).

2.1.2. Animal data

Data on toxicokinetics following inhalation or dermal exposure are not available. 2,6-DMA is readily absorbed after oral administration and distributed to organs and tissues. After feeding of male F344 rats a diet containing 3000 ppm 2,6-DMA (200226 mg/kg d, calculated by the authors from study-specific food consumption and body weight data) for one week, the concentration in blood reached 0.36 mg/l. At 300 ppm (27 mg/kg d), the concentration in blood was below the detection limit (0.02 pg/ml) (Yasuhara et al., 2000).

In rats treated with up to 10 doses of 63 mg 14C-2,6-DMA/kg d by gavage, accumulation of the radiolabel occurred. Animals having received 10 doses had higher radioactivity levels in blood and other tissues and the radioactivity disappeared more slowly than in rats treated once with the same dose. High concentrations of radioactivity were found in red blood levels, liver and kidneys but also in nasal tissues where the concentration 24 hours after dosing was 2.5fold higher than in the liver. High concentrations of radioactivity were also found in nasal tissues after intraperitoneal administration. Excretion of radioactivity was mainly through the kidneys with urine. The
increased retention after repeated administration was not due to an impairment of excretion but due to an increased binding in blood and tissue (NTP, 1990).

The metabolism of xylidines occurs via N-acetylation or N-oxidation, oxidation of one methyl group, and hydroxylation of the aromatic ring. The main metabolite of 2,6-DMA in dogs and rats was 4-hydroxy-2,6-dimethylaniline (4-amino-3,5-dimethylphenol). Dogs also excreted significant quantities of 2-amino-3-methylbenzoic acid and trace amounts of the corresponding glycine conjugate, of 2,6-dimethynitroso-benzene and of an unknown compound, possibly 3,5-dimethyl-4-imino-quinone (Lindström et al., 1963; Short et al., 1989a). N-Oxidation leads to N-hydroxy-2,6-DMA, which may be further oxidised to the nitroso compound and covalently bound to haemoglobin. The corresponding haemoglobin adducts have been detected in rats after administration of 2,6-DMA (and of lidocaine) (Bryant et al., 1994).

The covalent binding of several aromatic amines was compared (Sabbioni, 1994). Alkyl substitution of aniline reduced haemoglobin binding, and two methyl groups in ortho-position to the amino group as in 2,6-DMA almost abolished haemoglobin binding.

Bioactivation of 2,6-DMA to reactive metabolites which bind to tissue was shown to occur in the liver but also in the nasal olfactory mucosa and the upper alimentary and respiratory tract of rats. The formation of protein and DNA-adducts from 2,6-DMA in vitro in the presence of microsomal preparations from various respiratory and alimentary tissues of rats revealed that binding was highest with preparations from nasal olfactory mucosa and was about 10-fold higher than with preparations from liver (Tyden et al., 2004). The differences in binding may be related to differences in the N-acetyltransferase activity between olfactory mucosa and liver: Experimental data indicate that the N-acetyltransferase activity against 2,6-DMA in the olfactory mucosa is about 10-fold higher than in liver (Genter, 2004).

2.1.3. Biological monitoring

Haemoglobin adducts of 2,6-DMA can be found in patients treated with lidocaine. Lower levels of such 2,6-DMA-adducts are also found in smoking and non-smoking humans not exposed to lidocaine indicating environmental exposure to 2,6-DMA sources (Sabbioni, 1994; Bryant et al., 1994). Based on the adduct levels found in non-smokers a daily uptake of about 23 pg 2,6-DMA has been estimated (Sabbioni, 1992). However, due to considerable interindividual variability in the activity of the involved enzymes, large individual differences in binding are expected (Gan et al., 2001). No quantitative relationship has been established between exposure to 2,6DMA and Hb-adduct formation which could be used for biological monitoring at the workplace.

2.2. Acute toxicity

2.2.1. Human data

It is reported without details that 40 ppm xylidines (200 mg/m³, no specification of isomers) will cause severe intoxication within 60 minutes and that 10 ppm (50 mg/m³) may lead to symptoms of illness if exposure continues for more than a short period of time (Goldblatt, 1955).

Methaemoglobinemia has been observed in humans who had been treated with the local anaesthetic lidocaine. Since lidocaine is metabolised by a cleavage reaction producing 2,6-DMA, the methaemoglobinemia has been ascribed to the formation of N-hydroxy-2,6-DMA. Consistent with this, haemoglobin adducts of 2,6-DMA can be
found in patients treated with lidocaine (see above).

### 2.2.2. Animal data

No mortality occurred in rats exposed to a 2,6-DMA-saturated atmosphere at 20 °C (approx. 0.75 mg/l) for 7 hours. Apathy, closed eyes and slight secretion of the nose were noted at the end of exposure. Necropsy revealed no morphological changes 14 days after exposure (ECB, 2000). Further data regarding acute inhalation or dermal toxicity are not available.

Oral LD$_{50}$ of 840-1230 mg/kg for 2,6-DMA and of 2050 mg/kg for 2,6-DMA hydrochloride have been determined for male rats. Cyanosis was observed in animals severely intoxicated with 2,6-DMA, but methaemoglobinemia did not appear so pronounced as to be the cause of death. An oral LD$_{50}$ of 710 mg/kg has been determined for mice (NTP, 1990).

The methaemoglobinemic potential of all six xylidine isomers was compared in rats after a single oral administration of 4.8 mmol/kg (581 mg/kg) (Cauchon and Krishnan, 1997). 2,6-DMA and all other isomers except 3,5-xylidine failed to induce significant methaemoglobin (MHb) formation in vivo.

2,6-DMA also produced less than 3 % MHb after injection of 20 mg into the femur of male rats (Lindstrom et al., 1969). In cats which are known to be very susceptible to MHb-forming agents, a mean level of only 7.2 % was reached after i.v. administration of 0.25 mmol/kg 2,6-DMA (30.3 mg/kg). The potential of 2,6-DMA was very low compared to equimolar doses of other alkyl amines, e.g., aniline which caused 61.6 % MHb (McLean et al., 1969).

No deaths or systemic effects were reported in irritation studies with dermal application of 2,6-DMA (see below). Further data regarding toxicity following dermal exposure are not available.

### 2.3. Irritation and corrosivity

#### 2.3.1. Human data

Xylidine (no specification of isomers) has a weak, amine-like odour. An odour threshold of 0.024 mg/m$^3$ has been reported (Ruth, 1986).

Data regarding irritation or corrosivity are not available.

#### 2.3.2. Animal data

No data are available regarding respiratory tract irritation following inhalation of 2,6-DMA.

**Skin**

In 4 out of 5 tests (all but one according to OECD guideline 404) reported in ECB (2000), 2,6-DMA was rated slightly irritating or irritating to the skin of rabbits.

**Eyes**

In 3 out of 4 tests according to OECD guideline 405 reported in ECB (2000), 2,6-DMA was
rated moderately irritating or irritating to the eye of rabbits. Symptoms included conjunctivitis, slight erythema of the iris and corneal opacity; all effects were reversible within one week.

2.4. Sensitisation

2.4.1. Human data

No data are available.

2.4.2. Animal data

No data are available.

2.5. Repeated dose toxicity

2.5.1. Human data

No data are available.

2.5.2. Animal data

Inhalation

No data are available.

Oral

Effects of 2,6-DMA on liver and kidney were studied in Sprague-Dawley rats and Beagle dogs (Magnusson et al., 1971). Both species were treated with 2,6-DMA for 4 weeks. Dogs (1 male + 1 female/group) were given 0, 2, 10 or 50 mg/kg d in capsules. Rats received 0, 20, 100, and 500 mg/kg d by gavage, the highest dose was increased to 700 mg/kg d after 2 weeks. No gross or histological effects were seen on the kidney at any dose in both species. Dogs were much more sensitive than rats. Vomiting occurred in dogs at 10 and 50 mg/kg d. At the highest dose, the body weight was reduced and the dogs had to be sacrificed after 2 weeks. Fatty degeneration of the liver was seen in dogs at all dose levels with a dose-dependent increase in severity (LOAEL dogs: 2 mg/kg d). Further effects at the highest dose were increased liver weight, pale liver with some isolated necrotic liver cells, hypoproteinaemia, hyperbilirubinaemia and icteric tissues. In rats, mortality was increased at the highest dose. Rats also showed lowered haemoglobin levels and haematocrit, and hepatomegaly but only a slight fat accumulation with necrotic foci in the liver at the highest dose (NOAEL rat: 100 mg/kg d).

Oral administration of 400-500 mg/kg d of 2,6-DMA to rats for 4 weeks caused hepatomegaly which was attributed to the proliferation of the smooth endoplasmic reticulum. Hepatic glycogen content and glucose-6-phosphatase were decreased; the activity of glucuronyltransferase was increased but not of aniline hydroxylase activity and of cytochrome P450 content (Magnusson et al., 1979).

Haemosiderosis of the spleen was observed in F344 rats after gavage administration of 157 mg/kg d over a period of 20 days. No other effects could be observed (Short et al., 1983).
In a further subacute study, male F344 rats were fed a diet containing 300 ppm 2,6DMA (27 mg/kg · d) for one week or 3000 ppm 2,6-DMA (200-226 mg/kg · d) for 4 weeks (Yasuhara et al., 2000). Histological changes in nasal tissue, such as atrophy of Bowman’s glands and irregular arrangement of olfactory epithelial cells were observed at 3000 ppm but not at 300 ppm. Other organs were not assessed.

Feeding of 2,6-DMA hydrochloride to Osborne-Mendel rats for 26 weeks led to an increased relative kidney weight (LOAEL: 2500 mg/kg food; NOAEL 750 mg/kg food). At higher doses, reduced weight gain and renal lesions such as interstitial fibrosis and inflammation, tubular atrophy papillary oedema, cystic tubular dilation, and chronic congestion of the spleen were also observed (Lindström et al., 1963).

In a range-finding study including a reproductive toxicity part (see below), the body weight gain of female CD rats was reduced after feeding a diet containing 3,000 ppm 2,6-DMA for 10 weeks (NTP, 1990).

In a subacute and a subchronic study within the framework of the NTP carcinogenicity study (see below), F344 rats treated with 2,6-DMA by gavage for 4 or 13 weeks developed haematological alterations (NTP, 1990). Male rats were more sensitive than females. After 4 weeks, anisocytosis, poikilocytosis, and polychromasia of red blood cells, a generalised leukocytosis and a decrease in body weight gain were observed at 310 mg/kg · d (LOAEL, NOAEL: 160 mg/kg · d). After 13 weeks, total leucocyte counts were significantly decreased at 40 mg/kg · d (LOEL; NOEL: 20 mg/kg · d), additional haematological changes (decrease of lymphocyte and increase in neutrophil counts, decrease of haemoglobin level, red blood cell count and haematocrit) were observed at higher concentrations. However, the decreases in red blood parameters were not severe enough to be considered as anaemia.

Non-carcinogenic effects in the 2-year NTP carcinogenicity study with CD rats (see below) included a dose-dependent, significant increase of acute inflammations (rhinitis) of the nasal cavity at all dose levels in both sexes. In males, the incidence was 21 % in controls and increased to 38 %, 57 % and 75 % at dose levels of 300 ppm, 1,000 ppm and 3,000 ppm 2,6-DMA in food. In females, the corresponding incidences were 13 %, 25 %, 27 % and 68 %. Additionally, epithelial hyperplasia in the nasal cavity was observed in mid- and high-dose males and females, and squamous metaplasia developed in high-dose males and females. Hyper- and metaplasia were not observed in any animal fed 300 ppm or control diet. Decreases in red blood cell count, haemoglobin level and haematocrit occurred in males fed diets containing 3000 ppm 2,6-DMA (according to HCN (2002): 120 mg/kg b.w. · d, calculated using standard factors) after 18 months and in females at 1000 ppm (50 mg/kg · d) and 3000 ppm (150 mg/kg · d) after 12 months. These haematological changes were small and not considered to be of biological significance (NTP, 1990).

Dermal

There are no data available.

2.6. Genotoxicity

2.6.1. In vitro

2,6-DMA or its hydrochloride did not induce mutations in various tests with Salmonella typhimurium strains TA97, TA98, TA100, TA1535, TA1537 and TA1538 in the presence or absence of exogenous metabolic activation system. However, a weak positive
response was observed in preincubation and plate incorporation tests with TA100 in the presence of metabolic activation. The substance was not active in a DNA damage and repair assay with E. coli. K-12 343/113, uvrB-/-recA- and uvrB+/-recA+. 2,6-DMA induced mutations in mouse L5178Y lymphoma cells. The substance also induced sister chromatid exchange and chromosomal aberrations at cytotoxic concentrations in Chinese hamster ovary cells with and without exogenous metabolic activation (ECB, 2000; NLM, 2006). A weakly positive effect was also observed in a cell transformation assay with Balb/c 3T3 cells (ECB, 2000).

N-Hydroxy-2,6-DMA, the product of metabolic activation of 2,6-DMA in vivo, is mutagenic in S. typhimurium TA100 (Jeffrey et al., 2002). In a series of N-(hydroxymethyl)-, N-(hydroxydimethyl)- and N-hydroxyethyl-aniline derivatives, N-hydroxy-2,6-DMA was the most mutagenic compound when tested in S. typhimurium TA100 in the absence of metabolic activation (Nohmi et al., 1984; Marques et al., 1997).

The products of N-oxidation of 2,6-DMA form DNA adducts. Different DNA adducts resulting from the reaction of N-acetoxy-2,6-DMA were identified. Three of the adducts were reaction products of the exocyclic heteroatoms of deoxyadenosine and deoxyguanosine with the carbon atom in para position to the arylamine nitrogen. However, the fourth adduct resulted from reaction of the 2,6-DMA nitrogen with the C8 atom of deoxyguanosine (Marques et al., 2002). Such N-(deoxyguanosine-8-yl)-adducts are the same as those which are found in case of carcinogenic polycyclic amines (Greim, 1998). However, in contrast to the usual pattern obtained with aromatic amines, where C-8-substituted deoxyguanosine adducts predominate, the adduct profile for N-acetoxy-2,6-DMA was different as the other three adducts mentioned above were obtained with higher yields (Goncalves et al., 2001).

2.6.2. In vivo - Human data

No data are available.

2.6.3. In vivo - Animal data

No genotoxic effect an E. coli K12 was observed in a host-mediated assay after feeding of 2,6-DMA to mice (ECB, 2000). Feeding or injection of 2,6-DMA had no mutagenic effect in a sex-linked recessive lethal assay in Drosophila melanogaster (ECB, 2000). No increase of micronuclei was detected in the bone marrow of male mice after oral doses of up to 375 mg/kg d (Parton et al., 1988). 2,6-DMA did not induce unscheduled DNA synthesis in rat hepatocytes (Mirsalis et al., 1989). However, covalent binding of 2,6-DMA to DNA was observed in the nasal epithelium of rats after gavage administration of 2,6-DMA (Duan et al., 2004). In another study, binding in liver and ethmoid turbinates of rats were observed after oral treatment with 2,6-DMA, and the covalent binding index increased after 9 days of pre-treatment with 2,6-DMA (Short et al., 1989b). In this and in a further study (Jeffrey et al., 2002), the level of covalent binding was higher in ethmoid turbinates than in liver tissue.

2.7. Carcinogenicity

2.7.1. Human data

No data are available.

2.7.2. Animal data

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In an NTP study, CD-rats were fed diets containing 0, 300, 1,000, or 3,000 ppm 2,6-DMA before breeding, during pregnancy, and through the lactation period. The carcinogenicity of 2,6-DMA was studied in 56 male and 56 female offspring from each dose group which were treated with the same dose (corresponding to 0, 12, 40 or 120 mg/kg d in male and 0, 15, 50 or 150 mg/kg d in female rats, according to HCN (2002)) after weaning for 102 weeks. Body weight gain was reduced in both male and female offspring at the high dose during the study and survival was reduced in males at mid and high dose level. Lesions of the nasal epithelium were the main neoplastic and non-neoplastic effects. The incidences of both papillomas and carcinomas of the nasal cavity were significantly increased in both sexes at the high dose. The combined number of carcinomas and adenocarcinomas was 28/56 in high dose males, 24/56 in high dose females, and 1/56 in mid dose females. Papillary adenomas were observed in 10/56 high dose males, 2/56 mid dose males, and 6/56 high dose females. None occurred in the other groups. The carcinomas were very invasive and metastasis to the brain was present in 5/56 male and 7/56 females at the high dose. In two male and two female rats of the high dose group rhabdomyosarcomas of the nasal cavity were observed, a malignant tumour extremely rare in rats. Non-neoplastic lesions observed in the nasal cavity included acute inflammation (all doses), epithelial hyperplasia (>300 ppm), and squamous metaplasia (1,000 ppm, see above). Furthermore, dose-related increases in the incidence of subcutaneous tissue fibromas and fibrosarcomas were observed in both sexes and there was a significant positive trend for neoplastic nodules in the liver of female rats (NTP, 1990).

The occurrence and development of 2,6-DMA-related nasal tumours was studied in an initiation-promotion carcinogenesis mode with male F344 rats. Initiation was carried out by a single subcutaneous injection of N-bis(2-hydroxypropyl)nitrosamine (DHPN) followed by administration of 2,6-DMA with the diet (3000 mg/kg) for 52 weeks. While no untreated animals developed nasal neoplasias, animals treated with DHPN developed nasal adenomas and carcinomas and the number of tumourbearing animals was further increased in the group treated with DHPN and 2,6-DMA. The incidences or multiplicity of epithelial hyperplasia and dysplastic foci was also increased in this group. Detailed histopathological and ultrastructural investigations revealed that the lesions typically arose in the Bowman's glands of the olfactory mucosa and that dysplastic foci developing in this gland progress to carcinomas while proliferation of the glands lead to hyperplasia and adenomas. Atrophy of Bowman's glands was apparent after 4 weeks, proliferation of undifferentiated cells after 13 weeks. Hyperplasia, dysplastic foci and adenoma could be observed from 26 weeks on and carcinomas at 52 weeks (Koujifani et al., 1999, 2000, 2001).

2.8. Reproductive toxicity

2.8.1. Human data

There are no data available.

2.8.2. Animal data

Fertility

An increase in relative testes weight was observed in male rats after feeding 2,6DMA at a dietary level of 10,000 ppm for 26 weeks (Lindström et al., 1963). In a screening study with mice, 2,4-, 2,5- and 3,4-DMA, but not 2,6-DMA, inhibited testicular DNA synthesis in mice (ACGIH, 2002). DNA-adducts of 2,6-DMA were found in testes of rats after oral treatment with 2,6-DMA; adduct levels in testes were lower than in liver and ethmoid...
turbinates (Jeffrey et al., 2002).

In a range-finding study within the framework of the NTP-carcinogenicity study, weanling CD rats (5/sex and group) were fed diets containing 0; 100; 300; 1,000; 3,000; or 10,000 ppm 2,6-DMA for 10 weeks. Thereafter, males and females from each dose group were mated for two weeks; the females were then allowed to litter and nurse their offspring for 10 days. The number of live and dead pups were recorded on day 0 and 10, body weight of the litter was recorded and gross necropsy of parents and pups were performed on day 10. The results were only briefly summarised without detail. The mean body weight gain of the females were reduced at 3,000 and 10,000 ppm and of the males at 10,000 ppm. It is further reported that no other signs of toxicity were observed and “no noteworthy lesions were seen during gross necropsy” (NTP, 1990). Further data for 2,6-DMA are not available.

Developmental toxicity

There are no data available.

**Recommendation**

For 2,6-DMA, the critical effect is its carcinogenicity. For non-neoplastic endpoints, it is assumed that effects in the upper respiratory tract are of major concern, since they were observed after oral exposure in experimental animals and are expected to be more pronounced after inhalation. However, no appropriate Inhalation study is available. For systemic effects, the liver is a major target organ as evidenced by studies in beagle dogs. Only a weak potency for methaemoglobinemia was demonstrated.

As with other monocyclic aromatic amines, 2,6-DMA is N-oxidised to metabolites which principally may initiate methaemoglobin (MHB) formation and bind to haemoglobin. However, the ability of 2,6-DMA to induce MHB formation is very weak compared to other amines, especially aniline. 2,6-DMA did not significantly increase MHB formation in rats (Cauchon and Krishnan, 1997). Methaemoglobinemia has been observed in humans after treatment with the local anaesthetic lidocaine which is metabolised to 2,6-DMA, and also in rats. However, methaemoglobinemia did not appear so pronounced as to be the cause of death (NTP, 1990).

Covalent binding to haemoglobin (Hb) is also low compared to other aromatic amines (Sabbioni, 1994). Hb-adducts are found in the normal human population indicating exposure to environmental sources, increased Hb-adduct levels may be found in patients after lidocaine treatment (Bryant et al., 1994). No quantitative relationship has been established between exposure to 2,6-DMA and Hb-adduct formation which could be used for biological monitoring at the workplace.

Inhalation studies with repeated exposure to 2,6-DMA are not available. The liver appears to be a target organ after repeated oral administration of 2,6-DMA in dogs. A subacute study (Magnusson et al., 1971) gave a LOAEL of 2 mg/kg d for hepatic effects. Species differences are apparent with dogs being much more sensitive than rats in which no hepatic effects were seen at 100 mg/kg d in the same study. As other monocyclic aromatic amines, 2,6-DMA affects blood cells and spleen. A decrease of total leukocyte counts was observed in a subchronic study in rats (NOEL of 20 mg/kg d). However, this effect and other haematological alterations seen at higher concentrations after subchronic or chronic exposure were small and are not considered to be of biological significance (NTP, 1990).
In a 2-year carcinogenicity study with feeding of 2,6-DMA to rats, a dose-dependent, significant increase of inflammation of the nasal cavity was seen at all dose levels in both sexes (LOAEL: 12 mg/kg • d) (NTP, 1990).

2,6-DMA did not show mutagenic activity in most bacterial tests. A positive response was obtained in S. typhimurium TA100 in the presence of metabolic activation. 2,6-DMA is mutagenic and causes sister chromatid exchange and chromosomal aberrations in mammalian cells. N-Hydroxy-2,6-DMA, the product of metabolic activation of 2,6-DMA in vivo, is mutagenic in S. typhimurium TA100. The products of N-oxidation of 2,6-DMA form DNA adducts. One of these adducts was identified as N-(deoxyguanosine-8-yl)-adduct of 2,6-DMA. Such N-(deoxyguanosine-8-yl) adducts are also found in case of genotoxic carcinogenic polycyclic aryl amines.

2,6-DMA did neither induce micronuclei formation in vivo nor unscheduled DNA synthesis in hepatocytes. However, 2,6-DMA is bound covalently in liver and ethmoid turbinates of rats. Covalent binding is increased after repeated exposure and the level of covalent binding is higher in ethmoid turbinates of the nasal cavity than in liver tissue.

The epithelium of the nasal cavity is also the target for carcinogenic effects of 2,6-DMA in rats. Papillomas and carcinomas of the nasal cavity are seen in both male and female rats. The carcinomas are very invasive and frequently show metastasis to the brain. 2,6-DMA also causes rhabdomyosarcomas of the nasal cavity, a malignant tumour which is extremely rare in rats. In the carcinogenicity study, the tumour incidence was especially high at the highest dose level where inflammation of the nasal tissues was most severe. Tumours also developed at the mid-dose but not at the lowest dose where inflammatory reactions were weak.

In summary, 2,6-DMA is a carcinogen in rats causing invasive tumours of the nasal cavity. These tumours develop in tissues which show inflammatory processes at the same time and prior to the occurrence of tumours. It remains open whether inflammatory and toxic effects play a role in tumour development. However, 2,6-DMA is N-oxidised to genotoxic products, binds to DNA at the target tissue, and forms a type of DNA-adduct analogue to that observed with genotoxic carcinogenic polycyclic arylamines. Hence, the mode of action of nasal tumour development in experimental animals remains open. Under these conditions, no health-based OEL can be derived. Moreover, no inhalation study exists to derive quantitative risk estimates.

A STEL can not be assigned. Methemoglobinemia occurs only at concentrations well above those which are of concern for long-term average inhalation exposure. There are no data on developmental or reproductive toxicity in humans or animals.

According to theoretical calculations by Fiserova-Bergerova et al. (1990) the potential for dermal absorption and resulting toxicity of 2,6-DMA is significant. Thus, although no quantitative experimental data on dermal uptake were found on this substance, a skin notation is warranted.

No data are available regarding sensitisation in humans or animals.
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