Recommendation from the Scientific Committee on Occupational Exposure Limits for *N*-Methylaniline

SCOEL/SUM/178
December 2012

Employment, Social Affairs and Inclusion

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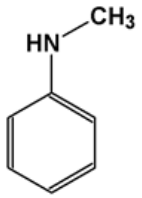
8-hour TWA:	0.2 ppm (0.89 mg/m^3)
STEL (15-min):	0.5 ppm (2.2 mg/m^3)
BLV:	-
Notation:	Skin

This document is based on Greim (1996), Greim (2001), ACGIH (2001), NLM (2006a), the references cited in these reviews and additional references from a Medline search (1966 to 2010).

1. Substance identification, physico-chemical properties

Chemical name: *N*-Methylaniline
Synonyms: Monomethylaniline; (methylamino)benzene; *N*-methylamino-benzene; *N*-methylbenzenamine
Structural formula:



CAS No.: 100-61-8
EC No.: 202-870-9
Annex I Index No.: 612-015-00-5
Molecular formula: C_7H_9N
Molecular weight: 107.15
Conversion factor 20 °C, 101.3 kPa: 1 ppm = 4.45 mg/m^3; 1 mg/m^3 = 0.225 ppm

EU classification:

Acute Tox. 3	H331	Toxic if inhaled.
Acute Tox. 3	H311	Toxic in contact with skin.
Acute Tox. 3	H301	Toxic if swallowed.
STOT RE 2	H373	May cause damage to organs through prolonged or repeated exposure.
Aquatic Acute 1	H400	Very toxic to aquatic life.
Aquatic Chronic 1	H410	Very toxic to aquatic life with long lasting effects.

N-methylaniline (NMA) is a colourless liquid that turns brown when left standing in air. NMA has a melting point of -57 °C and a boiling point of 195.9 °C. The vapour pressure at 20 °C is 0.4 hPa. NMA is slightly soluble in water (5.6 g/l at 25 °C) and soluble in alcohol and ether. A log P_{OW} of 1.66 is reported. The pk_a is 4.85 (25 °C), the density 0.989 g/cm^3 at 20 °C The substance has a flash point of 79.4 °C (closed cup) (ACGIH 2001, NLM 2006a).

2. Occurrence/use and occupational exposure

NMA is used in organic syntheses, as a solvent for organic reactions, and as an acid acceptor. NMA has been detected in foodstuff, e.g. in some vegetables and cheese, and in orange oil (NLM 2006a).

3. Health significance

3.1. Toxicokinetics

3.1.1. Human data

No data were available regarding the absorption, distribution, metabolism or excretion of NMA in humans.

It is expected that NMA, like aniline, is readily absorbed through the skin (ACGIH 2001, ICSC 1994, Greim 1996)

3.1.2. Animal data

Quantitative data on absorption were not available. However, the symptoms occurring following inhalation, oral or dermal exposure clearly indicate that NMA is well absorbed via all these routes of exposure. Especially, the lethality after dermal application of liquid NMA in rabbits indicates that NMA is readily absorbed through the intact skin (Treon *et al* 1949).

Data on the distribution of NMA were not available.

The biotransformation of NMA has been elucidated in studies on *N,N*-dimethylaniline. *In vitro* studies with liver tissue from rodents (rat, hamster, guinea pig) and rabbits have shown that *N,N*-dimethylaniline is mainly *N*-oxidised or is demethylated to NMA. NMA was shown to be further demethylated to aniline and/or ring-hydroxylated to *o*- and *p*-hydroxy derivatives by liver microsomes of rodents and rabbits. In addition to microsomal monooxygenases, demethylation of NMA to aniline can also be catalysed by prostaglandin synthase.

Very few data were available on the metabolism of NMA *in vivo*. Following i.v. administration of NMA in dogs, rapid (within 5 min) formation of nitrosobenzene could be detected in blood. The formation of nitrosobenzene from NMA (15 mg/kg) was faster and the nitrosobenzene concentration was higher (about 3 times at peak level, as read from figure) than after injection of a higher dose of aniline (100 mg/kg) (Kiese 1959).

After i.p. administration of 28 mg NMA/kg in rats, 85 % of the dose was excreted unchanged in urine within 24 hours (Greim 1996).

3.1.3. Biological monitoring

No data were available on the biomonitoring of NMA.

However, NMA, as an aromatic amine, induces the formation of methaemoglobin (MetHb), which may be a relevant parameter to monitor exposure and/or effects. A maximum MetHb-level of 5 % in blood has generally been regarded as tolerable for workers (Bolt *et al* 1985). Biological monitoring of MetHb is, however, hampered by the rapid alteration of MetHb concentrations in blood.

3.2. Acute toxicity

3.2.1. Human data

There were no data available regarding the acute toxicity of NMA in humans but it is expected that acute NMA poisoning will resemble that seen with aniline, including induction of MetHb with cyanosis, weakness, dizziness and severe headache (ACGIH 2001).

In most individuals, the background MetHb level is between 1 and 2 % and an increase up to 15 % will be without significant signs or symptoms. It is, however, probable, based on knowledge on CO poisoning, that much lower MetHb levels may not be tolerated in persons with latent coronary or arterial dysfunction (Bolt *et al* 1985). Clinical cyanosis will develop at about 15–20 % MetHb and more. Fatigue, anxiety, headache, weakness, dizziness, tachycardia, dyspnoea and syncope will occur at 30–45 % MetHb. Higher concentrations will cause a reduced level of consciousness and finally coma, heart failure and death at more than 60–70 % MetHb (NRC 2000, Henschler 1992, HSE 1997).

3.2.2. Animal data

The clinical toxicology of NMA in experimental animals resembles that of aniline. Cyanosis, dyspnoea, weight loss, prostration, albuminuria and sometimes terminal convulsions are the main signs of NMA intoxication.

Data on the inhalation toxicity of NMA after a single exposure were not available.

After oral administration by gavage (in corn oil) to rats, LD_{50}s between 700 and 800 mg/kg bw were determined in a study following current OECD guidelines (NIHS Japan 2010). The minimum lethal dose in this study was 640 mg/kg for both sexes. In an older study, lethal doses of NMA were determined for rabbits and cats via various routes of administration (Treon *et al* 1949). The minimum lethal oral dose of NMA for rabbits was 280 mg/kg.

When administered intravenously in rabbits or cats, the minimum lethal dose in both species was 24 mg/kg (Treon *et al* 1949). The formation of MetHb after a single administration of NMA was assessed in rats, cats and dogs. After a single i.v. injection of 18–24 mg NMA/kg in 3 cats, peak levels of MetHb were attained shortly after administration and amounted to 60–70 % MetHb (Treon *et al* 1949). In another study using i.v. injection, NMA was found more potent at producing MetHb than aniline, 30 mg NMA/kg lead to 68 % MetHb in cats whereas aniline induced only 30 % MetHb (Holzer and Kiese 1960). In rats, i.p. administration of a similar dose of NMA or aniline (3.23×10^{-4} mol/kg bw) showed that NMA was about twice more potent at producing MetHb than aniline (46 vs. 22 % maximum MetHb induction) (Lin *et al* 1972). While NMA caused cyanosis and death at 7.6 ppm (32.6 mg/m³) (Treon *et al* 1950), no deaths were observed in rats exposed to aniline in subacute studies at exposure up to 70.9 ppm (Pauluhn 2004) or 87 ppm (du Pont de Nemours 1982). In an early subchronic study, exposure of various species to 5 ppm aniline had no adverse health effect other than a doubtful marginal increase in MetHb (Oberst 1956). In rabbits, no increase in MetHb could be observed after i.v. administration of NMA. However, after oral administration of 180 mg/kg MetHb-formation occurred after 6 hours–2 days and usually peaked on the 3rd day reaching 23–45 % (Treon *et al* 1949).

The formation of Heinz bodies in erythrocytes was studied in rabbits and cats (Treon *et al* 1949). Intravenous administration of 54 mg NMA/kg in a rabbit was followed by the presence of Heinz bodies in 82 % of erythrocytes the next day; in another rabbit, 21 % of erythrocytes showed Heinz bodies after injection of 36 mg NMA/kg. No

consistent formation of Heinz bodies could be observed at injected doses of 24–36 mg/kg. In 5 rabbits treated once orally with 12–18 mg NMA/kg, 98 % of erythrocytes showed Heinz bodies. The abnormal erythrocytes disappeared from the blood within 10 days. Rabbits also became temporarily anaemic 5–6 days after i.v. injection of 24–36 mg NMA/kg or oral treatment with 180 mg NMA/kg. Large numbers of Heinz bodies were also found after i.v. injection of NMA in cats but no quantitative data were presented. Anaemia was observed after i.v. injection of 18 mg NMA/kg in cats.

Cyanosis and death were observed in all rabbits after dermal application on the intact skin of ≥ 3 000 mg/kg for 1 hour or longer (Treon *et al* 1949).

3.3. Irritation and corrosivity

3.3.1. Human data

An odour threshold of 1.7 ppm (7.4 mg/m³) has been reported (ACGIH 2001).

Data on irritation were not available.

3.3.2. Animal data

Skin

NMA is apparently not irritating to the skin. No signs of irritation were seen after dermal application of lethal doses to the intact skin of rabbits (Treon *et al* 1949).

Eyes

There were no data available.

3.4. Sensitisation

There were no human or animal data available.

3.5. Repeated dose toxicity

3.5.1. Human data

There were no data available.

3.5.2. Animal data

Inhalation

In early studies, Treon *et al* (1950) exposed several species (rats, rabbits, guinea pigs, dogs, cats and monkeys) to different concentrations of NMA for 7 hours/day, 5 days/week for a different number of total exposures. The cat is generally considered as the most sensitive species to MetHb formation, the rabbit is the least sensitive. Humans, cat and dogs have a roughly similar sensitivity (RIVM 2001). No signs of intoxication were seen at 2.4 or 2.3 ppm. Cyanosis, increased respiration, salivation, prostration, loss of body weight and death were observed at a concentration of 7.6 ppm (32.6 mg/m³) and above. At 86 ppm (371 mg/m³), animals displayed severe signs of poisoning and died.

The concentration of MetHb in blood was correlated to the concentration of NMA in air. In cats exposed at 2.4 ppm, MetHb was at or below 5 % (as read from figure). In rabbits exposed to 26.6, 7.6 or 2.4 ppm, the MetHb level reached 6.3 %, 5.2 % or 1.7 % (Treon *et al* 1950).

Formation of Heinz bodies was also observed. In 4 cats exposed to 2.4 ppm, Heinz bodies were found on average in 12–29 % of erythrocytes and disappeared slowly over a period of several weeks after cessation of exposure. No Heinz bodies were observed in rabbits at 2.3 and 7.6 ppm. However, decreased erythrocyte count and haemoglobin (Hb) concentration, and hyperplasia of the bone marrow were observed at 7.6 ppm in this species. At the same concentration, pathologic effects (oedema, congestion) were observed in heart, lung, liver, spleen and kidneys of all species. No such effects were observed in animals exposed to 2.4 ppm (Treon *et al* 1950). This level (2.4 ppm, 10.7 mg/m³) is probably a NOAEL in this study but the low number of animals examined limits its validity. It is also possible that dermal uptake may have contributed to the total dose received by the animals, and 2.4 ppm may represent an underestimate of the true NOAEL.

Oral

A slight decrease in erythrocyte counts and Hb level in blood, but no sign of illness or death were observed in rabbits after oral administration of 24 mg/kg/day NMA in propane-1,2-diol, 5 days/week for 20 weeks (Treon *et al* 1949).

In a subacute toxicity study, rats were treated for 28 days by gavage with 0, 5, 25 or 125 mg NMA in corn oil/kg/day (NIHS Japan 2010). In females, significant and dose-dependent decreases of several haematological parameters [Hb concentration, mean cell volume (MCV), Hb amount/red blood cell (MCH)] and an increase in serum creatinine were seen at all dose levels. At 25 and 125 mg/kg/day, methaemoglobinaemia, cyanosis, anaemia and yellowish brown urine were observed in male and female rats. Macroscopically, enlargement and black changes of the liver were also observed in both sexes at 25 or 125 mg/kg/day. Histopathologically, congestion, increase in haematopoiesis or deposition of pigment in the spleen, increase in haematopoiesis in the bone marrow, extramedullary haematopoiesis and deposits of pigments in the liver and proximal tubules of the kidney were observed in treated groups of both sexes. The absolute and relative spleen weights were also increased in both sexes at 125 mg/kg and in females at 25 mg/kg. Minimal spleen congestion was observed in all 5 necropsied male animals at every dose group but not in any of the control animals. In both sexes, the Hb content of the blood showed a dose-dependent decrease which was significant at all dose levels in females. The LOAEL in this study was 5 mg NMA/kg/day.

Dermal

Limited data were available from a study in rabbits which received repeated 1-hour applications of NMA on the shaved abdominal skin for 5 days/week. No death occurred after 50 doses of 100 mg/kg each (2 animals) and of 160 mg/kg (one animal). Two animals each died after 22 doses of 220 mg/kg or 12 doses of 380 mg/kg. At necropsy, degenerative alterations were seen in brain, heart, lung, liver and kidneys. One animal each survived 22 doses of 390 mg/kg or 27 doses of 720 mg/kg but both lost 18 % of their body weight (Treon *et al* 1949).

3.6. Genotoxicity

3.6.1. In vitro

N-methylaniline was not mutagenic in Ames tests with *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535 and TA1537 in the absence or presence of S9 mix from different species (NLM 2006b). A mutagenic effect was observed in an Ames test with strain TA98 and, to a lesser extent, with TA100 in the presence of metabolic activation and norharman, a co-mutagenic compound when mixed with aromatic amines (Greim 1996). NMA was not mutagenic in *Escherichia coli* WP2 uvrA

and did not induce unscheduled DNA-synthesis in primary cultures of rat hepatocytes. NMA induced structural chromosomal aberrations including gaps, but no polyploidy in Chinese hamster lung cells in the absence (no cytotoxicity) and in the presence (about 50 % growth inhibition) of exogenous metabolic activation (NIHS Japan 2010).

3.6.2. In vivo – Human and animal data

There were no data available.

3.7. Carcinogenicity

3.7.1. Human data

There were no data available.

3.7.2. Animal data

Two early studies were available in which the carcinogenicity of NMA was investigated. Haemorrhagic foci in the liver, but no tumours in this or any other organ were found in 20 male and 20 female Osborne-Mendel rats after oral treatment with 0.06 % NMA-hydrochloride in food for a period of 272–758 days. No control group was described in this study (White and Mori-Chavez 1952). In another study, 20 male and 20 female Swiss mice received NMA in food (1 950 mg/kg food) for 28 weeks followed by a post-treatment period of 12 weeks. The incidence of lung adenomas in the NMA-treated group was not different from control data. No other tumours were observed (Greenblatt *et al* 1971). Because of the short exposure period, small group size, and absence of control group in the rat study, no definite conclusions can be drawn from these results.

As a secondary amine, NMA may be converted to the corresponding *N*-nitroso compound under certain conditions. Many of these *N*-nitrosoamines are mutagenic. Corresponding data on the mutagenicity of *N*-nitroso-*N*-methylamine were not available. The incidence of lung adenomas was increased significantly when the NMA-treated animals also received sodium nitrite ($NaNO_2$) at 0.1 % in drinking water 5 times/week (Greenblatt *et al* 1971). It was concluded that carcinogenic nitrosamines were formed from NMA *in vivo*.

3.8. Reproductive toxicity

3.8.1. Human data

There were no data available.

3.8.2. Animal data

Fertility and developmental toxicity

There were no data available.

Aniline can cross the placenta and induce foetal methaemoglobinaemia; a similar property can be expected for *N*-methylaniline.

4. Recommendation

For the evaluation of the toxicity of NMA, the following effects must be taken into account:

- methaemoglobin (MetHb) formation
- toxic effects on the haematopoietic system with erythrocytotoxicity and effects on the spleen

Information is insufficient to assess the carcinogenic and reprotoxic capacity of NMA.

No human data were available regarding the haematological effects of NMA. In experimental animals, the critical effect of NMA (acute and chronic) is the formation of MetHb but the available database does not allow directly deriving a clear point of departure in studies conducted with NMA. Animal data indicate, however, that NMA is twice more potent at inducing MetHb than aniline, and this analogy may help to recommend an OEL. SCOEL proposed for aniline an 8-hour TWA OEL and a STEL of 0.5 and 1 ppm, respectively (SCOEL 2010). An *8-hour TWA OEL and a STEL of 0.2 and 0.5 ppm,* respectively, are therefore proposed for NMA.

Aniline is at worst considered as a very weak *in vivo* genotoxicant and no carcinogenic activity or reproductive toxicity can be expected at exposure levels that do not cause increased MetHb formation (SCOEL 2010). It can, therefore, be expected by analogy that the OEL proposed for NMA also protects against carcinogenicity and reproductive toxicity, if any.

Animal studies clearly indicate that NMA is well absorbed through the intact skin. Therefore, assignment of a "skin" notation is recommended. Data regarding sensitisation were not available.

The present Recommendation was adopted by SCOEL on 13 December 2012

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