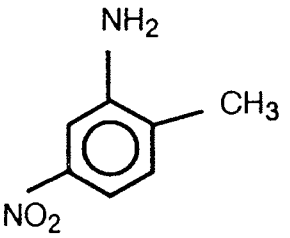
5-NITRO-*ortho*-TOLUIDINE

1. Chemical and Physical Data

1.1 Synonyms

Chem. Abstr. Services Reg. No.: 99-55-8
Chem. Abstr. Name: Benzenamine, 2-methyl-5-nitro-
IUPAC Systematic Name: 5-Nitro-*ortho*-toluidine
Colour Index No.: 37105
Synonyms: 1-Amino-2-methyl-5-nitrobenzene; 2-amino-4-nitrotoluene; azoic diazo component 12; 2-methyl-5-nitroaniline; 6-methyl-3-nitroaniline; 4-nitro-2-aminotoluene; 3-nitro-6-methylaniline; 5-nitro-2-methylaniline; 5-nitro-2-toluidine; PNOT

1.2 Structural and molecular formulae and molecular weight



$C_7H_8N_2O_2$ Mol. wt: 152.16

1.3 Chemical and physical properties of the pure substance

From Weast (1985), unless otherwise specified

(*a*) *Description*: Yellow monoclinic prisms (from ethanol)

(*b*) *Melting-point*: 107-108°C

(*c*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Pouchert, 1981, 1983, 1985; Weast & Astle, 1985).

(*d*) *Solubility*: Soluble in acetone, benzene, chloroform, diethyl ether and ethanol

1.4 Technical products and impurities

Trade Names: Amarthol Fast Scarlet G Base; Amarthol Fast Scarlet G Salt; Azoene Fast Scarlet GC Base; Azoene Fast Scarlet GC Salt; Azofix Scarlet G Salt; Azogene Fast Scarlet G; Dainichi Fast Scarlet G Base; Daito Scarlet Base G; Devol Scarlet B; Devol Scarlet G Salt; Diabase Scarlet G; Diazo Fast Scarlet G; Fast Red SG Base; Fast Scarlet G; Fast Scarlet G Base; Fast Scarlet GC Base; Fast Scarlet G Salt; Fast Scarlet J Base; Fast Scarlet J Salt; Fast Scarlet M 4NT Base; Fast Scarlet T Base; Hiltonil Fast Scarlet G Base; Hiltonil Fast Scarlet GC Base; Hiltonil Fast Scarlet G Salt; Kayaku Scarlet G Base; Lake Scarlet G Base; Lithosol Orange R Base; Mitsui Scarlet G Base; Naphthanil Scarlet G Base; Naphtoelan Fast Scarlet G Base; Naphtoelan Fast Scarlet G Salt; Scarlet Base Ciba II; Scarlet Base Irga II; Scarlet Base NSP; Scarlet G Base; Sugai Fast Scarlet G Base; Symulon Scarlet G Base

5-Nitro-*ortho*-toluidine is available commercially with a purity of 99% (Aldrich Chemical Co., 1988).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Several processes have been reported for the preparation of nitrotoluidines, including 5-nitro-*ortho*-toluidine. These include the reaction of nitrocresol with aqueous ammonia, the catalytic hydrogenation of aromatic nitro compounds in the presence of Raney copper, reacting aromatic nitro compounds with hydrogen sulfide in the presence of ammonia dissolved in dioxane, reacting 2,4-dinitrotoluene with hydrogen sulfide in a pyridine solution, and reacting 2,4-dinitrotoluene with carbon monoxide in the presence of cupric oxide and manganese dioxide (Scott, 1971).

5-Nitro-*ortho*-toluidine has also been synthesized by the nitration of *ortho*-toluidine and the monoreduction of 2,4-dinitrotoluene with alcoholic ammonium sulfide (Glinsukon *et al.*, 1975). It can be prepared by the electrolytic reduction of *ortho*-nitrotoluene to *ortho*-toluidine sulfate and subsequent nitration (Udupa *et al.*, 1984).

US production of 5-nitro-*ortho*-toluidine was reported to be 180 tonnes in 1972, with an additional 190 tonnes reported as azoic diazo component 12, salt (US Tariff Commission, 1974). US production of 5-nitro-*ortho*-toluidine in 1975 was reported to be 57 tonnes (US International Trade Commission, 1977).

No data were available on production elsewhere.

(*b*) *Use*

5-Nitro-*ortho*-toluidine has been used as an intermediate in the synthesis of Pigment Red 17 and Pigment Red 22. It has also been used as a precursor in the synthesis of a wide

assortment of azo dyes of various red, yellow, orange, violet and brown hues (National Cancer Institute, 1978).

As an azoic diazo component, 5-nitro-*ortho*-toluidine is used with naphthol derivatives to form azo dyes *in situ* on fabric and yarns. Dyeing with naphthol dyes takes place in two phases: the textile is first immersed in a solution of azoic coupling component, naphthol, and then allowed to react with an azoic diazonium component consisting of an aromatic amine converted first to a diazonium derivative (Priha *et al.*, 1988).

(*c*) *Regulatory status and guidelines*

No regulatory standard or guideline has been established for 5-nitro-*ortho*-toluidine.

2.2 Occurrence

(*a*) *Natural occurrence*

5-Nitro-*ortho*-toluidine is not known to occur as a natural product.

(*b*) *Occupational exposure*

No data were available to the Working Group.

(*c*) *Water and sediments*

5-Nitro-*ortho*-toluidine was identified as a product of the microbial transformation by *Mucrosporium* sp. of 2,4-dinitrotoluene, a compound common in water discharges and effluents from ammunition plants, ammunition loading facilities and sites for the destruction of stockpiled weapons (McCormick *et al.*, 1978). 5-Nitro-*ortho*-toluidine was identified as an intermediate in the anaerobic biotransformation of 2,4-dinitrotoluene added to a sample of municipal activated sludge (Liu *et al.*, 1984).

This compound was identified in samples of water effluent resulting from the continuous 2,4,6-trinitrotoluene manufacturing process. The concentration of 5-nitro-*ortho*-toluidine in samples collected over a one-year period from an effluent pipe ranged from 0.002-0.10 mg/l, with an occurrence rate of 7.6% (Spanggord *et al.*, 1982a).

(*d*) *Animals*

5-Nitro-*ortho*-toluidine is formed enzymatically from 2,4-dinitrotoluene by liver and lung microsomal fractions from mice (Schut *et al.*, 1985), by liver microsomal and cytosolic fractions from rats (Decad *et al.*, 1982; Mori *et al.*, 1984a) and by intestinal microorganisms from rats and mice (Guest *et al.*, 1982; Mori *et al.*, 1985).

5-Nitro-*ortho*-toluidine and its *N*-acetyl derivative were identified as minor metabolites of 2,4-^{14}C-dinitrotoluene when this compound was administered orally to male and female Fischer 344 rats at doses of 35, 63 or 100 mg/kg bw (Medinsky & Dent, 1983).

(*e*) *Humans*

Human intestinal microflora catalyse the reductive metabolism of 2,4-dinitrotoluene to 5-nitro-*ortho*-toluidine and 4-amino-2-nitrotoluene *via* nitroso intermediates (Guest *et al.*, 1982; Mori *et al.*, 1984b).

2.3 Analysis

A diazotization-coupling spectrophotometric technique has been applied to the determination of diverse aromatic amines, including 5-nitro-*ortho*-toluidine, using 8-amino-1-hydroxynaphthalene-3,6-disulfonic acid or *N*-(1-naphthyl)ethylenediamine as the coupling agent (Norwitz & Keliher, 1982).

A method for quantifying 5-nitro-*ortho*-toluidine in effluents from the production and purification of 2,4,6-trinitrotoluene involves gas chromatography with flame ionization for detection and gas chromatography with mass spectrometry for confirmation of identity (Spanggord *et al.*, 1982a).

The principal hazardous organic constituents in various streams from an incinerator facility, including 5-nitro-*ortho*-toluidine, can be detected by high-performance liquid chromatography with reverse-phase columns and ultraviolet spectrophotometric detection, with a detection limit of 1 ng (James *et al.*, 1983).

A method for the determination of 5-nitro-*ortho*-toluidine in activated sludge involves extraction of a broth sample with dichloromethane, transfer of the extract into hexane, evaporation and analysis by gas chromatography with flame ionization and electron capture detection (Liu *et al.*, 1984) .

5-Nitro-*ortho*-toluidine in air has been collected on a membrane filter, desorbed with water and analysed by high-performance liquid chromatography with ultraviolet detection, with a detection limit of 3 μg/sample (Eller, 1985).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Oral administration

Mouse: Groups of 49 or 50 male and 50 female B6C3F$_1$ mice, six weeks old, were fed 0.12% or 0.23% (time-weighted average concentration) 5-nitro-*ortho*-toluidine (considered to be of high purity) in the diet for 78 weeks and observed for up to 19-20 additional weeks. Groups of 50 males and 50 females served as untreated controls. Mean body weight depression was observed in treated mice of each sex; there was no significant difference in survival between treated and control animals. A significant increase in the incidence of hepatic tumours (all considered to be hepatocellular carcinomas; $p < 0.001$, Cochran-Armitage test) was observed in treated groups of each sex: in 12/50 control males, 12/44 low-dose males and 29/45 high-dose males; and in 2/47 control females, 7/46 low-dose females and 20/45 high-dose females (National Cancer Institute, 1978).

Rat: Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were fed 0.005% or 0.01% (time-weighted average concentration) 5-nitro-*ortho*-toluidine (considered to be of high purity) in the diet for 78 weeks and observed for up to 30 or 31 additional weeks.

Groups of 50 males and 50 females served as untreated controls. A slight depression in mean body weight was observed in high-dose males and in both high- and low-dose females; there was no significant difference in survival between the treated and control groups. A positive trend ($p = 0.039$, Cochran-Armitage test) in the incidence of hepatocellular carcinomas was observed in male rats (control, 0/47; low-dose, 0/41; high-dose, 3/46) (National Cancer Institute, 1978).

(*b*) *Intraperitoneal injection*

Mouse: In a screening study in strain A mice, based on the induction of lung tumours, groups of 20 female A/St mice, six to eight weeks of age, received intraperitoneal injections of 25, 50 or 100 mg/kg bw 5-nitro-*ortho*-toluidine [purity unspecified] in tricaprylin three times a week for eight weeks. A control group of 80 female mice was untreated and a group of 60 females received intraperitoneal injections of tricaprylin alone. All surviving animals were killed at 16 weeks, when lung adenomas were found in 8% of untreated controls, 11% of tricaprylin-treated controls, 18% of low-dose animals, 30% of mid-dose animals and 6% of high-dose animals (Maronpot *et al.*, 1986).

In a similar screening study, groups of 30 male A/J mice, six to eight weeks of age, received intraperitoneal injections of 40, 100 or 200 mg/kg bw 5-nitro-*ortho*-toluidine [purity unspecified] in corn oil three times a week for eight weeks. A control group of 20 male mice was untreated and a group of 30 males received intraperitoneal injections of corn oil alone. All surviving animals were killed at 16 weeks. No significant difference in the percentage of survivors with lung adenomas was observed compared to vehicle controls (Maronpot *et al.*, 1986).

3.2 Other relevant biological data

(*a*) *Experimental systems*

(i) *Absorption, distribution, excretion and metabolism*

No data were available to the Working Group.

(ii) *Toxic effects*

Methaemoglobin was detected in guinea-pigs and cats after intraperitoneal injection of 0.6-0.7 g/kg bw and 5-10 mg/kg bw 5-nitro-*ortho*-toluidine in vegetable oil, respectively (Reiter, 1948).

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Genetic and related effects* (see Appendix 1)

5-Nitro-*ortho*-toluidine was mutagenic to several strains of *Salmonella typhimurium* in the presence and absence of an exogenous metabolic system from Aroclor 1254-induced rat liver (Spanggord *et al.*, 1982b; Dunkel *et al.*, 1985; Couch *et al.*, 1987), mouse liver or Syrian hamster liver (Dunkel *et al.*, 1985). In one of the studies (Spanggord *et al.*, 1982b), positive results were obtained in TA1535 only in the absence of an exogenous metabolic system.

5-Nitro-*ortho*-toluidine was not mutagenic to *Escherichia coli* WP2*uvrA* in the presence or absence of an exogenous metabolic system (Dunkel *et al.*, 1985).

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

5-Nitro-*ortho*-toluidine is used as an intermediate in the production of a wide assortment of pigments and azo dyes. No data on occupational exposure levels were available.

4.2 Experimental carcinogenicity data

5-Nitro-*ortho*-toluidine was tested for carcinogenicity by oral administration in one strain of mice and in one strain of rats. It produced an increase in the incidence of hepatocellular tumours in mice of each sex and a marginal increase in the incidence of hepatocellular carcinomas in male rats.

4.3 Human carcinogenicity data

No data were available to the Working Group.

4.4 Other relevant data

5-Nitro-*ortho*-toluidine was mutagenic to bacteria in the presence and absence of an exogenous metabolic system.

4.5 Evaluation[1]

There is *limited evidence* for the carcinogenicity of 5-nitro-*ortho*-toluidine in experimental animals.

---

[1]For descriptions of the italicized terms and criteria for making the evaluation, see Preamble, pp. 25-29.

Summary table of genetic and related effects of 5-nitro-*ortho*-toluidine

Nonmammalian systems														**Mammalian systems**																													
Proka-ryotes		Lower eukaryotes				Plants			Insects				*In vitro*														*In vivo*																
													Animal cells								Human cells								Animals							Humans							
D	G	D	R	G	A	D	G	C	R	G	C	A	D	G	S	M	C	A	T	I	D	G	S	M	C	A	T	I	D	G	S	M	C	DL	A	D	S	M	C	A
	+																																								

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

In completing the table, the following symbol indicates the consensus of the Working Group with regard to the results for each endpoint:

\+ considered to be positive for the specific endpoint and level of biological complexity

No data were available from studies in humans on the carcinogenicity of 5-nitro-*ortho*-toluidine.

Overall evaluation

5-Nitro-*ortho*-toluidine is *not classifiable as to its carcinogenicity to humans (Group 3)*.

5. References

Aldrich Chemical Co. (1988) *1988-1989 Aldrich Catalog/Handbook of Fine Chemicals*, Milwaukee, WI, p. 1033

Couch, D.B., Abernethy, D.J. & Allen, P.F. (1987) The effect of biotransformation of 2,4-dinitrotoluene on its mutagenic potential. *Mutagenesis, 2*, 415-418

Decad, G.M., Graichen, M.E. & Dent, J.G. (1982) Hepatic microsomal metabolism and covalent binding of 2,4-dinitrotoluene. *Toxicol. appl. Pharmacol., 62*, 325-334

Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S. & Simmon, V.F. (1985) Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ. Mutagenesis, 7 (Suppl. 5)*, 1-248

Eller, P.M. (1985) *NIOSH Manual of Analytical Methods*, 3rd ed. (*DHHS (NIOSH) Publ. No. 84-1000*), Washington DC, US Government Printing Office, pp. 5013-1-5013-5

Glinsukon, T., Weisburger, E.K., Benjamin, T. & Roller, P.P. (1975) Preparation and spectra of some acetyl derivatives of 2,4-toluenediamine. *J. chem. Eng. Data, 20*, 207-209

Guest, D., Schnell, S.R., Rickert, D.E. & Dent, J.G. (1982) Metabolism of 2,4-dinitrotoluene by intestinal microorganisms from rat, mouse and man. *Toxicol. appl. Pharmacol., 64*, 160-168

James, R.H., Dillon, H.K. & Miller, H.C. (1983) Survey methods for the determination of principal organic hazardous constituents (POHCs). In: Schultz, D.W., ed., *Incineration and Treatment of Hazardous Waste: Proceedings of the Eighth Annual Research Symposium, Ft Mitchell, KY, 1982 (US EPA Report No. EPA-600/9-83-003; US NTIS PB83-210450)*, Cincinnati, OH, US Environmental Protection Agency, pp. 159-173

Liu, D., Thomson, K. & Anderson, A.C. (1984) Identification of nitroso compounds from biotransformation of 2,4-dinitrotoluene. *Appl. environ. Microbiol., 47*, 1295-1298

Maronpot, R.R., Shimkin, M.B., Witschi, H.P., Smith, L.H. & Cline, J.M. (1986) Strain A mouse pulmonary tumor test results for chemicals previously tested in the National Cancer Institute carcinogenicity tests. *J. natl Cancer Inst., 76*, 1101-1112

McCormick, N.G., Cornell, J.H. & Kaplan, A.M. (1978) Identification of biotransformation products from 2,4-dinitrotoluene. *Appl. environ. Microbiol., 35*, 945-948

Mcdinsky, M.A. & Dent, J.G. (1983) Biliary excretion and enterohepatic circulation of 2,4-dinitroltoluene metabolites in Fischer-344 rats. *Toxicol. appl. Pharmacol., 68*, 359-366

Mori, M.-A., Matsuhashi, T., Miyahara, T., Shibata, S., Izima, C. & Kozuka, H. (1984a) Reduction of 2,4-dinitrotoluene by Wistar rat liver microsomal and cytosol fractions. *Toxicol. appl. Pharmacol., 76*, 105-112

Mori, M.-A., Miyahara, T., Hasegawa, Y., Kudo, Y. & Kozuka, H. (1984b) Metabolism of dinitrotoluene isomers by *Escherichia coli* isolated from human intestine. *Chem. pharm. Bull. (Tokyo), 32*, 4070-4075

Mori, M.-A., Kudo, Y., Nunozawa, T., Miyahara, T. & Kozuka, H. (1985) Intestinal metabolism of 2,4-dinitrotoluene in rats. *Chem. pharm. Bull. (Tokyo), 33*, 327-332

National Cancer Institute (1978) *Bioassay of 5-Nitro-o-toluidine for Possible Carcinogenicity (CAS No. 99-55-8) (Technical Report No. 107; DHEW Publ. No. (NIH) 78-1357)*, Bethesda, MD, US Department of Health, Education, and Welfare

Norwitz, G. & Keliher, P.N. (1982) Spectrophotometric determination of aromatic amines by the diazotization-coupling technique with 8-amino-1-hydroxynaphthalene-3,6-disulfonic acid and *N*-(1-naphthyl)ethylenediamine as the coupling agents. *Anal. Chem., 54*, 807-809

Pouchert, C.J., ed. (1981) *The Aldrich Library of Infrared Spectra*, 3rd ed., Milwaukee, WI, Aldrich Chemical Co., p. 824

Pouchert, C.J., ed. (1983) *The Aldrich Library of NMR Spectra*, 2nd ed., Vol. 1, Milwaukee, WI, Aldrich Chemical Co., p. 1167

Pouchert, C.J., ed. (1985) *The Aldrich Library of FT-IR Spectra*, Vol. 1, Milwaukee, WI, Aldrich Chemical Co., p. 1365

Priha, E., Vuorinen, R., Schimberg, R. & Ahonen, I. (1988) *Tekstiilien Viimeistysaineet* (Textile Finishing Agents) (*Series on Working Conditions No. 65*), Helsinki, Institute of Occupational Health

Reiter, M. (1948) Methaemoglobin due to *p*-nitro-*o*-toluidine (Ger.). *Arch. exp. Pathol. Pharmakol., 205*, 327-331

Sadtler Research Laboratories (1980) *Standard Spectra Collection, 1980 Cumulative Index*, Philadelphia, PA

Schut, H.A.J., Dixit, R., Loeb, T.R. & Stoner, G.D. (1985) In vivo and in vitro metabolism of 2,4-dinitrotoluene in strain A mice. *Biochem. Pharmacol., 34*, 969-976

Scott, J.A. (1971) *Process for Preparing Toluidines (US Patent 3,578,713 (to Olin Corp.))*

Spanggord, R.J., Gibson, B.W., Keck, R.G., Thomas, D.W. & Barkley, J.J., Jr (1982a) Effluent analysis of wastewater generated in the manufacture of 2,4,6-trinitrotoluene. 1. Characterization study. *Environ. Sci. Technol., 16*, 229-232

Spanggord, R.J., Mortelmans, K.E., Griffin, A.F. & Simmon, V.F. (1982b) Mutagenicity in *Salmonella typhimurium* and structure-activity relationships of wastewater components emanating from the manufacture of trinitrotoluene. *Environ. Mutagenesis, 4*, 163-179

Udupa, H.V., Venkatachalapathy, M.S., Chidambaram, S.I., Srinivasan, R.K. & Balasubramanian, L. (1984) *An Electrochemical Process for the Production of 2-Amino 4-Nitro Toluene from o-Nitro Toluene (Indian Patent 154,403)*

US International Trade Commission (1977) *Synthetic Organic Chemicals, US Production and Sales, 1975 (USITC Publication 804)*, Washington DC, US Government Printing Office, p. 49

US Tariff Commission (1974) *Synthetic Organic Chemicals, US Production and Sales, 1972 (TC Publication 681)*, Washington DC, US Government Printing Office, pp. 21, 59

Weast, R.C., ed. (1985) *CRC Handbook of Chemistry and Physics*, 66th ed., Boca Raton, FL, CRC Press, p. C-526

Weast, R.C. & Astle, M.J. (1985) *Handbook of Data on Organic Compounds*, Boca Raton, FL, CRC Press, p. 380