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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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2-Nitro-4-methylaniline

No. 118

CAS No. 89-62-3

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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2-Nitro-4-methylaniline

1 Summary and assessment

2-Nitro-4-methylaniline is of low toxicity following single oral administration and single dermal application (LD_{50} rat oral > 5000 to 9890 mg/kg body weight; LD_{50} mouse oral > 3200 mg/kg body weight; LD_{50} rat dermal > 2000 mg/kg body weight; LD_{50} guinea pig dermal > 1000 mg/kg body weight), and the substance induces only slight methaemoglobin formation in rats and cats. In a study in rats, an oral LD_{50} of 1903 mg/kg body weight was found. On 4-week oral administration of 0 (controls), 40, 200 and 1000 mg/kg body weight to rats, 2-nitro-4-methylaniline caused minor anaemia in the females and males, starting at doses of 200 mg/kg body weight and 1000 mg/kg body weight, respectively. In the highest dose group there was an increase in the liver weights in both sexes and the females also had increased kidney weights, but there was no histopathological correlate in either case. The *no effect levels* are 200 mg/kg body weight and 40 mg/kg body weight for male and female rats, respectively.

In studies conducted in accordance with current relevant guidelines, the chemical has no primary irritant effect on the skin and the eye of the rabbit.

Salmonella/microsome assays with 2-nitro-4-methylaniline in the *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 1535, TA 1537, TA 1538, TA 2637 as well as *Escherichia coli* WP2uvrA have produced varying results. In the HPRT assay using Chinese hamster V79 cells 2-nitro-4-methylaniline did not prove genotoxic, whereas in a mouse lymphoma test with metabolic activation the substance caused gene mutations in a concentration-dependent manner. The in vitro DNA repair test in rat hepatocytes has not produced any evidence that 2-nitro-4-methylaniline causes damage to the DNA. Similarly, the results of a mouse micronucleus test were negative after intraperitoneal administration. In summary, 2-nitro-4-methylaniline possesses no relevant genotoxic potential.

Two cell transformation tests in mouse fibroblasts (BALB/3T3 and BALBc/3T3) produced positive results, whereas the result from a further such test was negative.

2 Name of substance

2.1	Usual name	2-Nitro-4-methylaniline
2.2	IUPAC name	1-Amino-2-nitro-4-methylbenzene
2.3	CAS No.	89-62-3
2.4	EINECS No.	201-924-9

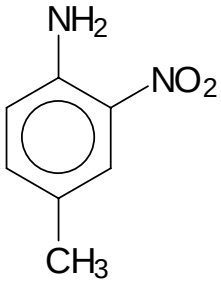
3 Synonyms, common and trade names

Amarthol Fast Red GL Base
Amarthol Fast Red GL Salt
1-Amino-2-nitro-4-methylbenzol
4-Amino-3-nitrotoluene
Azoamine Red A
Azobase NAT
Azoene Fast Red GL Salt
Azofix Red GL Salt
Azoic Diazo Component 8
Benzenamine, 4-methyl-2-nitro- (9CI)
C.I. 37110
Devol Red G
Devol Red Salt G
Diazo Fast Red GL
Fast Red Base GL
Fast Red Base JL
Fast Red G Base
Fast Red GL
Fast Red GL Base
Fast Red MGL Base
Fast Red 3NT Base
Fast Red 3NT Salt
HD Fast Red GL Base
Hiltonil Fast Red GL Base
Hiltosal Fast Red GL Salt
Lake Red G Base
Lithosol Scarlet Base M

Lithosol Scarlet Base MB
Lithosol Scarlet Base MBW
Lithosol Scarlet Base MW
4-Methyl-2-nitroanilin
4-Methyl-2-nitroaniline
Mitsui Red GL Base
MNPT
Naphthanil Red G Base
Naphtoelan Fast Red GL Base
3-Nitro-4-aminotoluene
3-Nitro-4-aminotoluol
2-Nitro-4-methylanilin
2-Nitro-4-toluidin
3-Nitro-4-toluidin
3-Nitro-p-toluidin
m-Nitro-p-toluidin
m-Nitro-p-toluidine
2-Nitro-p-toluidine
Ölbase
Ölbase TF
Red Base Ciba VII
Red Base Irga VII
Red Base NGL
Red G Base
Red G Salt
Red Salt Ciba VII
Red Salt Irga VII
Sanyo Fast Red GL Base
Shinnippon Fast Red GL Base
Tolabase Fast Red GL
p-Toluidine, 2-nitro- (8CI)
Toyo Fast Red GL Base

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_7H_8O_2N_2$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	152.15
5.2	Melting point, °C	114 (solidification temperature, dried) (Bayer, 1990 a) 116.3 (Lide and Frederikse, 1996) 117 (Booth, 1991)
5.3	Boiling point, °C	134 (at 2.6 hPa) (Booth, 1991)
5.4	Vapour pressure, hPa	2.96×10^{-5} (at 20 °C) 1.31×10^{-3} (at 50 °C) 4.02×10^{-3} (at 60 °C) (Bayer, 1990 a)
5.5	Density, g/cm³	1.16 (at 121 °C; melt) (Lide and Frederikse, 1996) 1.31 (at 15 °C) (Booth, 1991)
5.6	Solubility in water	ca. 0.16 g/l (at 20 °C) (Bayer, 1990 a)
5.7	Solubility in organic solvents	Soluble in ethanol and chloroform (Lide and Frederikse, 1996)
5.8	Solubility in fat	No information available
5.9	pH value	Ca. 6.5 at 0.1 g/l water (Bayer, 1990 a)
5.10	Conversion factor	1 ml/m³ (ppm) $\triangleq$ 6.21 mg/m³ 1 mg/m³ $\triangleq$ 0.16 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

As a diazo component in C.I. Pigment Yellow 1 and C.I. Pigment Red 3 (Booth, 1991).

7 Experimental results

7.1 Toxicokinetics and metabolism

No information available.

7.2 Acute and subacute toxicity

Acute toxicity

2-Nitro-4-methylaniline, formulated as a 25-percent suspension in starch gruel, was administered once by oral gavage to female SPF Wistar rats (weighing from 93 to 153 g) in doses ranging from 4000 to 15000 mg/kg body weight. Per dose 10 rats were used. The observation period was 7 days. The LD_{50} was determined as being 9890 mg/kg body weight. The clinical signs observed included lying on the abdomen and the side. The animals died after 2½ to 48 hours (Hoechst, 1967).

Five male Wistar-II rats (weighing from 160 to 180 g) were given 5000 mg 2-nitro-4-methylaniline/kg body weight once by oral gavage as a solution in polyethylene glycol 400. The observation period was 14 days. No symptoms or deaths occurred. Thus the LD_{50} was > 5000 mg/kg body weight (Bayer, 1978 a).

In a further study 2-nitro-4-methylaniline was administered to groups of 20 rats as single oral doses of 200 to 3200 mg/kg body weight in a 10-percent formulation in 0.5-percent guar gum. The symptoms observed included slight prostration, atypically coloured urine, mucous-like exudate around and over the eyes as well as stiffness, all of which were reversible after two days. The LD_{50} was found to be 1903 mg/kg body weight (ranging from 1355 to 2672 mg/kg body weight) (no further details; Eastman Kodak, 1979).

Another oral LD_{50} of 2-nitro-4-methylaniline reported for rats was given as 6860 mg/kg body weight (from 6470 to 7280 mg/kg body weight) (no further details; Marhold, 1972).

Mice (20 animals/group) received 2-nitro-4-methylaniline in single oral doses of 200 to 3200 mg/kg body weight (10 percent in guar gum). The symptoms observed included slight prostration and atypically coloured urine, both of which were reversible after one day. The LD_{50} was given as > 3200 mg/kg body weight (no further details; Eastman Kodak, 1979).

The acute dermal toxicity of 2-nitro-4-methylaniline (purity: 76.3%, 23.9% water) was tested in accordance with guideline 84/449/EEC. Groups of 5 male and 5 female Wistar rats (average weight at the beginning of the experiment was 248 and 209 g, respectively) were subjected once to occlusive exposure of the mechanically depilated dorsal and flank skin to 2000 mg/kg body weight as a mixture with Cremophor® EL (2:1). The exposure lasted for 24 hours and the post-exposure observation period was 14 days. No signs of systemic toxicity and no deaths occurred. In 2 female rats the application site was reddened from days 1 to 5 after application. Necropsy did not reveal any macroscopic findings. Thus the LD_{50} was > 2000 mg/kg body weight (Bayer, 1990 b).

For guinea pigs (3 animals/group) the dermal LD_{50} of solid, water-moistened 2-nitro-4-methylaniline was > 1000 mg/kg body weight following occlusive application. Slight local oedema and staining of the skin were seen 24 hours after application, and after one week desquamation and discoloration were observed, the latter persisting for two weeks (no further details; Eastman Kodak, 1979).

Methaemoglobin formation

Two groups of two male cats (weighing from 3.15 to 4 kg) each were treated by gavage with single oral doses of 25 mg and 100 mg 2-nitro-4-methylaniline/kg body weight as 0.625-percent and 2.5-percent suspensions in starch gruel, respectively. Methaemoglobin formation was investigated 1 hour, 3, 7 and 24 hours after administration. In the high dose group slight methaemoglobin formation of 1.4% and 1.8%, respectively, was observed 7 hours after administration (no further details; Hoechst, 1967).

In a study in rats 2-nitro-4-methylaniline also proved to possess only very low activity as a methaemoglobin former. Three rats were treated with a single oral dose of 1000 mg/kg body weight administered as 10-percent formulations in 0.5-percent guar gum. For comparison, two additional groups of 3 rats received m-dinitrobenzene (30 mg/kg body weight) and water, respectively. After 2 hours and 4 hours the methaemoglobin levels found in the animals treated with 2-nitro-4-methylaniline were 0.9% and 1.0%, respectively. The respective 2-hour and 4-hour levels in the m-dinitrobenzene group were 66.2 and 58.2%, compared with 0.1 and 0.5% in the water-treated group (no further details; Eastman Kodak, 1980).

Subacute toxicity

In a preliminary study to a 28-day study 3 male and 3 female Wistar rats received 2-nitro-4-methylaniline by oral gavage at a dose level of 1000 mg/kg body weight/day for 14 days. Both the males and females showed clinical signs including squatting posture, uncoordinated and ataxic gait, decreased spontaneous activity and mydriasis, which were reversible after 3 days. Body weight gain was not affected. At necropsy the size of the seminal vesicles in the males was found to be extremely reduced (no further details; Hoechst, 1993).

In the subsequent 28-day study conducted in accordance with OECD guideline No. 407 groups of 5 male and 5 female Wistar rats (Hoe:WISKf, initial age approx. 6 weeks) each received 2-nitro-4-methylaniline (purity: > 99%) by oral gavage as a suspension in sesame oil at daily (7 times per week) dose levels of 0 (controls), 40, 200 and 1000 mg/kg body weight. No deaths occurred. A symptom observed in the animals of the top dose group was increased salivation starting on day 21 of the study. Body weight gain and feed consumption were similar to the control group. The rats of the top dose group showed slightly increased consumption of drinking water and increases in urine volume from day 13 until the end of the study. The urine exhibited orange to reddish discoloration. In the top dose group both sexes showed decreases in erythrocyte counts, haemoglobin levels and haematocrit values, and in the mid dose group the female rats were found to have lowered haemoglobin levels and haematocrit values. The clinical chemistry results revealed that the males and females of the top dose group had elevated serum urea levels and that the females of the top dose group additio-

nally had elevated serum γ-glutamyl transpeptidase activity. The rats of the top dose showed increased absolute and relative liver weights (both sexes) and the female animals of this group had increased relative kidney weights in addition. Macroscopic and histopathological examination did not reveal any substance-related changes. The finding from the preliminary study that the seminal vesicles were extremely reduced in size (see above) was not reproducible in the main study. Thus the *no effect level* for male and female rats was 200 mg/kg body weight and 40 mg/kg body weight, respectively (Hoechst, 1993).

7.3 Skin and mucous membrane effects

The skin irritancy of 2-nitro-4-methylaniline (purity: 88.1%) was tested in 3 albino New Zealand rabbits (weighing from 2.7 to 3.6 kg) in accordance with OECD guideline No. 404. They were subjected to a single semi-occlusive 4-hour exposure of the mechanically depilated dorsal skin to 500 mg of the test substance (mixed into a paste with 0.45 ml of 0.9-percent saline solution). The skin was assessed 30 to 60 minutes as well as 24, 48 and 72 hours after removal of the patch. During the entire observation period no signs of irritation were seen. The surface of the skin only showed yellowish discoloration (Hoechst, 1989 a).

No irritation was observed in the two rabbits either which were studied after a single 24-hour exposure of the inner side of the ear to 500 mg 2-nitro-4-methylaniline followed by a 7-day post-exposure observation period (Bayer, 1978 b).

Groups of 5 rabbits (”Gelb-Silber”) were treated with 0.02 ml of oily suspensions containing 10, 5, 1, 0.01 and 0.001 percent 2-nitro-4-methylaniline by intracutaneous injection into the depilated skin of the flank. None of the injections elicited skin reactions. On application of 0.5 ml of 10-percent and 5-percent oily suspensions of 2-nitro-4-methylaniline 5 times on 5 days to the intact depilated skin of the flanks of 5 rabbits per group, desquamation of the skin was observed at the high concentration level at the end of the study, and the skin was slightly chapped in 2 out of the 5 rabbits. The 5-percent oily suspension did not cause to any signs of irritation (Hoechst, 1967).

The eye irritancy of 2-nitro-4-methylaniline (purity: 88.1%) was tested in 3 albino New Zealand rabbits (weighing from 3.0 to 4.3 kg) in accordance with OECD guideline No. 405. The animals received a single dose of 100 mg into the conjunctival sac of the eye. Assessment of the eyes was carried out 1 hour, 24, 48 and 72 hours following application. After one hour to 2 days the conjunctivae showed marked hyperaemia to the extent of a diffuse crimson-red colour and slight to marked swelling. In addition, in all 3 animals ocular discharge was observed which was discoloured on account of the test substance. The findings cleared up as of day 3 after instillation. The average numerical values were 0.33 for reddening of the conjunctiva and 0.0 for conjunctival swelling, iritis and clouding of the cornea (Hoechst, 1989 b). The substance therefore proved not to be irritating to the eye.

In a further study, no signs of irritation were seen following a single instillation of 50 mg 2-nitro-4-methylaniline into the conjunctival sac of the eye in 2 rabbits (White New Zealand) (Bayer, 1978 b).

On instillation of a few crystals of 2-nitro-4-methylaniline into the conjunctival sac of the rabbit eye slight irritation was observed, which cleared up after 48 hours. The observation period was 14 days (no further details; Eastman Kodak, year not specified).

To test the irritating potential to the eye, 0.1 ml of 10-percent and 5-percent oily suspensions of 2-nitro-4-methylaniline were instilled into the conjunctival sac of the rabbit eye. After 1 hour, 3, 7 and 24 hours the eyes were examined. No signs of irritation were observed (Hoechst, 1967).

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

No information available.

7.6 Genotoxicity

7.6.1 In vitro

2-Nitro-4-methylaniline (no indication of purity) was tested for mutagenic activity in the Salmonella/microsome assay (standard-plate incorporation test) using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The test concentrations were in the range from 0.5 to 5000 µg/plate. The highest concentration proved to be bacteriotoxic to strains TA 98 and TA 100. The revertant counts were not found to be increased either with or without metabolic activation. The test was not repeated for confirmation (LBI, 1979 a).

In a further Salmonella/microsome assay (standard-plate incorporation test) 2-nitro-4-methylaniline (no indication of purity) was investigated using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 as well as *Escherichia coli* WP2uvrA with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The concentrations used were in the range from 4 to 5000 µg/plate, with at least the highest concentration showing bacteriotoxic activity. The test substance proved to possess concentration-dependent mutagenic activity in strain TA 1537 in the presence of metabolic activation as well as in strains TA 98 and TA 1538 with and without metabolic activation (Hoechst, 1982).

A Salmonella/microsome assay with preincubation in the *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 1535, TA 1537, TA 1538 and TA 2637 as well as in *Escherichia coli* WP2uvrA and WP2uvrA/pKM (in the presence and the absence of norharman) gave a negative result (no further details; Shimizu and Takemura, 1984).

2-Nitro-4-methylaniline (no indication of purity) was tested in a further Salmonella/microsome assay with preincubation in the strains TA 98 and TA 100 with and without metabolic activation (S-9 mix from Kanechlor 500-induced rat liver). The concentrations ranged from 100 to 5000 µg/plate. In the TA 100 strain the substance tested negative and in strain TA 98 the result was weakly positive (no further details; Kawai et al., 1987).

In addition, 2-nitro-4-methylaniline (purity: 98%) was investigated using an HPRT test with an independent verification experiment carried out in Chi-

nese hamster V79 cells with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). Concentrations of 5, 15.8, 158 and 500 µg/ml were used. At the highest concentration level clearly visible precipitation of the test substance was observed. N-Methyl-N'-nitro-N-nitrosoguanidine (1 µg/ml) and 7,12-dimethylbenz[a]anthracene (10 µg/ml) served as positive control substances. The preincubation periods were 24 hours and 3 hours in the absence and the presence of metabolic activation, respectively. Neither in the absence nor in the presence of metabolic activation did 2-nitro-4-methylaniline increase the mutation frequency of V79 cells in this test system (Merck, 1997).

2-Nitro-4-methylaniline (no indication of purity) was also investigated in L5178Y mouse lymphoma cells with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). In the presence of metabolic activation concentrations ranging from 31.3 to 250 µg/ml were moderately toxic and caused a concentration-dependent increase in mutant frequency. A concentration of 500 µg/ml was cytotoxic. In the absence of metabolic activation the substance showed no effect up to the highest test concentration of 500 µg/ml (LBI, 1979 b). The experiment was not confirmed by repetition.

In the DNA repair test in primary rat hepatocytes, 2-nitro-4-methylaniline (no indication of purity) did not exhibit any damaging effect to DNA at concentration levels ranging from 10^{-6} M to 10^{-4} M (15.2 µg/ml) following a 20-hour incubation period. A concentration of 10^{-3} M (152 µg/ml) proved to be toxic (Yoshimi et al., 1988).

7.6.2 In vivo

In vivo 2-nitro-4-methylaniline (purity: 76.3%, 23.9% water) was studied in the mouse micronucleus test (OECD guideline No. 474). Groups of 5 male and 5 female NMRI-mice (weighing from 28 to 43 g) received a single intraperitoneal administration of 800 mg of the substance per kilogram body weight in a 0.5-percent Cremophor®/water emulsion. The dose was chosen on the basis of an initial range-finding toxicity study. Femoral bone marrow preparations were carried out 16, 24 and 48 hours after injection. The negative and positive controls (cyclophosphamide, 20 mg/kg body weight) were prepared after 24 hours. No alterations were seen in the ratio of poly-

chromatic to normochromatic erythrocytes at any time. At none of the preparation times were increased frequencies of micronucleated cells observed as compared with the negative control group. Thus no evidence was seen in this test to suggest a chromosome-damaging potential of 2-nitro-4-methylaniline (Bayer, 1990 c).

7.7 Carcinogenicity

In a cell transformation assay system using mouse fibroblasts (BALB/3T3) without metabolic activation, concentrations were tested in the range from 0.01 to 20.0 µg 2-nitro-4-methylaniline/ml (no indication of purity of the test substance). The concentrations were chosen on the basis of a preliminary experiment which showed concentration levels of more than 250 µg/ml to be lethal to the cells. The relative survival rates were 67.1% and 12% at 0.061 µg/ml and 125 µg/ml, respectively. At all concentration levels applied, transformation frequencies were significantly increased, with the highest values being observed at 1.0 and 20 µg/ml (0.73 and 0.79 foci/flask, respectively; the solvent control had 0.13 foci/flask and the positive control with 3-methylcholanthrene had 4.9 foci/flask). The survival rates in these concentration groups were 62% and 50%, respectively. 2-Nitro-4-methylaniline thus tested positive in this study (LBI, 1980 a).

A second experiment under the same experimental conditions, however, produced a negative result. In this case, 2-nitro-4-methylaniline (no indication of purity) was applied at concentration levels ranging from 0.1 µg/ml to 25.0 µg/ml. They were chosen on the basis of a preliminary cytotoxicity study in which concentrations of 250 µg/ml and above proved lethal to the cells. The relative survival rates were 79.4% and 10.2% at 0.12 µg/ml and 125 µg/ml, respectively. Up to a test substance concentration of 25.0 µg/ml no transformed foci were detected, whereas the positive controls showed 4.6 foci/flask (LBI, 1980 b).

In a further cell transformation test in the absence of a metabolic activation system 2-nitro-4-methylaniline (no indication of purity) was applied to BALBc/3T3 cells at concentration levels of 15 µg/ml to 220 µg/ml, which resulted in relative survival rates ranging from 87.2 to 31.5%. In a cytotoxicity study, however, a concentration of 250 µg/ml had caused a cell survival rate of as little as 5.8%. There was a significant concentration-dependent in-

crease in transformation frequency of 0.53 foci/flask at 15 µg/ml up to 3.13 foci/flask at 220 µg/ml (compared with the solvent (DMSO) control of 0.27 foci/flask and the 3-methylcholanthrene positive control of 5.4 foci/flask). The test substance exhibited a transforming potential in this study (Eastman Kodak, 1983).

7.8 Reproductive toxicity

No information available.

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

No information available.

8 Experience in humans

No information available.

9 Classifications and threshold limit values

No information available.

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