5-Nitro-ortho-anisidine and its hydrochloride: Human health tier II assessment

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Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Benzenamine, 2-methoxy-5-nitro-	99-59-2
Benzenamine, 2-methoxy-5-nitro-, monohydrochloride	67827-72-9

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to

human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemical, benzenamine, 2-methoxy-5-nitro-, monohydrochloride (CAS No 67827-72-9), is the hydrochloride salt of benzenamine, 2-methoxy-5-nitro- (CAS No 99-59-2). These two chemicals, benzenamine, 2-methoxy-5-nitro- (CAS No 99-59-2) and benzenamine, 2-methoxy-5-nitro-, monohydrochloride (CAS No 67827-72-9), are considered together in this assessment report. The speciation of these chemicals in biological fluids will be dependent on pH of the fluid, but independent of the original form.

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

International

The following international uses have been identified through the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; Galleria Chemica; the United States Environmental Protection Agency's (US EPA) Aggregated Computer Toxicology Resource (ACToR); the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and various international assessments (International Agency for Research on Cancer (IARC), 1982; Otutu, 2012; Colour Index).

The chemical has reported site-limited use as an intermediate in the manufacture of dyes and pigments.

Restrictions

Australian

No known restrictions have been identified.

International

No known restrictions have been identified.

Existing Worker Health and Safety Controls

Hazard Classification

The chemical is not listed on the Hazardous Chemicals Information System (HCIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

No hazard data are available for the hydrochloride salt of the parent chemical (CAS No 67827-72-9). However, the data for the parent chemical (CAS No 99-59-2), which will be referred to as 'the chemical' in the **Health Hazard Information** section, is considered relevant for the hazard assessment of the hydrochloride salt due to the structural similarity of these two chemicals. However, the hydrochloride salt may have different properties with regards to local irritation effects.

Toxicokinetics

The chemical is relatively lipophilic and is therefore likely to be absorbed through the epidermis. Since the chemical is sparingly soluble in water; it is unlikely to be cleared in the body.

The toxicity of aromatic amines arises from metabolic activation, including N-hydroxylation and N-acetylation to yield alkoxy amines. Metabolic activation leads to increased methaemoglobin levels in the blood, reducing the capacity of oxygen to bind to haemoglobin and resulting in hypoxia (IARC, 2010).

Acute Toxicity

Oral

Based on limited data available, the chemical has the potential to have low to moderate acute toxicity from oral exposure. The minimal reporting of the data is not sufficient to determine whether hazard classification for the chemical is warranted.

The LD50 was determined to be 2250 mg/kg bw in rats (PubChem; REACH). The LD50 in mouse was identified as 1060 mg/kg bw (HSDB). The IARC (1982) identified the median lethal dose (LD50) to be 704 mg/kg bw in rats. No other details were provided for these studies.

Dermal

No data are available.

Inhalation

No experimental data are available for the chemical.

The acute inhalation median lethal concentration (LC50; 4-hour) was estimated to be 218.23 mg/L based on Quantitative Structure-Activity Relationship (QSAR) modelling (REACH).

Corrosion / Irritation

Skin Irritation

No experimental data are available for the chemical.

The primary dermal irritation index (PDI), determined using QSAR modelling, was 3.2, indicating that the chemical was moderately irritating to the skin (REACH).

Eye Irritation

No experimental data are available for the chemical.

The mean maximum average (MMA) irritation score, estimated using QSAR modelling, was 24.5 indicating that the chemical was slightly irritating to the eyes (REACH).

Sensitisation

Skin Sensitisation

No human and animal data are available for this chemical.

The chemical contains a mechanistic structural alert for skin sensitisation (Michael-type addition) using the profiling functionality of the Organisation for Economic Co-operation and Development (OECD) QSAR Application Toolbox v.3.3.

Repeated Dose Toxicity

Oral

The chemical is not considered to cause serious damage to health from repeated oral exposure.

In dose range-finding studies conducted in Fischer 344 (F344) rats (n=50/sex/dose) and B6C3F1 mice (n=50/sex/dose), the chemical was administered in the diet at up to 8000 mg/kg bw/d in rats and up to 8000 mg/kg bw/d in mice for seven weeks. Decreased bodyweight gains (less than 10 %) were observed in both species. Effects in the liver, thyroid, gastric mucosa, spleen, and testes were observed in mice administered 8000 mg/kg bw/d (NCI, 1978; IARC, 1982).

Dermal

No data are available. Considering that the chemical is likely to be absorbed through the skin, repeat dose toxicity via dermal application cannot be ruled out. However, the toxicity results from oral application (see **Repeat dose toxicity** section) indicate that the chemical is not likely to have high toxicity.

Repeated dermal exposure to the chemical may lead to a change in haematological and hepatic parameters in rats (TOXNET).

Inhalation

No data are available.

Genotoxicity

Based on the limited data available for genotoxicity, a conclusion on the genotoxicity of the chemical cannot be made.

In vitro

In vitro genotoxicity studies showed both positive and negative results for the chemical:

- negative in an a bacterial reverse mutation assay in *Salmonella typhimurium* strains TA 100 and TA 1535 without metabolic activation at doses up to 3333 µg/plate (REACH);
- positive in an a bacterial reverse mutation assay in *S. tymphimurium* strain TA 98 without metabolic activation. Doses were not specified (IARC, 1982);
- negative in a sister chromatid exchange assay in Chinese hamster ovary (CHO) cells with metabolic activation, at 4000 µg/mL (REACH);
- positive in a sister chromatid exchange assay in CHO cells without metabolic activation, at 4000 µg/mL (REACH);
- negative in a mammalian chromosome aberration assay in CHO cells at doses up to 4000 µg/mL (REACH).

In vivo

Negative and equivocal results were found in a sex-linked recessive lethal (SLRL) assay following exposure of *Drosophila melanogaster* to the chemical (purity 99.9 %) in 1 % Tween-60 solvent (Zimmering et al., 1989).

Carcinogenicity

The IARC has classified the chemical as Group 3 (Not classifiable as to its carcinogenicity to humans), based on inadequate evidence of carcinogenicity from experimental animals. No studies are available to show carcinogenic effects in humans.

Mice (B6C3F1; n=50/sex/dose) were treated with the chemical in the diet at doses of 4000 mg/kg for 63 weeks, 8000 mg/kg for 78 weeks and 16000 mg/kg for 15 weeks. All the animals were observed 18 weeks after treatment. A statistically significant increase in the incidence of hepatocellular carcinomas was observed in both male and female groups. It was noted, however; that the incidences of liver tumours were within historical range for controls (IARC, 1982).

In a study conducted in F344 rats (n=50/sex/dose), the chemical was administered in the diet at concentrations of 0, 4000 or 8000 mg/kg bw/d) in rats for 78 weeks. Observation continued up to 28 weeks after dosing period. Increased incidences in tumours of the integumentary system were observed in rats. Increases in basal cell adenocarcinomas and sebaceous adenocarcinomas occurred in the skin of the high dose male rats. Significant increases in adenomas were observed in the clitoral gland of female rats. Carcinomas of the Zymbal's gland or on the skin of the ear were significant in high dose groups of males and females (NCI, 1978; IARC, 1982).

It was noted that for both the mice and rat studies, the control and exposed animals were received in different shipments and that low dose groups were from a different commercial source than the controls (IARC, 1982). Given that oral administration in rats produced skin tumours and the study in mice was considered inadequate for evaluation, an evaluation of the carcinogenic effect of the chemical could not be made (IARC, 1982).

The chemical contains a mechanistic structural alert for carcinogenicity (Nitro-aromatics) using the profiling functionality of the Organisation for Economic Co-operation and Development (OECD) QSAR Application Toolbox v.3.3. Nitroaniline derivatives, which includes the chemicals in this assessment, can be metabolically activated to reactive electrophiles as an initial step in a carcinogenic mechanism of action. This usually involves activating N-hydroxylamine metabolites and their enzymatic reaction, and eventually formation of the pro-carcinogenic nitrenium ions. The highly reactive nitrenium ions covalently bind to DNA, provided that they are sufficiently stable to not undergo further reactions immediately. The stability of the nitrenium ion is correlated with mutagenicity, for example in an Ames test with metabolic activation (Benigni & Bossa, 2011). However, the stability of the nitrenium ion depends on the type of substituents and the isomeric position of the reactive groups (Vance & Levin, 1984; Shimizu & Yano, 1986; Assman et al., 1997). This makes the determination of the carcinogenic potential of any tri-substituted aromatic amines unclear. The carcinogenic potential of the chemicals in this assessment will be addressed in the recommended Tier III assessment for 'Azo dyes that cleave to aromatic amines of potential toxicological concern' (NICNAS) (see **NICNAS Recommendation** section).

Reproductive and Developmental Toxicity

No data are available.

Risk Characterisation

Critical Health Effects

Given that the parent chemical was reported to be of toxicological concern due to its carcinogenicity potential (Bruschweiler et al., 2014). The critical health effect for risk characterisation includes the possibility of carcinogenicity.

Public Risk Characterisation

The chemicals could be used as an intermediate in the manufacture of dyes and pigments (see **International use** section) which may be used in textile dyes and it may then be regenerated by reductive cleavage of the azo dyes. As such, further regulatory controls for public health may be determined as part of a Tier III assessment for 'Azo dyes that cleave to aromatic amines of potential toxicological concern' (NICNAS).

Occupational Risk Characterisation

Based on the available data, the chemicals may be hazardous to human health. As such, further regulatory controls for worker health may be determined as part of a Tier III assessment.

NICNAS Recommendation

The chemicals are recommended for a Tier III assessment as part of the assessment of 'Azo dyes that cleave to aromatic amines of potential toxicological concern' (NICNAS).

Regulatory Control

Public Health

The need for regulatory control for public health will be determined as part of the Tier III assessment.

Work Health and Safety

The need for regulatory control for worker health will be determined as part of the Tier III assessment.

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal and inhalation exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker’s health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;

- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals has not been undertaken as part of this assessment.

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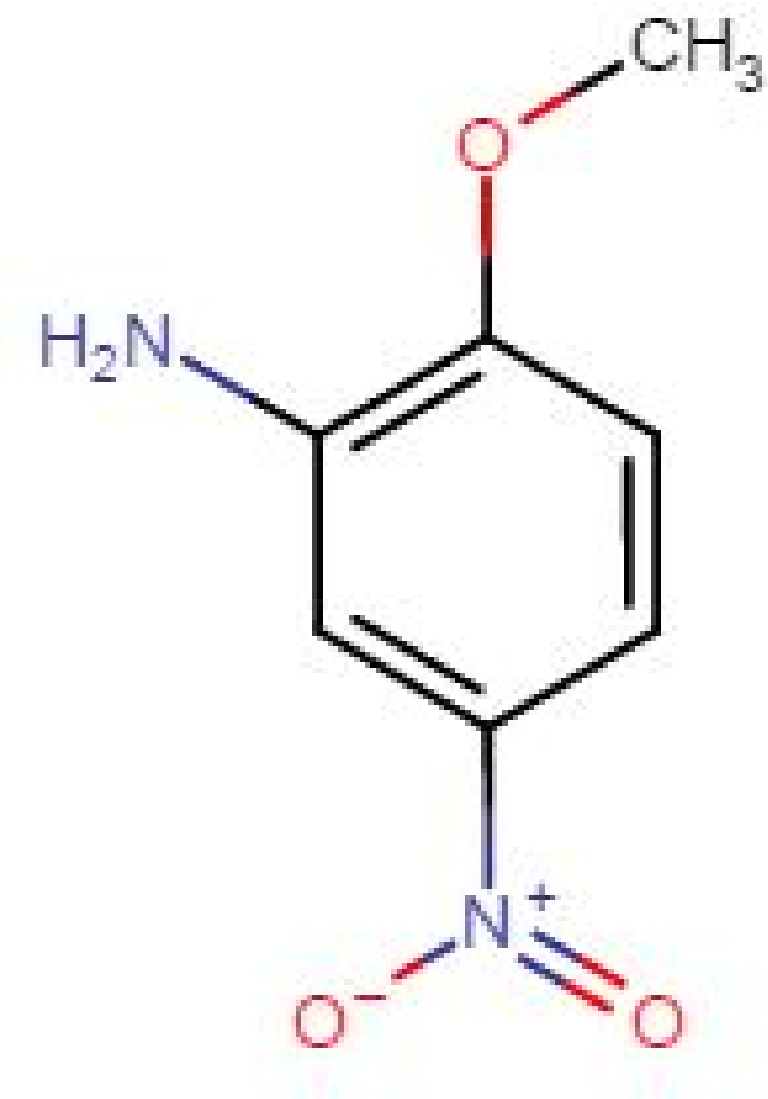
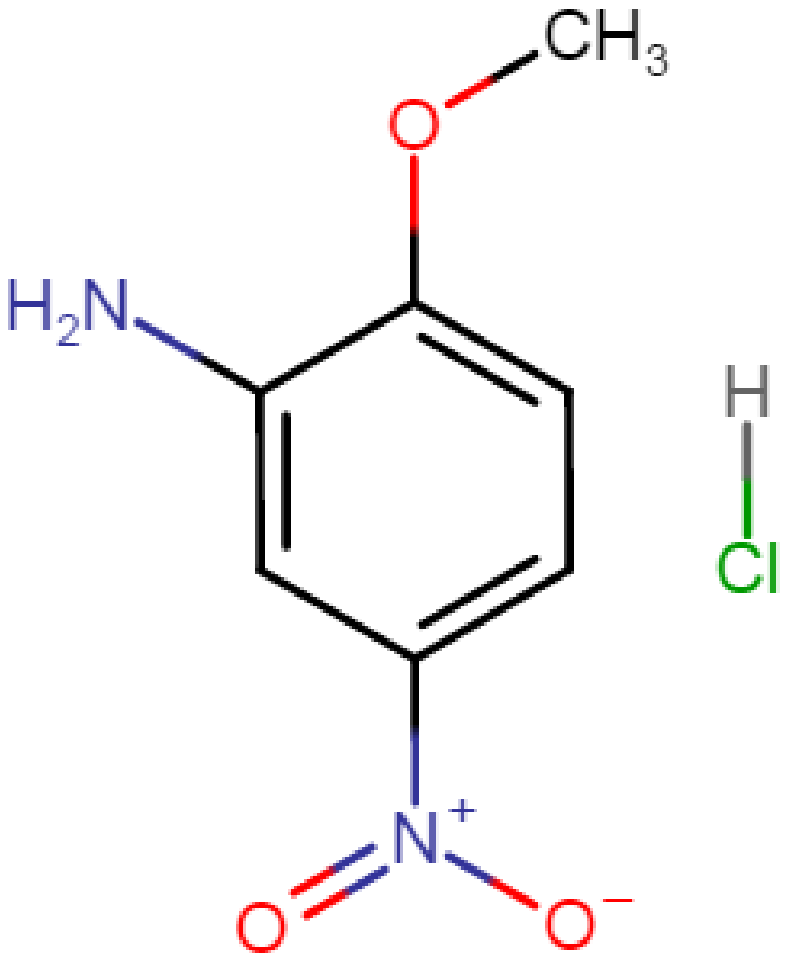
Chemical Identities

Chemical Name in the Inventory and Synonyms	**Benzenamine, 2-methoxy-5-nitro-** 5-nitro-o-anisidine 2-methoxy-5-nitroaniline
CAS Number	99-59-2
Structural Formula	

	
Molecular Formula	C7H8N2O3
Molecular Weight	168.15

Chemical Name in the Inventory and Synonyms	**Benzenamine, 2-methoxy-5-nitro-, monohydrochloride** 2-Methoxy-5-nitrobenzenamine, hydrochloride
CAS Number	67827-72-9
Structural Formula	

	
Molecular Formula	C7H8N2O3.ClH
Molecular Weight	204.61

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