The MAK Collection for Occupational Health and Safety

N-Methylaniline

MAK Value Documentation, addendum – Translation of the German version from 2017

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Keywords: N-methylaniline; MAK value; maximum workplace concentration; peak limitation; carcinogenicity; skin absorption; methaemoglobin; mechanism of action; read across

Citation Note: Hartwig A, MAK Commission. N-Methylaniline. MAK Value Documentation, addendum – Translation of the German version from 2017. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2019 Jul;4(3):1146-1170]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/mb10061e6319_w

Republished (online): 08 Aug 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.mb10061e6319

Addendum completed: 11 Dec 2014

Published (online): 25 Jul 2019

The commission established rules and measures to avoid conflicts of interest.

1146

N-Methylaniline

MAK value documentation

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DOI: 10.1002/3527600418.mb10061e6319

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the maximum concentration at the workplace (MAK value) of *N*-methylaniline [100-61-8] considering all toxicity endpoints. Available publications and unpublished study reports are described in detail. Critical effect is the formation of methaemoglobin (MetHb). Adequate data in humans to derive a MAK value are not available; thus, data with rats are used. In rats, *N*-methylaniline forms MetHb 2.7 times more efficiently than aniline. Based on the MAK value for aniline of 2 ml/m^3, the MAK value for *N*-methylaniline of 0.5 ml/m^3 is confirmed. As the critical effect is systemic, Peak Limitation Category II is retained; as the half-life in blood is unknown, the default excursion factor of 2 is also retained. *N*-Methylaniline remains in Pregnancy Risk Group D because developmental toxicity studies are lacking. The substance does not induce mutations in bacteria, but is clastogenic in mammalian cells. Studies on genotoxicity in vivo have not been performed. Adequate data to assess the carcinogenic potential of *N*-methylaniline are not available. Data for the metabolite aniline are used to assess the carcinogenic potential of *N*-methylaniline. Aniline induces spleen tumours in rats, but not in mice. The putative mode of action is an indirect tumour development by induction of MetHb and haemolytic effects which result in erythrocyte toxicity and its consequences, perturbations in iron metabolism in the spleen. The metabolites phenylhydroxylamine and nitrosobenzene are mainly responsible for the erythrocyte toxicity of aniline. As nitrosobenzene is also a metabolite of *N*-methylaniline, a similar mode of action has to be assumed for *N*-methylaniline. Therefore, a non-genotoxic mode of action is of prime importance and genotoxic effects play at most a minor part provided the MAK and BAT values are observed. Thus, *N*-methylaniline is classified as a suspected carcinogen in Carcinogen Category 3B. Skin contact may contribute significantly to systemic toxicity and the "H" notation is retained. There are no clinical data concerning the sensitizing activity of the substance. Results of a local lymph node assay in mice were negative.

Keywords

N-methylaniline; anilinomethane; (methylamino)benzene; N-methylaminobenzene; N-methylphenylamine; monomethylaniline; N-phenylmethylamine; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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N-Methylaniline

[100-61-8]

Supplement 2017

MAK value (1987)	**0.5 ml/m³ (ppm) ≙ 2.2 mg/m³**
Peak limitation (2001)	**Category II, excursion factor 2**
Absorption through the skin (1969)	**H**
Sensitization	**–**
Carcinogenicity (2016)	**Category 3B**
Prenatal toxicity (1994)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BAT value	**–**
1 ml/m³ (ppm) ≙ 4.45 mg/m³	**1 mg/m³ ≙ 0.225 ml/m³ (ppm)**

Documentation for *N*-methylaniline was published in 1987 (documentation "N-Methylaniline" 1993), followed by a supplement for peak limitation in 2001 (supplement "N-Methylanilin" 2001, available in German only).

The MAK value for *N*-methylaniline, which was provisionally established at 0.5 ml/m³ in 1987, was derived by analogy with aniline. Aniline has a MAK value of 2 ml/m³ (documentation "Aniline" 1993).

New studies have since been published that have made a supplement necessary.

N-Methylaniline is used in organic syntheses, as a solvent for organic reactions, and as an acid acceptor (SCOEL 2012).

1 Toxic Effects and Mode of Action

Aniline may form in the organism as a metabolite after the demethylation of *N*-methylaniline.

Like aniline, *N*-methylaniline is an indirect methaemoglobin former. Therefore, as in the case of aniline, further critical effects are erythrocyte toxicity and the subsequent effects on the spleens of rats. A 28-day inhalation study with *N*-methylaniline in Wistar rats revealed reduced haemoglobin levels, increased reticulocyte counts and the increased accumulation of brown pigment in the spleen at the low concentration of 13 mg/m³ and above. Methaemoglobin was increased in male and female rats at the *N*-methylaniline concentration of 45 mg/m³ and above. When gavage doses

of *N*-methylaniline of 5 mg/kg body weight and day and above were given to rats for 28 days, mild congestion was observed in the spleen, and in the females haemoglobin levels were reduced and creatinine concentrations were increased. Changes in the haematocrit and erythrocyte counts and histological changes in the spleen, liver and kidneys were observed at 25 mg/kg body weight and day and above.

N-methylaniline accelerated the rate of methaemoglobin formation to about twice that for aniline after single intravenous injections in cats and intraperitoneal injection in rats. The difference was about 2.7-fold in short-term inhalation studies in rats.

In rabbits, *N*-methylaniline did not cause irritation of the skin. The substance was found to cause irritation of the eyes in the isolated chicken eye test.

There are no clinical findings of sensitization, but negative results are available from a valid local lymph node assay in mice.

N-Methylaniline was not mutagenic in bacteria either with or without the addition of a metabolic activation system. *N*-Methylaniline induced structural chromosomal aberrations in Chinese hamster lung cells both with and without the addition of a metabolic activation system.

None of the available studies are suitable for evaluating the carcinogenic potential of *N*-methylaniline. As aniline is a metabolite of *N*-methylaniline and aniline caused splenic tumours in rats, but not in mice, *N*-methylaniline is suspected to cause carcinogenicity.

Studies of fertility, developmental toxicity or in vivo genotoxicity are not available.

2 Mechanism of Action

Like aniline, *N*-methylaniline is an indirect methaemoglobin former.

The methaemoglobin-forming and haemolytic properties of aniline are induced mainly by the metabolites phenylhydroxylamine and nitrosobenzene (supplement "Aniline" 2010).

In a co-oxidation process, phenylhydroxylamine is oxidized to nitrosobenzene in the erythrocytes and methaemoglobin is formed from haemoglobin (SCOEL 2010). Although methaemoglobin is reduced to haemoglobin by various intra-erythrocytic mechanisms, 95% of this activity is attributed to methaemoglobin reductase (= NADH-dependent cytochrome b5 reductase). In a redox cycle with NADPH, reactive phenylhydroxylamine may in turn be formed from nitrosobenzene, which, together with phenylhydroxylamine, is another *N*-oxidized metabolite of aniline. The higher level of exposure to this metabolite limits its formation from nitrosobenzene because of the consumption of NADPH in the erythrocytes (supplement "Aniline" 2010).

Following injection of *N*-methylaniline, methaemoglobin formation was so accelerated in cats that it could not be caused only by nitrosobenzene (and phenylhydroxylamine) in the blood. Therefore, other methaemoglobin-forming derivatives must have been formed from *N*-methylaniline (no other details; Holzer and Kiese 1960).

In short-term studies in cats and rats, the rate of methaemoglobin formation was about twice as high after exposure to *N*-methylaniline than after exposure to aniline (see Section 5.1.4; Holzer and Kiese 1960; Lin et al. 1972). In short-term inhalation studies in rats, it was about 2.7 times as high (see Section 6).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

There are no specific studies available.

Effects on the blood and spleen were observed after inhalation, oral administration and dermal application. Therefore, it is assumed that *N*-methylaniline is readily absorbed via these routes of absorption. Particularly the mortality observed after dermal application of liquid *N*-methylaniline to the skin of rabbits suggests high dermal absorption (SCOEL 2012). By applying the algorithms of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), it is calculated that 950, 68 and 76 mg, respectively, of *N*-methylaniline is absorbed through the skin from a saturated aqueous solution (exposure: 1 hour; exposed area: 2000 cm^2; solubility in water: 5620 mg/l (SRC 2013 a); log Kow: 1.66 (SRC 2013 a); molar mass: 107.16 g/mol).

Data for the absorption of *N*-methylaniline by inhalation are not available. About 90% of aniline is absorbed by inhalation via the respiratory tract (supplement "Aniline" 2010). *N*-Methylaniline and aniline differ in their solubility in water (*N*-methylaniline: 5620 mg/l, aniline: 36 000 mg/l at 25 °C (SRC 2013 b); 6.4-fold); the lower solubility of *N*-methylaniline in water allows it to penetrate more deeply than aniline into the lower respiratory tract after inhalation.

In rats given a single intraperitoneal injection of an *N*-methylaniline dose of 28 mg/kg body weight, 85% of the dose was eliminated unchanged with the urine within 24 hours (documentation "N-Methylaniline" 1993).

3.2 Metabolism

Studies of the metabolism of *N*,*N*-dimethylaniline provided information about the metabolism of *N*-methylaniline. *N*,*N*-Dimethylaniline was mainly *N*-oxidized or was demethylated to *N*-methylaniline in the liver tissue of rodents and rabbits. *N*-Methylaniline was *N*-demethylated to aniline or ring-hydroxylated to *o*-hydroxy or *p*-hydroxy derivatives by the liver microsomes of rodents and rabbits. The latter two metabolites were shown to likewise undergo further demethylation (see Figure 1 in documentation "N-Methylaniline" 1993; SCOEL 2012). In vitro the erythrocyte cytosol of male Wistar rats catalysed the enzymatic *N*-demethylation of *N*-methylaniline (Stecca and Duverger-van Bogaert 1989). The demethylation activity was linked with oxidized haemoglobin and was increased by the addition of NADH and an NADH-methaemoglobin reductase system (Stecca et al. 1992). *N*-Demethylation of *N*-methylaniline was catalysed also by prostaglandin synthase (documentation "N-Methylaniline" 1993; SCOEL 2012).There was also evidence of *N*-glucuronidation in primary cultures of rat hepatocytes that had been treated with *N*-methylaniline (US EPA 2005).

Aniline and nitrosobenzene were identified in the blood of 3 dogs 10 minutes after the single intravenous injection of an *N*-methylaniline-HCl dose of 15 mg/kg body weight. The peak level of nitrosobenzene (2.2 µg/ml) was reached after 10 minutes and that of aniline (1.4 µg/ml) after 20 minutes (according to a figure) (Holzer and Kiese 1960). After a single intravenous injection of *N*-methylaniline-HCl of 15 mg/kg body weight, the formation of nitrosobenzene was more rapid than after

the injection of 100 mg aniline-HCl/kg body weight and led to about 3 times more nitrosobenzene. Thus, nitrosobenzene was not formed from *N*-methylaniline via the oxidation of aniline, an intermediate formed by the demethylation of *N*-methylaniline, but by the direct oxidation of *N*-methylaniline to nitrosobenzene (Kiese 1959).

At least 3 cats were given single equimolar intravenous injections of *N*-methylaniline-HCl or aniline-HCl in 0.9% saline (0.209 mmol/kg body weight; *N*-methylaniline: 30 mg/kg body weight; aniline: 27 mg/kg body weight) After 10 to 20 minutes, the maximum nitrosobenzene concentrations in the blood were reached with both substances. The nitrosobenzene concentration was about 20% to 30% higher after exposure to *N*-methylaniline (1.3 µg/ml blood) than after exposure to aniline, but the difference was not reliable in view of the number of animals in the test and the scatter range (a statistical evaluation was not carried out). According to the authors, nitrosobenzene "may" have formed somewhat faster from *N*-methylaniline than from aniline. The rate of aniline formation from *N*-methylaniline corresponded approximately to the rate of nitrosobenzene formation. Therefore, the fraction of aniline formed from *N*-methylaniline via nitrosobenzene or phenylhydroxylamine may have been larger than was assumed on the basis of the low concentration of nitrosobenzene in the blood. There was a relatively rapid decrease in the nitrosobenzene concentration within 100 minutes. The aniline concentration was about 2.1 µg/ml blood 20 minutes after the administration of *N*-methylaniline and remained at this level for about 100 minutes (Holzer and Kiese 1960).

There was evidence that nitrosobenzene formed in the liver microsomes of rodents and rabbits that had been treated with *N*-methylaniline (Kampffmeyer and Kiese 1964; documentation "N-Methylaniline" 1993). Liver microsomes *N*-hydroxylated *N*-methylaniline faster than aniline (no other details; Kiese 1974).

In an in vitro cytochrome P450 (CYP) model system with *N*-methylaniline and synthetically produced polypeptide-bound porphyrinatoiron(III), *N*,*N*′-dimethyl-*N*,*N*′-diphenylhydrazine was identified as an unexpected metabolite in the presence of rat liver microsomes. The CYP inhibitors SKF-525A and metyrapone suppressed the formation of *N*,*N*′-dimethyl-*N*,*N*′-diphenylhydrazine. Therefore, the authors attributed the *N*,*N*′-coupling reaction to catalysis by CYP (Doi et al. 1993).

4 Effects in Humans

Clinical cyanosis develops at about 15% to 20% methaemoglobin. Fatigue, anxiety, headache, weakness, dizziness, tachycardia, dyspnoea and syncope occur at 30% to 45% methaemoglobin. Higher concentrations cause a reduced level of consciousness and finally coma, heart failure and death at more than 60% to 70% methaemoglobin (SCOEL 2012).

In humans, an increase in the methaemoglobin level above 1.5% is regarded as an exposure marker and indicates exposure to methaemoglobin formers. Health effects arising from methaemoglobin itself are not expected up to a methaemoglobin level of 5% (Leng and Bolt 2016).

4.1 Single exposures

There are no data available.

The odour threshold is 1.7 ml/m³ (ACGIH 2001).

It is assumed that acute intoxication with *N*-methylaniline resembles that with aniline, including methaemoglobin formation and the resulting cyanosis, weakness, dizziness and severe headache (SCOEL 2012).

4.2 Repeated exposure

Examinations of workers in the manufacture of monomethylaniline semi-finished products revealed an increased incidence of diseases of the internal organs, moderate methaemoglobinaemia, functional changes of the cardiovascular system, disorders of the pulmonary ventilation function and changes in metabolic processes (no other details; Kaminskaia et al. 1989). This information was taken from the English abstract of the Russian publication.

As aniline is a metabolite of *N*-methylaniline, a volunteer study with aniline is used for the evaluation of *N*-methylaniline.

A MAK value of 2 ml/m³ was established for aniline in 1983. A volunteer study examined methaemoglobin formation and elimination at this concentration. In this study (Käfferlein et al. 2014), 19 non-smoking volunteers (10 men, 26 to 59 years, and 9 women, 23 to 53 years; 15 slow acetylators and 4 fast acetylators) were exposed to an aniline concentration of 2 ml/m³ in a whole-body exposure chamber for 6 hours. During the exposure, the volunteers exercised on a bicycle ergometer for 3 intervals lasting 20 minutes. This increased the methaemoglobin level in the blood from 0.72 ± 0.19% before exposure to 1.21 ± 0.29% at the end of the 6-hour exposure period (range: 0.80% to 2.07%). The maximum level of the individuals was 2.07% methaemoglobin. After 24 hours the methaemoglobin levels had returned to the levels of the volunteers not exposed. Taking into account the mean baseline value of 0.72% methaemoglobin, the maximum increase in methaemoglobin induced by aniline was 1.35% (2.07%–0.72% = 1.35%). As the methaemoglobin level did not reach a plateau during the exposure, linear extrapolation of the experimental 6-hour exposure to 8-hour exposure corresponding to a working day was necessary. Therefore, the increase in methaemoglobin expected after 8 hours is 1.35% × 8/6 = 1.80%. The 8-hour increment found under the experimental conditions of exposure to aniline at the level of the MAK value is lower than the maximum permissible increase in methaemoglobin of 4% by a factor of 2.22 when the general baseline value is taken into account (Bolt et al. 2017). This shows that the MAK value of 2 ml/m³ for aniline provides protection against adverse methaemoglobin formation at the workplace (Käfferlein et al. 2014).

4.3 Local effects on skin and mucous membranes

There are no data available.

4.4 Allergenic effects

No reports of sensitization after contact with *N*-methylaniline are available. When patients with existing sensitization to *p*-phenylenediamine were patch tested, positive reactions to 5% *N*-methylaniline in yellow petrolatum were not observed in any of the 40 persons tested in the study (Kleniewska 1975). Unlike aniline or the toluidines, *N*-methylaniline did not cause cross-reactions with *p*-phenylenediamine in this cohort.

4.5 Reproductive and developmental toxicity

There are no data available.

4.6 Genotoxicity

There are no data available.

4.7 Carcinogenicity

There are no data available.

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

When gavage doses (in corn oil) were given to rats in a study carried out according to OECD Test Guideline 401, the oral LD_{50} was 782 mg/kg body weight for males and 716 mg/kg body weight for females. Hypoactivity and cyanosis were observed in all animals. Brown urine, increased salivation or lacrimation and abdominal or lateral position were found in several animals (NIHS Japan undated a, b). In rabbits, the minimum lethal dose was 280 mg/kg body weight (documentation "N-Methylaniline" 1993; Treon et al. 1949).

In rabbits given oral doses of 180 mg/kg body weight, maximum methaemoglobin levels of 23% to 45% were observed on day 3 after treatment (documentation "N-Methylaniline" 1993; Treon et al. 1949).

5.1.3 Dermal application

New data are not available.

5.1.4 Intravenous and intraperitoneal injection

After single intravenous injections of equimolar doses of *N*-methylaniline or aniline (0.209 mmol/kg body weight; *N*-methylaniline: 30 mg/kg body weight; aniline: 27 mg/kg body weight), the maximum methaemoglobin concentrations in the blood of cats (methaemoglobin was determined via the increase in the extinction of the blood solution after the addition of cyanide) were about 2.2 times higher after exposure to *N*-methylaniline than after exposure to aniline (according to a figure) (documentation "N-Methylaniline" 1993; Holzer and Kiese 1960).

When dogs were given 15 mg/kg body weight (0.104 mmol/kg body weight) by intravenous injection, *N*-methylaniline-HCl led to a methaemoglobin level of 47% after 1 to 2 hours (documentation "N-Methylaniline" 1993; Holzer and Kiese 1960).

When rats were given single intraperitoneal injections of equimolar doses of *N*-methylaniline and aniline (0.323 mmol/kg body weight), about 2.1 times more methaemoglobin was formed by *N*-methylaniline than by aniline, with a maximum methaemoglobin level of 37% (documentation "N-Methylaniline" 1993; Lin et al. 1972).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

The results from an inhalation study carried out in Wistar rats according to OECD Test Guideline 412 are shown in Table 1. The details of the haematological examination and of the histopathological examination of the spleen are shown in Table 2.

In this subacute inhalation study carried out according to OECD Test Guideline 412, reduced haemoglobin values, increased reticulocyte counts and the increased accumulation of brown pigment in the spleen of male and female rats were observed at the low concentration of 13 mg/m^3 and above. The erythrocyte counts were reduced in relation to the dose in the females at 13 mg/m^3 and above and in the males at 40 mg/m^3 and above. Methaemoglobin was increased in relation to the dose in male and female rats at the *N*-methylaniline concentration of 40 mg/m^3 and above. At 40 mg/m^3, 2 of 5 females developed cyanosis shortly after exposure on day 16 of treatment. The methaemoglobin level on this day is not known because methaemoglobin was determined only on the last day of exposure. According to the authors, the decrease in methaemoglobin observed in the males at 13 mg/m^3 was incidental. The LOAEC (lowest observed adverse effect concentration) was 13 mg/m^3; it was not possible to derive a NOAEC (no observed adverse effect concentration) (Rompetrol SA 2011).

Table 1 Effects of *N*-methylaniline after repeated inhalation

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 5 ♂, 5 ♀	**OECD Test Guideline 412**, **28 days**, corresponding to 20 treatment days, nose-only, vapour, nominal concentration: 0, 15, 45, 135 mg/m³, analysed concentration: 0, 13, 40, 116 mg/m³ (0, 2.9, 9.0, 26.1 ml/m³), 6 hours/day, 5 days/week, purity: > 93%, MetHb determined from blood taken from the tip of the tail on the last day of exposure at the end of the exposure because of the short half-life of MetHb	**no NOAEC**; **13 mg/m³ (2.9 ml/m³) and above**: blood: Hb ↓ (♂: 6%, ♀: 5%, at 40 mg/m³: not statistically significant), reticulocytes ↑ (13, 40, 116 mg/m³: ♂: 70%, 377%, 252%; ♀: 164%, 77%, 363%); spleen: increased accumulation of brown pigment; ♂: plasma: total bilirubin ↑; ♀: blood: RBC ↓ (13, 40, 116 mg/m³: 6%, 7%, 16%), MCV ↑, spleen: absolute weights ↑ (13, 40, 116 mg/m³: 13%, 11%, 71%); **40 mg/m³ (9.0 ml/m³)**: ♀: cyanosis (2/5 animals); **40 mg/m³ (9.0 ml/m³) and above**: respiratory frequency ↓ (40 mg/m³: observed twice at 2 different observation times in this dose group, no other details, 116 mg/m³: 3 times), blood: MetHb ↑; spleen: absolute weights ↑ (40, 116 mg/m³: ♂: 27%, 34%) and relative weights ↑ (40, 116 mg/m³: ♂: 24%, 34%, ♀: 125%, 73%); ♂: blood: RBC ↓ (40, 116 mg/m³: 10%, 11%), MCV ↑; spleen: extramedullary haematopoiesis; **116 mg/m³ (26.1 ml/m³)**: spleen: mild expansion of the red pulp; ♂: bone marrow: slight accumulation of brown pigment; ♀: plasma: total bilirubin ↑, creatinine ↓; spleen: extramedullary haematopoiesis; survival, no substance-induced change in body weights or feed consumption	Rompetrol SA 2011; ECHA 2015

Hb: haemoglobin; MCV: mean erythrocyte volume; MetHb: methaemoglobin; RBC: erythrocyte count; NOAEC (no observed adverse effect concentration)

Table 2 Results of the haematological examination and histopathological findings in the spleen obtained from the 28-day inhalation study in rats at the end of the study (Rompetrol SA 2011)

Organ parameter or findings	Controls	13 mg/m^3	40 mg/m^3	116 mg/m^3
blood (#)	♂: 5 ♀: 5	♂: 5 ♀: 5	♂: 5 ♀: 5	♂: 5 ♀: 5
RBC (10^{12}/l)	♂: 9.774 ± 0.302 ♀: 9.160 ± 0.118	♂: 9.322 ± 0.464 ♀: 8.636 ± 0.154$^{**a)}$	♂: 8.818 ± 0.124$^{**a)}$ ♀: 8.490 ± 0.099$^{**a)}$	♂: 8.696 ± 0.142$^{**a)}$ ♀: 7.716 ± 0.203$^{**a)}$
Hb (mmol/l)	♂: 10.28 ± 0.23 ♀: 9.80 ± 0.16	♂: 9.68 ± 0.41$^{**b)}$ ♀: 9.34 ± 0.18$^{**a)}$	♂: 9.32 ± 0.11$^{**b)}$ ♀: 9.60 ± 0.10	♂: 9.64 ± 0.09$^{*b)}$ ♀: 8.84 ± 0.21$^{**a)}$
MetHb (%)	♂: 2.118 ± 0.640 ♀: 1.890 ± 0.298	♂: 0.835 ± 0.632$^{*b)}$ ♀: 1.863 ± 1.461	♂: 3.602 ± 0.523$^{**b)}$ ♀: 4.150 ± 0.499$^{**b)}$	♂: 6.348 ± 0.633$^{**b)}$ ♀: 10.802 ± 1.398$^{**b)}$
PCV (l/l)	♂: 0.4818 ± 0.0133 ♀: 0.4536 ± 0.0064	♂: 0.4572 ± 0.0226$^{*a)}$ ♀: 0.4360 ± 0.0091$^{**a)}$	♂: 0.4460 ± 0.0071$^{**a)}$ ♀: 0.4468 ± 0.0060	♂: 0.4602 ± 0.0087 ♀: 0.4094 ± 0.0065$^{**a)}$
MCV (fl)	♂: 49.30 ± 0.26 ♀: 49.52 ± 0.28	♂: 49.05 ± 0.31 ♀: 50.49 ± 0.47$^{*b)}$	♂: 50.58 ± 0.13$^{**b)}$ ♀: 52.63 ± 0.52$^{**a)}$	♂: 52.92 ± 0.81$^{**b)}$ ♀: 53.07 ± 0.62$^{**a)}$
reticulocytes (%)	♂: 0.364 ± 0.048 ♀: 0.382 ± 0.168	♂: 0.620 ± 0.089$^{**a)}$ ♀: 1.008 ± 0.300$^{**a)}$	♂: 1.736 ± 0.255$^{**a)}$ ♀: 0.678 ± 0.136$^{*b)}$	♂: 1.280 ± 0.228$^{**a)}$ ♀: 1.768 ± 0.646$^{**a)}$
total bilirubin (plasma)	♂: 1.72 ± 0.04 ♀: 2.24 ± 0.56	♂: 2.00 ± 0.07$^{**b)}$ ♀: 2.56 ± 0.13	♂: 2.12 ± 0.19$^{**b)}$ ♀: 2.44 ± 0.11	♂: 2.46 ± 0.32$^{**b)}$ ♀: 3.70 ± 0.27$^{**b)}$

Table 2 (continued)

Organ parameter or findings	Controls	13 mg/m³	40 mg/m³	116 mg/m³
spleen (#)	♂: 5 ♀: 5	♂: 5 ♀: 5	♂: 5 ♀: 5	♂: 5 ♀: 5
extramedullary haematopoiesis				
very mild	♂: 0 ♀: 0	♂: 0 ♀: 0	♂: 0 ♀: 0	♂: 0 ♀: 2 (40%)
mild	♂: 0 ♀: 0	♂: 0 ♀: 0	♂: 5 (100%) ♀: 0	♂: 5 (100%) ♀: 3 (60%)
total	♂: 0 ♀: 0	♂: 0 ♀: 0	♂: 5 (100%)** ♀: 0	♂: 5 (100%)** ♀: 5 (100%)**
slight expansion of the red pulp	♂: 0 ♀: 0	♂: 0 ♀: 0	♂: 0 ♀: 0	♂: 5 (100%)** ♀: 5 (100%)**
increased accumulation of brown pigment				
very slight	♂: 0 ♀: 0	♂: 5 (100%) ♀: 0	♂: 0 ♀: 0	♂: 0 ♀: 0
mild	♂: 0 ♀: 0	♂: 0 ♀: 5 (100%)	♂: 5 (100%) ♀: 5 (100%)	♂: 5 (100%) ♀: 5 (100%)
total	♂: 0 ♀: 0	♂: 5 (100%)** ♀: 5 (100%)**	♂: 5 (100%)** ♀: 5 (100%)**	♂: 5 (100%)** ♀: 5 (100%)**

#: number of animals examined;
Hb: haemoglobin; MCH: mean corpuscular haemoglobin; MCV: mean erythrocyte volume; MetHb: methaemoglobin; PCV: haematocrit; RBC: erythrocyte count
[a)] Dunnett's test, two-sided
[b)] Dunnett's test, non-parametric, two-sided
in the case of histopathological changes: Fisher's exact test, two-sided
*$p \leq 0.05$; **$p \leq 0.01$

In 1 cat and 1 dog that were exposed to an *N*-methylaniline concentration of 86 ml/m³ by inhalation (50 times for 7 hours), a methaemoglobin level of 37% was observed; in rabbits exposed to 2.4, 7.6 or 26.6 ml/m³ (130 times for 7 hours), the corresponding values were 1.7%, 5.2% and 6.3%. Heinz bodies developed in the erythrocytes after exposure to concentrations as low as 2.4 ml/m³ and above; they only disappeared several weeks after the end of exposure. In rabbits, the erythrocyte counts and the haemoglobin levels of the blood were reduced and bone marrow hyperplasia was observed at the concentration of 7.6 ml/m³ and above. This effect was less marked in guinea pigs and not detected in cats at all. Glycosuria and slight haematuria were observed in all species. In rabbits, guinea pigs, rats, cats, dogs and monkeys, concentrations of 7.6 ml/m³ and above induced pathological changes such as oedema and congestion in the heart, lungs, liver, spleen and kidneys of all test animals; exposure to 2.4 ml/m³ produced no effects. The 4 rabbits exposed to 2.3 ml/m³ survived for 88, 100 and 130 days without signs of intoxication (no other details; Treon et al. 1950; documentation "N-Methylaniline" 1993). The procedure and documentation of the study do not comply with current standards.

5.2.2 Oral administration

The results of a study in rats with repeated oral administration of *N*-methylaniline are shown in Table 3.

In this study, in which gavage doses of *N*-methylaniline were given to rats for 28 days, mild congestion was observed in the spleen of all 5 males and haemoglobin levels were reduced and creatinine concentrations were increased in the females at 5 mg/kg body weight and day and above. The authors (NIHS Japan undated a, c) and the US EPA (US EPA 2005) considered this dose to be the NOAEL (no observed adverse effect level) because both effects were only mild and changes in the haematocrit and erythrocyte counts as well as further pathological changes in the spleen, liver and kidneys were observed only at 25 mg/kg body weight and day and above. The original study was published in Japanese and the tables were published in English. The Commission considers the low dose of 5 mg/kg body weight and day to be the LOAEL (lowest observed adverse effect level). Although the effects on the blood and spleen were only slight, they are regarded as adverse because the blood is the target organ of the toxicity of *N*-methylaniline.

In rabbits, oral *N*-methylaniline doses of 24 mg/kg body weight and day administered for 20 weeks (5 days/week; vehicle: 10% propylene glycol) induced a very slight decrease in the erythrocyte count and haemoglobin level (no other details; documentation "N-Methylaniline" 1993; Treon et al. 1949). This study and other oral studies with *N*-methylaniline that were described in Section 5.7 have not been included in the evaluation because of the small number of parameters examined, the inadequate documentation of the study and the use of only one dose.

5.2.3 Dermal application

After repeated application of *N*-methylaniline (no details, presumably semi-occlusive) for 1 hour, 5 times a week, rabbits survived exposure to 50 doses of 100 (2 animals) and 160 mg/kg body weight (1 animal), 22 doses of 390 mg/kg body weight

Table 3 Effects of *N*-methylaniline after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, Crj:CD(SD), 5, 25 mg/kg body weight: 5 ♂, 5 ♀, 0, 125 mg/kg body weight: 10 ♂, 10 ♀, recovery period 125 mg/kg body weight: 5 ♂, 5 ♀	**28 days**, 0, 5, 25, 125 mg/kg body weight and day, gavage, in corn oil, purity: 99.4%, 14-day recovery period: 0, 125 mg/kg body weight and day, according to the Japanese guidelines Toxicity Testing of Chemicals	**no NOAEL;** **5 mg/kg body weight and above:** ♂: spleen: mild congestion (5/5, severity ↑, but not dose-dependent); ♀: blood: Hb ↓ (4.1%), MCV ↓ (not dose-dependent), MCH ↓ (not dose-dependent); serum: creatinine ↑, spleen: pigment deposits (5/5 animals, control: 2/5 animals, not statistically significant); **25 mg/kg body weight and above:** blood: haematocrit ↓, RBC ↓; spleen: enlargement, black discoloration, haematopoiesis ↑ (5/5 animals, severity ↑, but not dose-dependent), kidneys: hyaline droplets; ♂: blood: Hb ↓, spleen: pigment deposits (5/5 animals, severity ↑, but not dose-dependent); ♀: spleen: absolute and relative weights ↑, congestion, bone marrow: haematopoiesis ↑; **125 mg/kg body weight**: cyanosis, liver: pigment deposits, extramedullary haematopoiesis ↑, kidneys: pigment deposits; ♂: urine: volume ↑, yellowy-brown discoloration, spleen: absolute and relative weights ↑, liver, kidneys: black discoloration, bone marrow: haematopoiesis ↑; **recovery period 125 mg/kg body weight**: mild anaemia, spleen: absolute and relative weights ↑, enlargement, black discoloration, congestion, pigment deposits, liver: pigment deposits, kidneys: pigment deposits, hyaline droplets; no substance-induced changes in survival or body weights, MetHb not determined	NIHS Japan undated a, c; US EPA 2005

NOAEL: no observed adverse effect level; Hb: haemoglobin; MCH: mean corpuscular haemoglobin; MCV: mean erythrocyte volume; MetHb: methaemoglobin; RBC: erythrocyte count

(1 animal) and 27 doses of 720 mg/kg body weight (1 animal). Body weight losses of 18% were observed in the animals of the two high dose groups. After 12 doses of 380 mg/kg body weight, 1 animal died, and another died after 22 doses of 220 mg/kg body weight. Pathological examinations revealed degenerative changes in the brain, heart muscle, lungs, liver and kidneys (documentation "N-Methylaniline" 1993; Treon et al. 1949).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In rabbits, repeated dermal applications of *N*-methylaniline to the shaved dorsal skin in 5 ml portions at intervals of 30 minutes did not induce local irritation (no other details; documentation "N-Methylaniline" 1993; Treon et al. 1949).

5.3.2 Eyes

In the isolated chicken eye test, 30 µl undiluted *N*-methylaniline was applied for 10 seconds followed by rinsing with 20 ml saline. The substance induced mild swelling of the cornea, mild to moderate opacity and mild fluorescein retention. The irritation score was calculated to be 65 of a maximum of 200. Therefore, the substance was assessed as causing irritation of the eyes (ECHA 2015).

5.4 Allergenic effects

In a valid local lymph node assay, *N*-methylaniline was not found to be sensitizing up to a concentration of 50%. In this study, the application of 10%, 25% or 50% *N*-methylaniline did not triple lymphocyte proliferation (ECHA 2015).

5.5 Reproductive and developmental toxicity

There are no data available.

5.6 Genotoxicity

5.6.1 In vitro

In vitro genotoxicity studies are shown in Table 4.

Table 4 Genotoxicity of *N*-methylaniline in vitro

End point	Test system	Concentration	Cytotoxicity	Result		Comments	References
				–m. a.	+m. a.		
bacteria							
gene mutation, pre-incubation	Salmonella typhimurium TA98	no data, in DMSO, purity: commercial grade (no other details)	no data	–	+ with norharman – without norharman	116 000 revertants/µmol	Wakabayashi et al. 1982; documentation "N-Methylaniline" 1993
	Salmonella typhimurium TA100	no data, in DMSO, purity: commercial grade (no other details)	no data	–	+ with norharman – without norharman	289 revertants/µmol	Wakabayashi et al. 1982; documentation "N-Methylaniline" 1993
gene mutation, plate incorporation	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	2.5–5000 µg/plate or up to cytotoxic concentrations, in DMSO, purity: commercial grade (no other details)	no data	–	–	3 plates/test, 2 replicates, the table only includes results at 50 µg/plate	Ho et al. 1981
gene mutation, plate incorporation	Salmonella typhimurium TA98, TA100	up to 5000 µg/plate, 5 concentrations, in DMSO, purity: 99.8%	up to 5000 µg/plate: –	–	–	increase in the number of revertants with S9 mix from hamsters, but not more than 2-fold	Le et al. 1985

Table 4 (continued)

End point	Test system	Concentration	Cytotoxicity	Result		Comments	References
				–m. a.	+m. a.		
gene mutation, pre-incubation	Salmonella typhimurium TA97, TA98, TA100, TA1535	–m. a.: up to 1000 µg/plate, +m. a.: up to 3333 µg/plate or up to cytotoxic concentrations, in DMSO, purity: 99%	+m. a. in the case of TA100 and TA1535: 3333 µg/plate and above, in the case of TA98: 10 000 µg/plate	–	–		Zeiger et al. 1988
gene mutation, pre-incubation	Salmonella typhimurium TA98, TA100, TA102, TA104, TA1535, TA1537, Escherichia coli WP2 uvrA, Escherichia coli WP2 uvrA/PKM101	0.0763–1250 µg/plate, in DMSO, purity: no data	no data	–	–	original study not available	NLM 2006
gene mutation, plate incorporation	Salmonella typhimurium TA98, TA100, TA1535, TA1537, Escherichia coli WP2 uvrA	–m. a.: 0, 156.3, 312.5, 625, 1250, 2500, 5000 µg/plate, +m. a.: 0, 312.5, 625, 1250, 2500, 5000 µg/plate, in DMSO, purity: 99.5%	5000 µg/plate	–	–	according to the Japanese guidelines Screening Mutagenicity Testing of Chemicals, 3 plates/test, 2 tests	NIHS Japan undated a, d

Table 4 (continued)

End point	Test system	Concentration	Cytotoxicity	Result		Comments	References
				−m. a.	+m. a.		
mammalian cells							
UDS	primary rat hepatocytes	0.001–1 mM (0.1–107 µg/ml), in DMSO, purity: commercial grade (no other details)	–	–	not determined		Yoshimi et al. 1988
CA	CHL	−m. a., 24 and 48 hours: 0, 300, 600, 1100 µg/ml, +m. a., 24 hours: 0, 300, 600, 1100 µg/ml, in DMSO, purity: 99.5%	growth inhibition: −m. a., 48 hours: cell growth in % of the controls: 140 µg/ml: 71%, 280 µg/ml: 54%, 550 µg/ml: 62%, 1100 µg/ml: 45%; −m. a., 6 hours: −; +m. a., 6 hours: 1100 µg/ml	+ 600 µg/ml and above, 24 hours: ≥ 15.0%[a)], 48 hours: ≥ 13.5%[a)]	+ 1100 µg/ml, 6 hours: 12.4%[a)]	according to the Japanese guidelines Screening Mutagenicity Testing of Chemicals, 2 plates/test, no induction of polyploid cells	NIHS Japan undated a, e

[a)] structural chromosomal aberrations: chromatid breaks, chromatid exchanges, without gaps

CA: chromosomal aberrations; CHL: Chinese hamster lung cells; DMSO: dimethyl sulfoxide; UDS: autoradiographic assay for induction of DNA repair synthesis

N-Methylaniline was not mutagenic in several tests in bacteria either with or without the addition of a metabolic activation system (NML 2006; Ho et al. 1981; Le et al. 1985; NIHS Japan undated a, d; Wakabayashi et al. 1982; Zeiger et al. 1988; documentation "N-Methylaniline" 1993). The substance induced mutagenicity only in the presence of norharman (Wakabayashi et al. 1982; documentation "N-Methylaniline" 1993).

The mutagenic effects induced by aniline with the addition of norharman were attributed to the mutagenic reaction product aminophenylnorharman rather than to aniline itself (supplement "Aniline" 2010). This is also assumed for *N*-methylaniline because aniline is a metabolite of *N*-methylaniline.

The results of the study of Shimizu and Takemura (1984) cited in the 1987 documentation (documentation "N-Methylaniline" 1993) agree with those obtained by Wakabayashi et al. (1982). As the data are not available in the original, they have not been included in the evaluation.

N-Methylaniline did not induce unscheduled DNA synthesis in primary rat hepatocytes (Yoshimi et al. 1988). In Chinese hamster lung cells, the substance caused structural chromosomal aberrations, such as chromatid breaks and chromatid exchanges, without and with the addition of a metabolic activation system at 600 µg/ml and above and at 1100 µg/ml, respectively (NIHS Japan undated a, e).

5.6.2 In vivo

There are no data available.

Summary

N-Methylaniline was not mutagenic in bacteria either with or without the addition of a metabolic activation system (Ho et al. 1981; Le et al. 1985; NIHS Japan undated a, d; NLM 2006; Wakabayashi et al. 1982; Zeiger et al. 1988; documentation "N-Methylaniline" 1993). In Chinese hamster lung cells, *N*-methylaniline caused structural chromosomal aberrations, such as chromatid breaks and chromatid exchanges, with and without the addition of a metabolic activation system (NIHS Japan undated a, e). Likewise, aniline induced chromosomal aberrations in CHO-K1 cells at 444 µg/ml and above without the addition of a metabolic activation system (supplement "Aniline" 2010).

Studies of in vivo genotoxicity are not available for *N*-methylaniline.

5.7 Carcinogenicity

Two studies in rats (US EPA 2005; White and Mori-Chavez 1952; documentation "N-Methylaniline" 1993) are not suitable for the evaluation of carcinogenicity because of the following shortcomings: inadequate documentation, no control animals, short exposure periods, small group sizes, the use of only one dose and a limited scope of histopathological examinations. A study in Swiss mice (Greenblatt et al. 1971; documentation "N-Methylaniline" 1993) is not suitable for the evaluation of carcinogenicity because only one organ, namely the lungs, was examined and the study period was too short.

There was evidence of *N*-nitrosomethylaniline formation after the oral administration of *N*-methylaniline together with sodium nitrite (documentation "N-Methylaniline" 1993). The List of MAK and BAT Values includes a footnote for *N*-methylaniline that draws attention to the fact that the reaction with nitrosating agents may lead to the formation of the carcinogenic *N*-nitrosomethylaniline.

Summary

The studies did not yield evidence of carcinogenicity; however, they are not suitable for the evaluation of the carcinogenic effects of *N*-methylaniline because of inadequate documentation or an inadequate treatment period.

6 Manifesto (MAK value/classification)

As is the case for aniline, methaemoglobin formation is the critical effect of *N*-methylaniline in humans.

In rats, erythrocyte toxicity in females and anaemic effects in males and females are the most sensitive end points of *N*-methylaniline.

MAK value. The MAK value for *N*-methylaniline should be derived from human data because, as in the case of aniline, the critical effect of *N*-methylaniline in humans is methaemoglobin formation. The MAK value for aniline was likewise derived from human data (supplement "Aniline" 1993; supplement "Aniline" 2010) because of the great differences between the species dogs, rats and humans with regard to the extent of methaemoglobin formation after inhalation exposure (SCOEL 2010). In addition, marked differences between species were found in the methaemoglobin reductase activity, which is responsible for the regeneration of the functioning haem from methaemoglobin. The activity of this enzyme is 5 and 10 times higher in the erythrocytes of rats and mice, respectively, than in human erythrocytes (EU 2004; Smith 1986).

A volunteer study with exposure to aniline at the level of the MAK value of 2 ml/m^3 demonstrated that humans are protected from adverse methaemoglobin formation at the workplace (Käfferlein et al. 2014). As the extrapolated methaemoglobin increment is 2.22 times lower than that permissible it is also taken into account that the respiratory volume of 10 m^3 at the workplace is higher than that in the volunteer study.

As none of the human data available for *N*-methylaniline can be used to derive a MAK value, data in rats are used for comparing the critical parameter of methaemoglobin formation. A comparison of the 28-day inhalation study with *N*-methylaniline (Rompetrol 2011) with the 14-day inhalation study with aniline (Pauluhn 2004) revealed the following:

- Methaemoglobin formation is a suitable parameter for comparing *N*-methylaniline with aniline because it is an "acute" and direct parameter; the different exposure periods used in the two studies are of only minor relevance. This pathophysiological assessment of the time course is substantiated by a flat time – response relationship for the methaemoglobin values induced by aniline taking into account the standard deviations.

- The sampling time is an important factor for determining methaemoglobin – in both studies, blood samples were taken shortly after or at the end of exposure (aniline: retro-orbitally; *N*-methylaniline: from the tip of the tail). In the case of aniline, the samples taken to determine the methaemoglobin levels were prepared within 5 minutes; in the case of methylaniline, the blood samples were specifically taken from the tip of the tail towards the end of exposure on the last day of exposure because of the short half-life of methaemoglobin; no data are provided regarding the preparation time after blood sampling. The methods are thus not identical, but they were adapted for the specific case of determining methaemoglobin. Thus, although a degree of uncertainty remains when comparing the methaemoglobin determinations of the two studies, it is only slight.
- By comparing the methaemoglobin levels after exposure to aniline at 116 mg/m^3 with those after exposure to *N*-methylaniline at 96.7 mg/m^3 and taking the baseline values (*N*-methylaniline: 6.4%–2.1% = 4.3%; aniline: 2.8%–1.2% = 1.6%; 4.3/1.6 = 2.7-fold) into account, *N*-methylaniline was found to be about 2.7 times more effective than aniline.

Considering this difference and based on the MAK value for aniline, a MAK value of 0.5 ml/m^3 is calculated for *N*-methylaniline in line with the preferred value approach.

In rats, the 28-day inhalation study carried out according to OECD Test Guideline 412 showed that the most sensitive end points were the toxic effects on the erythrocytes in females and the anaemic effects in males and females. The LOAEC for *N*-methylaniline was 13 mg/m^3 (2.9 ml/m^3); it was not possible to derive a NOAEC (Rompetrol SA 2011). On the basis of the LOAEC of 13 mg/m^3 and the data from rats, a MAK value of 0.1 ml/m^3 would be derived by taking into consideration a factor of 2 for the extrapolation from the LOAEC with slight effects to the NOAEC, a factor of 4 for time, a factor of 2 for the increased respiratory volume and a factor of 2 for the extrapolation from animals to humans. Thus, the MAK value would be far lower if it were derived from the data in rats.

Considered together, the extent of methaemoglobin formation, the reduction of methaemoglobin to haemoglobin by the NADH-dependent methaemoglobin reductase and the subsequent erythrocyte toxicity represent a multistage process. In addition, this effect is largely dependent on the availability of anti-oxidative substances (Pauluhn 2004). Glutathione reductase activities in human erythrocytes are about 5.5 times higher than those in rats; catalase activities are about 2.5 times higher (Godin and Garnett 1992). Unlike human haemoglobin, rat haemoglobin tetramer contains additional cysteines, which are localized at 125β and 13α; the first is exposed to a small extent and the latter is completely inaccessible in the polypeptide chain. Cys125β has a low pKa value and better accessibility, which increases its reactivity to a level that is about 3 orders of magnitude higher than human Cys93β. The higher reactivity of Cys125β is the reason why more HbSSG than GSSG is formed in rat erythrocytes under oxidative stress (Colombo et al. 2010). The MAK value should therefore not be derived from animal data, not only because of the species differences in methaemoglobin formation, but also because of the physiological differences in the erythrocytes of rats and humans.

Inhalation studies in rats revealed a 2.7-fold difference in the potency of *N*-methylaniline and aniline in forming methaemoglobin. Therefore, in view of the MAK

value of 2 ml/m³ for aniline, the MAK value of 0.5 ml/m³ for *N*-methylaniline is confirmed.

Peak limitation. Systemic effects are the critical effects for deriving the MAK value; therefore, *N*-methylaniline remains classified in Peak Limitation Category II. The half-life of the substance in human blood is not known. In line with the procedure of the Commission (see documentation "Limitation of exposure peaks and short term exposures" 2017), the default excursion factor of 2 has been retained.

Prenatal toxicity. No developmental toxicity studies are available for *N*-methylaniline. In 2006, aniline was classified in Pregnancy Risk Group C (supplement "Aniline" 2010). Although *N*-methylaniline is demethylated to aniline in the organism, metabolites without demethylation at the nitrogen atom are also possible, such as derivatives hydroxylated on the ring in the *o*-position or *p*-position (see Figure 1 in documentation "N-Methylaniline" 1993). A quantitative assessment of the metabolites cannot be made because studies of the toxicokinetics of *N*-methylaniline have not been performed. Conclusions can thus not be drawn by analogy to aniline. Therefore, classification in Pregnancy Risk Group D has been retained for *N*-methylaniline.

Carcinogenicity. None of the available studies of *N*-methylaniline are suitable for the evaluation of its carcinogenicity. *N*-Methylaniline was not mutagenic in bacteria either with or without the addition of a metabolic activation system. In Chinese hamster lung cells, *N*-methylaniline caused structural chromosomal aberrations both with and without the addition of a metabolic activation system. Studies of in vivo genotoxicity are not available.

As aniline is a metabolite of *N*-methylaniline, the carcinogenic potential of *N*-methylaniline was evaluated on the basis of the data for aniline.

In 2003, the toxicity, genotoxicity and carcinogenicity of monocyclic aromatic amino and nitro compounds were reviewed as a contribution to classify these substances in carcinogen categories. It was concluded that it is very unlikely that the carcinogenicity of *N*-methylaniline can be different from that of aniline (and of *N*,*N*-dimethylaniline, which is classified in Carcinogen Category 2) (documentation "Monocyclic aromatic amino and nitro compounds" 2005).

Aniline was classified in Carcinogen Category 4 in 2006. In animal studies, aniline caused splenic tumours in rats but not in mice. Numerous mechanistic studies suggest an indirect mechanism for tumourigenicity. Accordingly, the splenic tumours in rats have been causally related to the toxic effects of aniline on the erythrocytes and their sequelae. An increase in tumour incidences is therefore not expected if damage to the erythrocytes is avoided (supplement "Aniline" 2010). Marked species differences were found as regards the methaemoglobin reductase activity, which is responsible for the regeneration of the functioning haem from methaemoglobin. Reductase levels are higher in mice than in rats. Therefore, mice are considerably less susceptible than rats to substances that induce haematological changes and splenic lesions as a result of methaemoglobin formation (Srivastava et al. 2002).

In the case of aniline, the methaemoglobin-forming and haemolytic properties were induced mainly by the metabolites phenylhydroxylamine and nitrosobenzene (supplement "Aniline" 2010). Because of the indirect mechanism described above,

which results in splenic tumours developing in rats from damage to the erythrocytes, the metabolites phenylhydroxylamine and nitrosobenzene are primarily responsible for tumour formation. As toxicity studies found that aniline caused genotoxic effects only at doses that led to marked lesions of the haematopoietic system, it is assumed that genotoxicity is of subordinate relevance as long as the MAK value is not exceeded at the workplace (supplement "Aniline" 2010). Genotoxicity is of minor relevance both for aniline and *N*-methylaniline.

In the absence of toxicokinetic studies with *N*-methylaniline, a quantitative assessment of the extent of the formation of the toxic metabolites phenylhydroxylamine and nitrosobenzene from *N*-methylaniline cannot be made. However, a similar mechanism of action is assumed because there was evidence of the aniline metabolite nitrosobenzene and of aniline in the blood of dogs and cats (Holzer and Kiese 1960). A carcinogenic potential is therefore suspected for *N*-methylaniline and the substance is classified in Carcinogen Category 3B.

Germ cell mutagenicity. *N*-Methylaniline was not mutagenic in bacteria either with or without the addition of a metabolic activation system. In Chinese hamster lung cells, *N*-methylaniline caused structural chromosomal aberrations, such as chromatid breaks and chromatid exchanges, both with and without the addition of a metabolic activation system. Studies of in vivo genotoxicity are not available. In genotoxicity tests, aniline led to similar results and is not classified in a germ cell mutagen category (supplement "Aniline" 2010). *N*-Methylaniline has therefore not been classified in any of the categories for germ cell mutagens.

Absorption through the skin. *N*-Methylaniline is readily absorbed through the intact skin. Under standard conditions (exposure of 2000 cm^2 of skin for 1 hour), between 68 mg and 951 mg is absorbed from diluted aqueous solutions. Exposure to a concentration at the level of the MAK value of 0.5 ml/m^3 for 8 hours leads to the absorption of about 22 mg via inhalation. Absorption through the skin may thus substantially contribute to toxicity. Therefore, designation of *N*-methylaniline with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts) has been retained.

Sensitization. Clinical findings of sensitization were not obtained, but negative results are available from a valid local lymph node assay in mice. Therefore, *N*-methylaniline has not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

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completed December 11, 2014