

2-Methoxy-4-nitroaniline

97-52-9

OVERVIEW

2-Methoxy-4-nitroaniline came to the attention of the NCI Division of Cancer Biology following a review of chemicals that do not meet the criteria for inclusion in the United States (U.S.) Environmental Protection Agency (EPA) HPV Challenge Program even though their production volume in 1998 exceeded 1 million pounds. According to industry information, 10 - 1,000 metric tons of 2-methoxy-4-nitroaniline were also produced or imported annually in the European Union in the timeframe of 1990 to 1994.

2-Methoxy-4-nitroaniline is used in dyeing textiles such as cotton, as a dye in the printing industry, and as an intermediate in the synthesis of azo dyes that have applications in tattoo inks, emulsion paints, and toy enamels. Dye manufacture and textile dyeing involve batch processing, a type of operation that can be difficult to control. Worker exposure to dye dust is particularly likely during manual transfer of powder dyes. There is limited evidence of the carcinogenicity of the structurally related chemical, 2-methoxy-5-nitroaniline (BG Chemie, 1995a; IARC, 1982). The International Agency for Research on Cancer (IARC) has listed the structurally related chemical, *o*-anisidine, as a Group 2B chemical (IARC, 1999). Despite these findings, large quantities of 2-methoxy-4-nitroaniline appear to be in use worldwide.

Given the concerns about other anisidines, findings that 2-methoxy-4-nitroaniline is mutagenic when metabolically activated raises concerns about the carcinogenic potential of this compound. 2-Methoxy-4-nitroaniline was mutagenic to *Salmonella typhimurium* and *Escherichia coli* and produced chromosome aberrations in Chinese hamster lung cells. The urine of rats administered 2-methoxy-4-nitroaniline also produced mutations in the TA98 strain of *S. typhimurium*. Toxicity prediction programs have projected that 2-methoxy-4-nitroaniline is a carcinogen in rodents, but there is no actual data on the chronic toxicity or carcinogenicity of this chemical. In a 28-day repeat dose study in rats, histopathological examination showed myocardial necrosis. It has also been suggested that 2-methoxy-4-nitroaniline produces methemoglobinemia.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

In comments provided on January 25, 2006, Dr. John Walker supplied the following post-meeting information on Interagency Testing Committee (ITC) activities regarding 2-methoxy-4-nitroaniline. This chemical had production volumes greater than and less than a million pounds in 1998 and 2002, respectively. In its 56th Report (70 FR 61520, October 24, 2005), the ITC stated that it had not determined whether to conduct a data-availability study on approximately 237 chemicals that were high production chemicals only in the 1998 Inventory Update Rule (IUR), but not in the 1990, 1994, or 2002 IURs. IUR data for 2006 will be available later this year.

DATA GAPS IDENTIFIED BY NCI

Given the significant potential for human exposure to 2-methoxy-4-nitroaniline and the suspicion of carcinogenic activity based on structure-activity relationships, the following studies are warranted:

- A complete toxicological characterization in a chronic rodent study, with interim evaluations of this chemical's effects on preneoplastic lesions and the blood. Particular emphasis should be given to the heart as a potential target organ.
- An environmental assessment to determine the extent that 2-methoxy-4-nitroaniline is released into the environment and the hazards associated with this release.

NOMINATION OF 2-METHOXY-4-NITROANILINE TO THE NTP

Based on a review of the available literature and the recommendations of the Chemical Selection Working Group (CSWG) on December 15, 2005, NCI nominates 2-methoxy-4-nitroaniline for testing by the National Toxicology Program (NTP) and forwards the following information:

- The attached Summary of Data for Chemical Selection
- Copies of references cited in the Summary of Data for Chemical Selection and Dr. Walker's post-meeting comments
- CSWG recommendations to:
 - (1) Conduct a complete toxicological characterization in a chronic rodent study, with

interim evaluations of this chemical's effects on preneoplastic lesions and the blood.
(2) Conduct developmental and reproductive effect testing.

The CSWG assigned the priority for testing this chemical as high and requested that special attention be given to concerns about potential cardiotoxicity and hepatotoxicity as well as carcinogenicity.

Comments:

Because the CSWG also requested that an environmental assessment as outlined under Data Gaps be conducted, the NCI is forwarding this request to the ITC for action.

Although the NCI is now aware that the ITC has not made a decision regarding a data availability study because of uncertainties regarding current production levels, this would not influence the request for carcinogenicity testing by the NTP. The potential for worker exposure in the past is amply demonstrated and chemically-induced cancer is a disease with a very long latency.

Testing protocols developed by NTP should be done in consultation with the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health to ensure that the information collected is adequate to protect workers in the future and provide monitoring of previously exposed workers, as needed by the testing results.

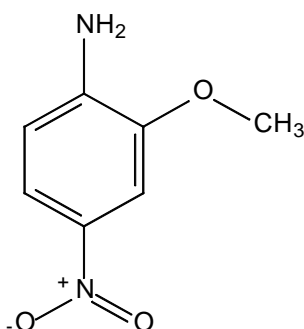
Should industry have unpublished data available on the toxicity of 2-methoxy-4-nitroaniline, this information may be submitted to the NTP as a public comment during the nomination process.

SUMMARY OF DATA FOR CHEMICAL SELECTION

CHEMICAL IDENTIFICATION

<u>CAS Registry Numbers:</u>	97-52-9
<u>Chemical Abstracts Service Name:</u>	Benzenamine, 2-methoxy-4-nitro- (9CI)
<u>Synonyms and Trade Names:</u>	2-Methoxy-4-nitroaniline; 2-amino-5-nitroanisole; Azoamine Pink O; C.I. 37125; C.I. azoic diazo component 5; EINECS 202-588-6; Fast Red B Base; 4-nitro- <i>o</i> -anisidine (ChemFinder, 2004; ChemIDplus, 2004)
<u>Structural Class:</u>	Substituted benzenamine

Structure, Molecular Formula, and Molecular Weight:



C₇H₈N₂O₃

Mol. wt.: 168.15

Chemical and Physical Properties:

<u>Description:</u>	Deep yellow powder (Sigma-Aldrich MSDS, 2004)
<u>Melting point:</u>	141 °C (Lide, 2005)
<u>Solubility:</u>	Slightly soluble in water; soluble in alcohol, acetone, and DMSO (BG Chemie, 1995b; Fisher Scientific MSDS, 2003; Lide, 2005)
<u>Density/Specific Gravity:</u>	1.211 @ 20 °C (BG Chemie, 1995b)

Reactivity: Incompatible with acids, acid anhydrides, acid chlorides, chloroformates, and strong oxidizing agents. Hazardous decomposition products include carbon monoxide, carbon dioxide, nitrogen, and nitrogen oxides (Fisher Scientific MSDS, 2003; Sigma-Aldrich MSDS, 2004)

O/W Partition Coefficient: 0.71 (predicted) (Accelrys, Inc., 2004)

Technical Products and Impurities: 2-Methoxy-4-nitroaniline is available from Fisher Scientific and Sigma-Aldrich and at a purity of 99% and 98%, respectively (Fisher Scientific, 2005; Sigma-Aldrich, 2005).

EXPOSURE INFORMATION

Production and Producers:

Manufacturing Process. Two methods for the production of 2-methoxy-4-nitroaniline were found in the literature. The first method produces this compound by nitration of *o*-acetanisidide with subsequent hydrolysis (Chudgar, 1992). The second process utilizes acetylation and subsequent nitration of 2-methoxyaniline to form an intermediate, which is then deacetylated to form 2-methoxy-4-nitroaniline (Mitchell & Waring, 2003).

Producers and Importers. Chemical Sources International (2004) lists ten U.S. suppliers of 2-methoxy-4-nitroaniline. ChemACX lists four U.S. suppliers selling four products; online vendors include Alfa Aesar, Lancaster, Oakwood, and TCI (ChemACX, 2005).

According to recent issues of chemical directories, 2-methoxy-4-nitroaniline is manufactured or distributed in the U.S. by Aceto Corporation; City Chemical LLC; and SK Energy & Chemical (ChemBuyersGuide.com, 2005; Chemweek Buyer's Guide, 2005; OPD Search, 2005). There are over 20 international producers of 2-methoxy-4-nitroaniline located mainly in China and India (DWCP, 2004).

Production/Import Levels:

2-Methoxy-4-nitroaniline is listed in the EPA Toxic Substances Control Act (TSCA) Inventory (ChemIDplus, 2004).

The EPA's Inventory Update Rule reports nonconfidential production ranges of chemicals every four years. The production levels of 2-methoxy-4-nitroaniline during 1986 to 2002 are listed in Table 1.

Table 1. Production Levels of 2-Methoxy-4-nitroaniline

Year	Production Range (lbs.)
1986	10,000 - 500,000
1990	10,000 - 500,000
1994	> 500,000 - 1,000,000
1998	> 1,000,000 - 10,000,000
2002	> 500,000 - 1,000,000

Source: EPA (2005a)

2-Methoxy-4-nitroaniline is listed as an LPV chemical in the European Union, meaning that annual production of 10 - 1,000 metric tons was reported and the chemical was produced or imported between 1990 and 1994. European producers are Bayer AG, CHEMAG Aktiengesellschaft, Cerdec Corporation, Clariant GmbH, DyStar, ROHNER AG, and Wychem Limited (DWCP, 2004; European Chemicals Bureau, 2005).

A search for the amount of 2-methoxy-4-nitroaniline imported into the U.S. was conducted using the Port Import/Export Reporting Service (PIERS) database. For the 17-month period from April 2004 to August 2005, the Port Import/Export Reporting Service (PIERS) database reported 4 imports of 2-methoxy-4-nitroaniline and 4 imports of Fast Red B Base with a combined cargo weight of 299,297 pounds. Given the substantial use in the U.S. and the number of overseas producers, it is likely that this value underestimates the actual amount imported since it may be imported under its Color Index name and/or one or more of its many common or trade names (Dialog Information Services, 2005).

2-Methoxy-4-nitroaniline is shipped in 25 kg plastic bags or 100 kg drums (Hengshui Taocheng Chemical Auxiliary Co., Ltd, 2005).

Use Pattern:

2-Methoxy-4-nitroaniline has applications in dyeing textiles such as cotton and is a chromogenic agent in printing. It is mainly used as an intermediate in the manufacture of Pigment Yellow 74 (C.I. 11741). Pigment Yellow 74 is utilized in yellow tattoo inks,

emulsion paints, toy enamels, printing inks, and is a component of pigment pastes for paper and adhesives. A major market for Pigment Yellow 74 powder is traffic paints (BG Chemie, 1995b; Colour Index, 2005; Cui *et al.*, 2004; Hengshui Taocheng Chemical

Auxiliary Co., Ltd., 2005; HSDB, 2003a; Merck Safety Data Sheet, 2004; Sunye Chemical Co., Ltd., 2005).

An examination of the internet suggests that 2-methoxy-4-nitroaniline may have a minor use in histological staining (GCC Diagnostics, 2005).

A search for 2-methoxy-4-nitroaniline or 5-nitro-2-anisidine in the U.S. patent database from 1976 to September 6, 2005 indicated that 159 patents cited one of these chemicals with the majority of patents pertaining to dyes, pigments, or inks (U.S. Patent and Trademark Office, 2005).

Human Exposure:

Occupational Exposure. In dye handling operations, the manual transfer of powder dyes from bulk containers to smaller process containers can generate significant amounts of dust. Workers in powder dye handling operations are often poorly protected from dust exposures. Most powder dyes are shipped in drums that range in height from 30 to 36 inches. When manually transferring dye from these drums, many workers must lean forward and place their heads inside the drum to scoop out dye near the bottom. In this position, the worker is greatly exposed to airborne dye dust, even in a ventilated booth. Worker exposure to dye dust through breathing or skin contact can result in adverse health effects such as occupational asthma, eczema, and allergic reactions (NIOSH, 1998).

2-Methoxy-4-nitroaniline, as cited above, is an intermediate in the synthesis of the azo dye, Pigment Yellow 74, but it may also be used to manufacture acid dyes. The production of intermediates and dyes is carried out in bomb-shaped reaction vessels with capacities of 500-10,000 gallons. The final stage of dye manufacturing is grinding or milling, which typically generates a considerable amount dust, although well-established methods have

been implicated to control this problem (Gregory, 1993). Another potential source of exposure to 2-methoxy-4-nitroaniline in the workplace is during the dyeing of textile materials.

The National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 198 workers, including 80 female workers, were potentially exposed to 2-methoxy-4-nitroaniline in the workplace (RTECS, 2003). Mixing and blending machine operators and medical scientists were the occupations identified as having exposure to this compound (NOES, 2005a). The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed therein.

Since the production volume of 2-methoxy-4-nitroaniline appears to have increased significantly after the NOES information was collected, this survey may not accurately reflect exposure to this compound at the present time. New applications for this chemical in the dyes, textiles, and printing industries would result in additional sources of occupational exposure to 2-methoxy-4-nitroaniline.

Production of Pigment Yellow 74, an unsponsored addition to the 1994 HPV Challenge List, may also result in exposure to 2-methoxy-4-nitroaniline since 2-methoxy-4-nitroaniline is used as an intermediate to produce Pigment Yellow 74 (EPA, 2005b; Hengshui Taocheng Chemical Auxiliary Co., Ltd, 2005).

NOES estimates that 54,867 workers including 11,681 females were exposed to Pigment Yellow 74. Individuals working in the following industries potentially had the most exposure to Pigment Yellow 74: apparel and other textile products, printing and publishing, wholesale trade - durable goods, and special trade contractors (NOES, 2005b).

Consumer Exposure. No data were found in the available literature documenting consumer exposure to 2-methoxy-4-nitroaniline. The general population may be exposed to small quantities of this chemical from its use in consumer end products such as textiles and

printing inks, particularly if the dye is capable of leaching from the consumer product (e.g., from washing clothing containing this dye).

Environmental Exposure. Human exposure to 2-methoxy-4-nitroaniline may occur from its release into the environment during the manufacture, use, and disposal of this product and products made from this compound (e.g., Pigment Yellow 74).

Environmental Occurrence:

2-Methoxy-4-nitroaniline has not been reported to occur naturally. No information was found in the available literature identifying this compound in environmental media. However, no studies appear to have been conducted; some quantity contained in wastewater from the production of Pigment Yellow 74 and from the production and use of 2-methoxy-4-nitroaniline would be expected.

Although no quantitative data on the ecological effects of this product are available, no appreciable bioaccumulation potential is expected (Merck, 2004).

Regulatory Status:

No standards or guidelines have been set by NIOSH or the Occupational Safety and Health Administration (OSHA) for occupational exposure to or workplace allowable levels of 2-methoxy-4-nitroaniline. 2-Methoxy-4-nitroaniline was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made.

2-Methoxy-4-nitroaniline is not regulated under SARA Section 302 (Extremely Hazardous Substances), SARA Section 313 (Toxic Chemical Release Inventory), or CERCLA Section 103 (Hazardous Substances) (Fisher Scientific MSDS, 2003).

TOXICOLOGICAL INFORMATION

Human Data:

No epidemiological studies or case reports investigating the association of exposure to 2-methoxy-4-nitroaniline with cancer risks in humans were identified in the available literature.

Twenty-six Indian dye-stuff workers with contact dermatitis underwent patch testing with 0.2% solutions of various dyestuffs. Fourteen of the 26 workers showed positive reactions, with only one individual showing sensitization to 2-methoxy-4-nitroaniline. This individual also gave positive reactions to two other chemicals, 5-chloro-*o*-anisidine hydrochloride and *m*-chloroaniline hydrochloride (Mathur *et al.*, 1985).

Animal Data:

Acute Toxicity. The LD₅₀ values for 2-methoxy-4-nitroaniline are given in Table 2.

Table 2. Acute Toxicity Values for 2-Methoxy-4-nitroaniline

Species	Route	LD₅₀ (mg/kg)
Rat	Oral	997
Male rat	Oral (given in PEG 400)	2,000
Female rat	Oral (given in PEG 400)	1,260
Sprague-Dawley rat	Dermal	> 2,000

PEG = polyethylene glycol

Source: BG Chemie, 1995b; RTECS, 2003

The irritant effects of 2-methoxy-4-nitroaniline were examined in several assays in rabbits. These assays were conducted in accordance with FDA guidelines or with OECD guideline No. 404. 2-Methoxy-4-nitroaniline was evaluated as not irritating. The irritant effects of 2-methoxy-4-nitroaniline on the rabbit eye was also conducted in accordance with FDA guidelines and OECD guideline No. 405. This chemical was evaluated as not irritating (BG Chemie, 1995b).

2-Methoxy-4-nitroaniline failed to induce a sensitization reaction in Dunkin-Hartley guinea pigs in the Magnusson and Kligman maximization test. Induction treatment involved an intradermal injection of a 7.5 percent (w/w) solution in Alembicol D, a coconut oil triglyceride, and dermal application at 75 percent in water, with challenges of 75 and 40 percent solutions (w/w) in distilled water (BG Chemie, 1995b).

Prechronic/Subchronic Studies. In a 28-day repeat dose study, five male and female Crj:CD rats were gavaged with 0, 30, 100, or 300 mg/kg 2-methoxy-4-nitroaniline in corn oil daily. Animals were sacrificed on either day 29 or 43. Decreases in hematocrit levels, hemoglobin levels, red blood cell counts, and increases in platelet and reticulocyte counts were observed in high-dose males and females; increases in total cholesterol, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and total bilirubin levels were also noted. At the highest dose, treated males and females had slightly lower mean body weights and absolute thymus weights but had increases in absolute and relative spleen and relative liver weights compared to controls. Histopathological examination showed increases in myocardial necrosis and extramedullary hematopoiesis in the spleen in high-dose males and females. Pigment deposits in the spleen of high-dose females were also noted (Global Information Network on Chemicals, 2005).

Chronic/Carcinogenicity Studies. No 2-year carcinogenicity studies on 2-methoxy-4-nitroaniline in animals were identified in the available literature.

Short-term Tests:

A search of the literature revealed that the genotoxicity of 2-methoxy-4-nitroaniline has been evaluated in a number of test systems. A summary of these studies is listed in Table 3.

Table 3. *In vitro* Genotoxicity Studies of 2-Methoxy-4-nitroaniline

Test system	Study details	Result	Reference
<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537 and <i>E. coli</i> WP2 uvrA	Preincubation, with rat liver S-9, 313-5,000 µg/plate	Positive in TA 100, TA1535, TA98, TA1537 & <i>E. coli</i>	Global Information Network on Chemicals, 2005
<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537 and <i>E. coli</i> WP2 uvrA	Preincubation, without rat liver S-9, 313-5,000 µg/plate	Positive in TA100, TA98, & TA1537	Global Information Network on Chemicals, 2005
<i>S. typhimurium</i> TA98	Standard, with and without rat liver S-9, 100-500 µg/plate	Positive	Koovi <i>et al.</i> (1987) as cited in CCRIS, 2005a
<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	Plate incorporation, with rat liver S-9, 0.8-2,500 µg/plate	Positive	Hoechst Res Tox, 1986
<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	Plate incorporation, without rat liver S-9, 0.8-2,500 µg/plate	Negative	Hoechst Res Tox, 1986
Chinese hamster V79 cells	HPRT test, with and without rat liver S-9, 50-350 µg/ml	Negative	BG Chemie, 1995b
Chinese hamster lung cells	Chromosomal aberration assay, 6 hr treatment, 18 hr recovery and 24 hr and 48 hr continuous treatment, w/o rat liver S-9	Negative	Kusakabe <i>et al.</i> , 2002
Chinese hamster lung cells	Chromosomal aberration assay, 6 hr, 18 hr recovery, with rat liver S-9	Structural changes	Kusakabe <i>et al.</i> , 2002

The lyophilized urine of treated rats given an i.p. dose of 15 mg 2-methoxy-4-nitroaniline was more mutagenic in strain TA98 than the urine of untreated rats with and without S-9. The investigators also included the metabolites of 2-methoxy-4-nitroaniline in their study and concluded that the reduction of the nitro group increased mutagenic activity, while O-demethylation and increasing N-acetylation weakened or abolished mutagenic activity (BG Chemie, 1995b).

Metabolism:

Following an i.p. injection of 2-methoxy-4-nitroaniline at a dose of 20 mg/rat in male and female Wistar rats, metabolites identified in urine collected over a 48-hour period included 2,5-diacetylamino-1-methoxybenzene (~2.5%), 2,5-diacetylamino-phenol, 2-amino-5-

nitrophenol (~0.4%), and traces of 2-acetylamino-1-methoxy-5-nitrobenzene and 2-acetylamino-1-methoxy-5-aminobenzene (BG Chemie, 1995b).

The metabolism prediction program, METEOR, was used to determine potential breakdown products of 2-methoxy-4-nitroaniline. METEOR indicated that two possible metabolic pathways were the oxidative O-demethylation to form 2-amino-5-nitrophenol or the reduction of the aromatic nitro group to form 2,5-diaminoanisole (LHASA Ltd., 2004).

Other Biological Effects:

Methemoglobin formation. A Material Safety Data Sheet lists the blood as a target system of 2-methoxy-4-nitroaniline and notes that absorption into the body leads to the formation of methemoglobin, which at sufficient concentrations causes cyanosis (Sigma-Aldrich MSDS, 2004). However, an unpublished report cited in the BG Chemie evaluation of 2-methoxy-4-nitroaniline states that no methemoglobin formation or increases in Heinz bodies were detectable in a cat 3, 7, and 24 hours after oral administration of 10 mg/kg body weight of the substance (purity at least 60 percent) in polyethylene glycol. Insufficient information was available to resolve this discrepancy.

Enzyme induction. 2-Methoxy-4-nitroaniline, following an i.p. injection of 0.11 or 0.22 mmol/kg into male F344 rats, selectively increased the levels of CYP1A2 protein in the livers of rats; the levels of CYP1A1 were also elevated but to a lesser extent. Northern blot analysis showed that this compound induced the expression of CYP1A2 mRNA (Degawa *et al.*, 1995).

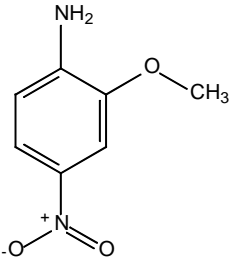
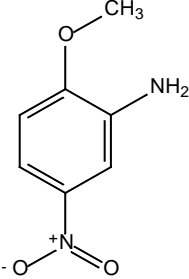
Structure/Activity Relationships:

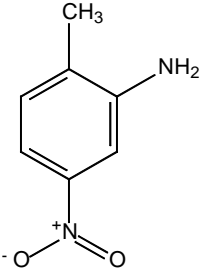
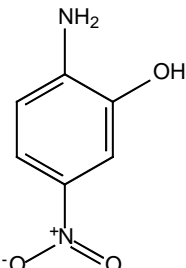
Several dye intermediates that are structurally related to 2-methoxy-4-nitroaniline have been tested for carcinogenicity and produced positive results in mice and rats. Metabolites of 2-methoxy-4-nitroaniline were also chosen as representatives to assess the mutagenicity and carcinogenicity of 2-methoxy-4-nitroaniline. The chemical structure similarity search tool in ChemIDplus was also utilized in finding additional chemicals for analysis.

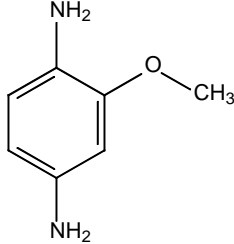
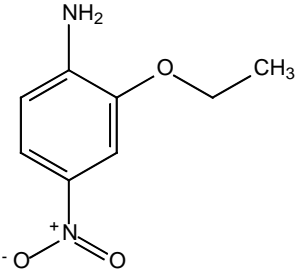
Information on the carcinogenicity and genotoxicity of these selected compounds is

summarized in Table 4.

Table 4. Toxicity Information on Chemicals Structurally Related to 2-Methoxy-4-nitroaniline

Compound/CAS No.	Mutagenicity	Carcinogenicity
<p>2-Methoxy-4-nitroaniline (CAS No. 97-52-9)</p> 	<p>Positive in <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 w/ S-9; mixed results w/o S-9 (Global Information Network on Chemicals, 2005; Hoechst Res Tox, 1986; Koovi <i>et al.</i>, 1987 as cited in CCRIS 2005a)</p> <p>Positive in <i>E. coli</i> WP2 uvrA w/ S-9 (Global Information Network on Chemicals, 2005)</p> <p>Negative in the HPRT test in Chinese hamster V79 cells w/wo S-9 (BG Chemie, 1995b)</p> <p>Positive for CA in Chinese hamster lung cells w/ S-9; negative w/o S-9 (Kusakabe <i>et al.</i>, 2002)</p>	<p>No data found</p>
<p>5-Nitro-<i>o</i>-anisidine (CAS No. 99-59-2)</p> 	<p>Positive in <i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537 with and without S-9 (NTP, 2005a)</p> <p>Positive in the <i>Drosophila</i> SLRL test (NTP, 2005a)</p> <p>Positive for SCE in CHO cells w/ S-9 but negative w/o S-9; negative for CA in CHO cells w/wo S-9 (NTP, 2005a)</p> <p>Negative for CA in Chinese hamster V79 cells (BG Chemie, 1995a)</p> <p>Negative for UDS in primary rat hepatocytes (BG Chemie, 1995a)</p>	<p>F344 rats (50/dose/sex) were fed diets of 0.4 and 0.8% for 78 weeks and observed for an additional 28 weeks. Male rats had an increased incidence of skin tumors and tumors of the Zymbal gland. Female rats had an increased incidence of carcinomas of the Zymbal gland, the clitoral gland, and the skin of the ear (NTP, 2005a)</p> <p>B6C3F₁ mice (50/sex/dose) were fed diets of 0.8 and 1.6%, but after 15 weeks, the high-dose group was lowered to 0.4%. After 78 weeks of administration, mice were observed up to an additional 19 weeks. Female mice had a significant increase in the incidence of hepatocellular carcinomas. A marginal increase in liver tumors was observed in male mice (NTP, 2005a)</p>

Compound/CAS No.	Mutagenicity	Carcinogenicity
<p>5-Nitro-2-toluidine (CAS No. 99-55-8)</p> 	<p>Mixed results in <i>S. typhimurium</i> TA98 and TA100 w/wo S-9 (Couch <i>et al.</i>, 1987, Dunkel <i>et al.</i>, 1985 & Sayama <i>et al.</i>, 1991 as cited in CCRIS, 2005d; HSDB, 2003b; NTP, 2005b)</p> <p>Negative in <i>E. coli</i> WP2 uvrA w/wo S-9 (Dunkel <i>et al.</i>, 1985 as cited in CCRIS, 2005d)</p> <p>Mixed results for morphological transformations in Syrian hamster embryo cells (Holen <i>et al.</i>, 1990; Kerckaert <i>et al.</i>, 1998)</p> <p>Positive for CA and SCE in CHO cells w/ S-9 but negative w/o S-9 (NTP, 2005b)</p>	<p>F344 rats (50/dose/sex) were fed diets of 0.005 and 0.01% for 78 weeks and observed for an additional 30 to 31 weeks. No association between treatment and tumor incidence was found (NCI/NTP, 1978; NTP, 2005b)</p> <p>B6C3F₁ mice (50/sex/dose) were fed diets of 0.12 and 0.23% for 78 weeks and observed up to an additional 20 weeks. Hepatocellular carcinomas and hemangiosarcomas were increased in both sexes (NCI/NTP, 1978; NTP, 2005b)</p>
<p>2-Amino-5-nitrophenol (CAS No. 121-88-0)</p> 	<p>Mixed results in the Ames assay w/wo S-9 (Gee <i>et al.</i>, 1998, Koovi <i>et al.</i>, 1987, NCI/NTP, 1988 & Zeiger <i>et al.</i>, 1987 as cited in CCRIS, 2005c)</p> <p>Positive in the mouse lymphoma assay (NCI/NTP, 1988 as cited in CCRIS, 2005c)</p> <p>Negative in the dominant lethal test in rats (GENETOX, 1995)</p> <p>Negative for CA in <i>Neurospora crassa</i> (GENETOX, 1995)</p> <p>Positive for CA and SCE in CHO cells w/wo S-9 (NTP, 2005c)</p>	<p>F344 rats (50/sex/dose) were gavaged with 0, 100, or 200 mg/kg for 2 years. Mean body weights were decreased in both sexes and survival of male rats was significantly lower than controls, with the high-dose group being insufficient to detect the presence of neoplasms. The incidence of pancreatic acinar cell adenomas was increased in low-dose male rats and a marginal increase in the combined incidences of preputial or clitoral gland adenomas and carcinomas occurred in low-dose males and females, respectively. Neoplasms of the intestinal tract were observed but were not considered to be a result of chemical exposure (NTP, 2005c)</p> <p>B6C3F₁ mice (50/sex/dose) were gavaged with 0, 400, or 800 mg/kg for 2 years. Mean body weights were affected by treatment and the poor survival of high-dose animals of both sexes were inadequate to evaluate a carcinogenic response. No increase in tumor incidence was found in low-dose animals (NTP, 2005c)</p>

Compound/CAS No.	Mutagenicity	Carcinogenicity
<p>2,5-Diaminoanisole (CAS No. 5307-02-8)</p> 	<p>Positive in <i>S. typhimurium</i> TA98 w/wo S-9 (Koovi <i>et al</i>, 1987 as cited in CCRIS, 2005b)</p> <p>Negative in the dominant lethal test in rats (GENETOX, 1991)</p> <p>Positive for inducing UDS (HSDB, 2002)</p> <p>Sulfated form of this compound produced mixed results in <i>Saccharomyces cerevisiae</i> (Mayer & Goin, 1980)</p>	<p>No data found</p>
<p>2-Ethoxy-4-nitrobenzenamine (CAS No. 16383-89-4)</p> 	<p>No data found</p>	<p>No data found</p>

CA = chromosomal aberration; CHO = Chinese hamster ovary cell; SCE = sister chromatid exchange; UDS = unscheduled DNA synthesis

Two SAR-based computer software programs were used as tools to assess the toxicity of 2-methoxy-4-nitroaniline. One program, named TOPKAT, uses robust, cross-validated models based on experimental data to calculate a probability value from 0.0-1.0 that a chemical will be positive for a certain endpoint. This program also incorporates a validity diagnostic that indicates if the predicted toxicity values may be accepted with confidence. Another SAR-based model, DEREK, uses structure alerts to predict the toxicity of a compound. The toxicity predictions made for 2-methoxy-4-nitroaniline by TOPKAT and DEREK are shown in Table 5.

Table 5. Toxicity Predictions for 2-Methoxy-4-nitroaniline Using SAR-based Programs

Toxicity Endpoint	Toxicity Prediction
TOPKAT	
Carcinogenicity (male rat, NTP model)	1.000 - Probable
Carcinogenicity (female rat, NTP model)	1.000 - Probable
Carcinogenicity (male mouse, NTP model)	0.996 - Probable
Carcinogenicity (female mouse, NTP model)	0.946 - Probable
Carcinogenicity (male rat, FDA model)	0.000 - Unlikely
Carcinogenicity (female rat, FDA model)	0.872 - Probable
Carcinogenicity (male mouse, FDA model)	0.907 - Probable
Carcinogenicity (female mouse, FDA model)	0.000 - Unlikely
Weight of Evidence Carcinogenicity Call	1.000 - Probable
Mutagenicity in the Ames assay	0.999 - Probable
Developmental Toxicity	0.001 - Unlikely
Skin Irritation	0.908 - Probable
Skin Sensitization	0.931 - Probable
DEREK	
Carcinogenicity	Plausible for mammalian carcinogenicity
Mutagenicity	Plausible for mutagenicity in the Ames assay
Skin Sensitization	Plausible as mammalian skin sensitizer

Source: Accelrys, Inc., 2004; LHASA Ltd., 2004

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