Benzenamine, 2-methoxy-4-nitro-: Human health tier II assessment

10 March 2017

CAS Number: 97-52-9

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.

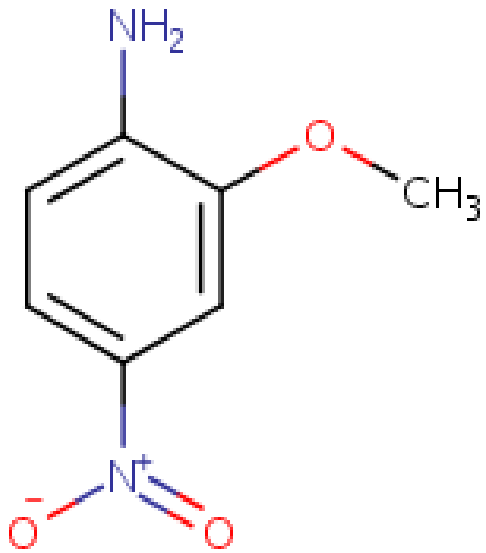
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	4-nitro-o-anisidine 2-methoxy-4-nitroaniline
Structural Formula	
Molecular Formula	C7H8N2O3
Molecular Weight (g/mol)	168.151
Appearance and Odour (where available)	Deep yellow powder
SMILES	c1(N)c(OC)cc(N(=O)=O)cc1

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 Substances and Preparations in Nordic countries (SPIN) database; the United States (US) Environmental Protection Agency's (EPA) Aggregated Computer Toxicology Resource (ACToR); and the nomination information for the chemical from the US National Toxicology Program (NTP) database.

The chemical has reported commercial uses including:

- as a textile dye;
- as a chromogenic agent in printing; and
- as a component of pigment pastes for paper and adhesives.

The chemical has reported site-limited use as an intermediate in the manufacture of Pigment Yellow 74 (Butanamide, 2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxo-; CAS No. 6358-31-2) which is used in tattoo inks, emulsion paints, amd toy enamels.

Restrictions

Australian

No known restrictions have been identified.

International

No known restrictions have been identified.

Existing Work Health and Safety Controls

Hazard Classification

The chemical is not listed on the Hazardous Chemical 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

Toxicokinetics

In a toxicokinetic study, male and female Harlan Sprague Dawley rats and B6C3F(1)/N mice were administered with the radiolabelled chemical at 2, 15, or 150 mg/kg by various routes (oral, dermal, and intravenous). The radiolabelled chemical was readily absorbed orally, with the amount of total radioactivity highest in the liver (43 %) and the red blood cells (30 %). Radioactivity was excreted mainly in urine (75-79 and 55-68 % of the applied dose in rats and mice, respectively, excreted 72 hours after dose administration). Minimal amount (less than 1 %) of radioactivity was detected in the tissues after 72 hours. Dermal absorption is moderate, 5.5 % in rats and 10 % in mice, 24 hours post dosing. The main metabolic pathway was by hydroxylation of the phenyl ring, with the sulfate and glucuronide conjugates of the hydroxylated chemical detected as the major metabolites (Matthews et al., 2012).

In a metabolism study, 20 mg of the chemical in tricaprylin vehicle was administered intraperitoneally to male and female Wistar rats. Urine was collected over a 48-hour period. The urinary metabolites, identified by thin-layer chromatography (TLC), were: 2,5-diacetylamino-1-methoxybenzene (approximately 2.5 % of the applied dose); 2-amino-5-nitrophenol (approximately 0.4 % of the applied dose); and small amounts of 2-acetylamino-1-methoxy-5-nitrobenzene and 2-acetylamino-1-methoxy-5-aminobenzene (US NTP, 2006; REACH).

The nomination information submitted to the US NTP reported metabolic pathways using the metabolism prediction program METEOR: oxidative o-demethylation of the chemical to form phenol, 2-amino-5-nitro- (CAS No. 121-88-0); or the reduction of the aromatic nitro group of the chemical to form 1,4-benzenediamine, 2-methoxy- (CAS No. 5307-02-8; not listed in the Australian Inventory of Chemical Substances (AICS)) (US NTP, 2006).

Acute Toxicity

Oral

The chemical has low to moderate acute toxicity based on results from animal tests following oral exposure, warranting hazard classification (see **Recommendation** section). The median lethal dose (LD50) values in rats is ranged from 997 to 2286 mg/kg bw.

In an acute oral toxicity study conducted similarly to the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 401, the chemical (vehicle: starch in water) was administered by gavage to female Wistar rats (n=10) at doses up to 5000 mg/kg bw. The effects observed include disturbance of gait, narcosis, and yellow discolouration of skin. The LD50 was determined to be 1517 mg/kg bw (REACH).

The following are results from other acute oral toxicity studies:

- male and female Wistar rats administered the chemical in polyethylene glycol (PEG) 400 vehicle by gavage showed effects including poor general condition, sedation, ruffled fur, narcosis, and reduced body weight. The LD50s determined were 2000 and 1260 mg/kg bw in males and females, respectively (US NTP, 2006; REACH);
- female Wistar rats administered the chemical—LD50 determined was 1750 mg/kg bw. Observed effects include sedation, coma, analgesis, staggered gait, piloerection, and disturbed respiration (REACH);
- female Wistar rats administered the chemical in sesame oil vehicle at doses up to 4000 mg/kg bw—LD50 determined was 2286 mg/kg bw. No other details were reported (REACH); and

- rats administered the chemical—LD50 was determined as 997 mg/kg bw. No other details were reported (US NTP, 2006).

Dermal

The chemical has low acute toxicity based on results from animal tests following dermal exposure. The reported LD50 in rats is >2000 mg/kg bw.

In an acute dermal toxicity study conducted in accordance with OECD TG 402, a single dose of 2000 mg/kg bw of the chemical (concentration 88.9 % suspended in water) was applied under occlusion to male and female Sprague Dawley rats (n=5/sex). There were no observed effects on clinical signs, bodyweight, gross pathology, or mortality. The LD50 was determined to be >2000 mg/kg bw (US NTP, 2006; REACH).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

The chemical produced no skin irritation in studies that were conducted in accordance with or equivalent to OECD TG 404.

There were no skin irritation effects observed in the following studies (US NTP, 2006; REACH):

- semiocclusive application of the chemical in PEG 400 vehicle to shaved skin of New Zealand White (NZW) rabbits (study reportedly compliant with the principles of good laboratory practice (GLP));
- occlusive application of 1 and 10 % of the chemical in sesame oil vehicle to shaved and abraded skin of Himalayan rabbits (study non-GLP compliant);
- occlusive application of the neat chemical to shaved and abraded skin of Himalayan rabbits (study non-GLP compliant);
- semiocclusive application of the neat chemical to shaved skin of NZW rabbits (GLP compliance not specified for the study).

Eye Irritation

The chemical produced no eye irritation in studies that were conducted in accordance with or equivalent to OECD TG 405.

There were no eye irritation effects observed in three separate applications of the neat chemical to the eyes of Himalayan rabbits (all studies were conducted prior to GLP) (US NTP, 2006; REACH).

Sensitisation

Skin Sensitisation

The chemical was not found to induce dermal sensitisation when tested according to OECD TG 406.

In a guinea pig maximisation test (OECD TG 406; GLP compliance not specified), female Dunkin Hartley guinea pigs (n=20/dose) were administered induction treatments of 7.5 % chemical in Alembicol D vehicle (intradermal injection) and 75 %

chemical in water (dermal application), and challenge treatments of 75 and 40 % chemical in distilled water (US NTP). No skin reactions were observed (US NTP, 2006; REACH).

Observation in humans

Patch testing of 0.2 % solution of various dyes was conducted in 26 'tie and dye' industry workers with contact dermatitis in India. Positive reactions were observed in 14 workers, wherein one individual reportedly reacted to the chemical. The individual also had positive reactions to benzenamine, 5-chloro-2-methoxy-, hydrochloride (CAS No. 4274-03-7) and benzenamine, 3-chloro-, hydrochloride (CAS No. 141-85-5) (Mathur et al., 1985; US NTP, 2006).

Repeated Dose Toxicity

Oral

Based on the limited information available, the chemical does not cause serious damage to health following repeated oral exposure.

In a 28-day study, male and female Crj:CD rats (n=5/sex/dose) were administered a daily dose of 0, 30, 100, or 300 mg/kg bw/day of the chemical in corn oil by gavage. At the highest dose tested, the following effects were observed (Global Information Network on Chemicals (2005), as cited in US NTP, 2006):

- decreased levels of haematocrit, haemoglobin, and red blood cells (both sexes);
- increased levels of platelets and reticulocytes (both sexes);
- increased levels of total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (both sexes);
- pigment deposition in the spleen (females only);
- slight reduction in mean body weights and absolute thymus weights (both sexes);
- increased absolute and relative spleen weight (both sexes), with associated histopathological observations of increased myocardial necrosis and extramedullary hematopoiesis; and
- increased relative liver weight (both sexes).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the weight of evidence from the available data, genotoxicity cannot be discounted. Experimental in vitro genotoxicity information showed both positive and negative results. No experimental in vivo genotoxicity data are available. A simulated metabolite of the chemical was predicted positive for in vivo genotoxicity using Quantitative Structure-Activity Relationship (QSAR) modelling.

In vitro

The following results are available for the chemical (US NTP, 2006; REACH):

- positive with metabolic activation and negative without metabolic activation in a bacterial reverse mutation assay in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) at varying test doses to a maximum dose of 2500 µg/plate;
- positive with and without metabolic activation in bacterial reverse mutation assays in four strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537) and *Escherichia coli* WP2 uvrA at varying test doses up to a maximum dose of 5000 µg/plate;
- strongly positive with metabolic activation and weakly positive without metabolic activation in a bacterial reverse mutation assay in *S. typhimurium* TA98 strain at varying test doses up to a maximum dose of 500 µg/plate;
- negative with and without metabolic activation in a mammalian cell gene mutation assay (OECD TG 476; GLP-compliant) in Chinese hamster lung fibroblasts (V79) at varying test doses up to a maximum dose of 350 µg/mL; and
- negative with and without metabolic activation in a mammalian chromosome aberration test (OECD TG 473; GLP-compliant) in Chinese hamster lung fibroblasts (V79) at test doses of 30–350 µg/mL.

In vivo

No in vivo genotoxicity data are available.

QSAR

The chemical has structural alerts for binding to deoxyribonucleic acid (DNA) using the mechanistic profiling functionality of the OECD QSAR Application Toolbox (OECD QSAR Toolbox v.3.4).

Additionally, QSAR predictions using OASIS-TIMES indicate that the chemical is negative for micronucleus test and negative for liver clastogenicity. One of the simulated metabolites of the chemical, 1,4-benzenediamine, 2-methoxy- (CAS No. 5307-02-8; not listed in the AICS), was predicted positive for both these tests. All the predictions are within the applicability domain of the in vivo genotoxicity models (OASIS-TIMES v.2.27.19).

Carcinogenicity

No animal data are available. Based on the available mechanistic information and QSAR modelling, the chemical is potentially carcinogenic.

The chemical has a structural alert for carcinogenicity using the endpoint-specific profiling functionality of the OECD QSAR Application Toolbox (OECD QSAR Toolbox v.3.4). Nitroaniline derivatives, which include the chemical, 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). 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). Based on the structure of the chemical, the combination and positioning of constituents may lead to carcinogenic effects due to enhanced generation of reactive oxygen species (ROS) and oxidative damage to DNA. Additionally, the chemical is an ortho-substituted aniline containing two electron-donating groups, $–OCH_3$ and $–NO_2$, which enables the stabilisation of the nitrenium ions (OECD QSAR Toolbox v.3.4).

The nomination information submitted to the US NTP reported QSAR model predictions as follows: "probable" for carcinogenicity as predicted by TOPKAT; and "plausible for mammalian carcinogenicity" as predicted by DEREK (US NTP, 2006).

Reproductive and Developmental Toxicity

No animal data are available.

The chemical has a QSAR model prediction of "unlikely" for developmental toxicity using TOPKAT based on the nomination information submitted to the US NTP (US NTP, 2006).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include the possibility of genotoxicity and carcinogenicity. It has systemic acute effects (acute toxicity from oral exposure).

The chemical was reported to be of potential toxicological concern due to its carcinogenicity potential (Bruschweiler et al., 2014).

Public Risk Characterisation

The chemical could be used as an intermediate in the manufacture of azo dyes and pigments (see **International use** section) which may be used in tattoo inks and 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

Given the critical potential systemic long-term and systemic acute health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise exposure are implemented. The chemical should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the Hazardous Chemical Information System (HCIS) (Safe Work Australia) (see **Recommendation** section).

NICNAS Recommendation

The chemical is 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 chemical is recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Harmful if swallowed - Cat. 4 (H302)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for industry

Control measures

Control measures to minimise the risk from oral exposure to the chemical 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 chemical is used. Examples of control measures that could minimise the risk include, but are not limited to:

- 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 chemical.

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 chemical 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 the chemical has not been undertaken as part of this assessment.

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