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

30 June 2017

CAS Number: 100-01-6

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.

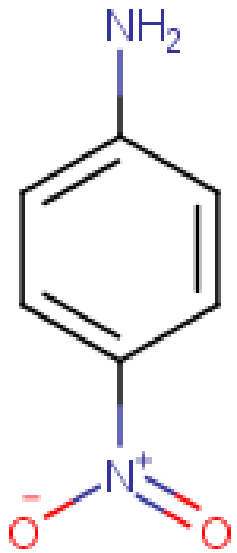
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	4-nitroaniline p-nitroaniline 1-amino-4-nitrobenzene Fast Red GG Base PNA
Structural Formula	
Molecular Formula	C6H6N2O2
Molecular Weight (g/mol)	138.12
Appearance and Odour (where available)	Bright yellow, crystalline powder. Slight ammonia-like odour.
SMILES	c1(N)ccc(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 United States (US) National Library of Medicine's Hazardous Substances Data Bank (HSDB); and the US National Toxicology Program (NTP) technical report on the chemical (NTP, 1993):

The chemical has reported site-limited uses including as:

- synthesis of organic products
- an intermediate in the production of dyes, including azo-based, and pigments;
- a gum inhibitor;
- a corrosion inhibitor; and
- an intermediate for antioxidants.

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 classified as hazardous, with the following hazard categories and hazard statements for human health in the Hazardous Chemical Information System (HCIS) (Safe Work Australia):

- Acute toxicity – category 3; H331 (Toxic if inhaled) H311 (Toxic in contact with skin) H301 (Toxic if swallowed)
- Specific target organ toxicity (repeated exposure) – category 2; H373 (May cause damage to organs through prolonged or repeated exposure)

Exposure Standards

Australian

The chemical has an exposure standard of 7.6 mg/m^3 (2 ppm) time weighted average (TWA) (Galleria Chemica).

International

The following exposure standards are identified (Galleria Chemica; RTECS).

An occupational exposure limit (OEL) of 1–6 mg/m^3 (0.2–1 ppm) TWA in different countries such as Bulgaria, Canada (Alberta, British Columbia, Quebec and Saskatchewan), China, Denmark, Estonia, France, Greece, Hungary, Iceland, Ireland, Japan, Mexico, Norway, Poland, South Africa, Spain, Switzerland, Taiwan and the United States of America (Hawaii and Vermont).

A short-term exposure limit (STEL) of 6 mg/m^3 in Canada (Saskatchewan) and 10 mg/m^3 in Poland (Galleria Chemica).

Health Hazard Information

Toxicokinetics

Based on the studies available, the gastrointestinal absorption of the chemical was near complete and was not affected by dose in the study (2–100 µmol/kg) (REACH). The chemical, when administered orally or intravenously, was rapidly distributed throughout body tissues and showed no affinity for any particular tissue (REACH).

Radiolabelled p-nitroaniline was rapidly cleared by metabolism into nine metabolites which are found to be excreted primarily in the urine and to a lesser extent in faeces (REACH). The major metabolites were determined to be p-phenylenediamine and 2-amino-5-nitrophenol (NTP, 1993).

The elimination of the chemical was reported to follow a two-component decay curve. The first component half-life was one hour, with approximately 80 % of radioactivity cleared. The second component half-life was 16–72 hours, depending on the tissue, wherein a small amount of radioactivity was cleared. The main excretion routes were urinary and faecal, with approximately 77% and 12–14% of the administered radioactivity recovered in urine and faeces, respectively, within three days (NTP, 1993).

Acute Toxicity

Oral

The chemical is classified as hazardous with hazard category 'Acute toxicity – category 3' and hazard statement ‘Toxic if swallowed’ (H301) in the HCIS (Safe Work Australia). The available data support this classification.

The following median lethal dose (LD50) values were reported for the chemical (RTECS, HSDB):

- 450 mg/kg bw in guinea pigs;
- 810 mg/kg bw in mice; and
- 750 mg/kg bw in rats.

No other details were provided.

Dermal

The chemical is classified as hazardous with hazard category 'Acute toxicity – category 3' and hazard statement ‘Toxic in contact with skin’ (H311) in the HCIS (Safe Work Australia). There is insufficient evidence to support a recommendation to amend this classification.

The median lethal dose (LD50) in guinea pigs (strain and sex not specified) is >500 mg/kg bw in a standard acute method (REACH). No other details were available.

Inhalation

The chemical is classified as hazardous with hazard category 'Acute toxicity – category 3' and hazard statement ‘Toxic if inhaled’ (H331) in the HCIS (Safe Work Australia). No data are available to evaluate this classification.

Corrosion / Irritation

Skin Irritation

Limited information is available. According to Aggregated Computational Toxicology Resource (ACToR) database; the chemical was not found to be irritating on the skin of rabbit.

Eye Irritation

No data are available.

Sensitisation

Skin Sensitisation

No experimental data for the chemical are available. Based on the available information on the metabolites of the chemical and Quantitative Structure-Activity Relationship (QSAR) modelling, the chemical is considered a skin sensitiser and warrants hazard classification (see **Recommendation** section).

The chemical has structural alerts for protein binding based on the mechanistic profiling functionality of the Organisation for Economic Co-operation and Development (OECD) QSAR Application Toolbox (OECD QSAR Toolbox v3.4). The chemical was predicted as a weak sensitiser based on metabolite information using the skin sensitisation with autooxidation model of OASIS-TIMES v.2.27.19, with the prediction within the applicability domain of the model.

The major metabolites of the chemical, p-phenylenediamine and 2-amino-5-nitrophenol (NTP, 1993), are considered to be skin sensitisers based on the available experimental animal data (NICNASa; NICNASb).

Repeated Dose Toxicity

Oral

The chemical is classified as hazardous with hazard category ‘Specific target organ toxicity (repeated exposure) – category 2’ and hazard statement 'May cause damage to organs through prolonged or repeated exposure' (H373) in the HCIS (Safe Work

Australia).

Groups of B6C3F1 mice (20 per sex per dose) received the chemical in corn oil by gavage at doses of 0, 1, 3, 10, 30, or 100 mg/kg bw/day, 5 days per week for 13 weeks. No mortalities were associated with exposure to the chemical, and final mean body weights of treated mice were similar to those of the control animals. Absolute and relative spleen weights of male and female mice receiving 30 and 100 mg/kg were significantly greater than those of controls at 7 and 13 weeks. Additional observations reported at these doses include: increased methaemoglobin levels; decreased haematocrit levels and haematocrit erythrocyte counts; transitional absolute and relative liver weight increases (NTP, 1993).

In a carcinogenicity study, groups of Sprague-Dawley (SD) rats (60 per sex per dose) were administered the chemical at doses of 0, 0.25, 1.5, or 9 mg/kg bw/day for two years. No treatment-related effects in the survival and final mean bodyweights were seen. Significant increases in absolute and relative spleen weights, and relative spleen weights were observed in the high-dose males and mid-dose males, respectively, at the end of the study (Nair et al (1990), as cited in NTP (1993)).

In another carcinogenicity study, groups of B6C3F1 mice (70 per sex per dose) were administered the chemical in corn oil by gavage at doses of 0, 3, 30, or 100 mg/kg bw/day, 5 days per week for up to 103 weeks. The mean body weights of male and female mice administered the chemical were similar to the control mice throughout the study. No clinical findings were observed and the survival of the dosed animals was similar to that of controls. The two year survival rates were 33/50 (control), 32/50 (3 mg/kg bw), 36/50 (30 mg/kg bw) and 39/50 (100 mg/kg bw). Haematological findings were similar to those in the 13-week study described above (NTP, 1993).

Dermal

The chemical is classified as hazardous with hazard category ‘Specific target organ toxicity (repeated exposure) – category 2’ and hazard statement 'May cause damage to organs through prolonged or repeated exposure' (H373) in the HCIS (Safe Work Australia). No data are available to evaluate this classification.

Inhalation

The chemical is classified as hazardous with hazard category ‘Specific target organ toxicity (repeated exposure) – category 2’ and hazard statement 'May cause damage to organs through prolonged or repeated exposure' (H373) in the HCIS (Safe Work Australia). There is insufficient evidence to support a recommendation to amend this classification.

Groups of SD rats (10 per sex per dose) were exposed to the chemical at concentrations of 0, 5, 15 or 45 mg/m^3 for 6 hours a day, 5 days per week for 4 weeks. There was no mortality or body weight reductions in the test animals; however, mean spleen weights were increased in all group exposed to the chemical (NTP, 1993).

Genotoxicity

There is insufficient evidence to evaluate the genotoxicity potential of the chemical. Insufficient data are available to evaluate the in vivo genotoxicity potential of the chemical.

The genotoxic potential of the chemical was tested in two laboratories (NTP, 1993). Assays were conducted with and without metabolic activation (unless otherwise indicated):

- Gene mutations were noted in *Salmonella typhimurium* strain TA98 when exposed to the chemical at up to 6,666 µg/plate, with and without metabolic activation. Negative results were obtained under similar conditions in strains TA97, TA100, TA1535 and TA1537.
- Sister chromatid exchanges in Chinese hamster ovary cells in vitro were positive at an effective dose range between 1,600 to 3,000 µg/mL with activation, and equivocal results without activation.
- Mouse lymphoma gene mutation studies were negative with activation and positive without activation. Dose levels were not specified.

- Chromosomal aberrations in Chinese hamster ovary cells in vitro were positive with activation and weakly positive without activation. One lab reported that an effective does of 1,600 µg/mL of the chemical led to weakly positive results without activation while the second lab produced negative results with an effective dose of 800 µg/mL.

- Sex-linked recessive lethal mutations in *Drosophila melanogaster* were negative when administered by feed (5,000 ppm) or injection (1,000 ppm) to adult males, or by feeding (100 ppm) to larvae.

Carcinogenicity

The available animal data for the carcinogenicity of the chemical does not support a recommendation for the chemical to be classified as hazardous.

In a previously described carcinogenicity study (see **Repeated Dose Toxicity: Oral**) in B6C3F1 mice, up to 10 mice per dose were randomly selected for lesion evaluations after 9 and 15 months of exposure to the chemical. Lesions were observed in the spleen, liver, and bone marrow of mice treated with 30 or 100 mg/kg bw/day at the stated observation periods and at the end of the study. At the 100 mg/kg bw/day dose, a slight marginal increase in incidences of haemangiosarcoma of the liver and haemangioma or haemangiosarcoma at all sites were seen in male mice. Also at this dose, a significant decrease in the incidence of hepatocellular adenoma or carcinoma at all sites were detected in male mice. Other effects observed include increased incidence or severity of congestion, haematopoiesis, and hemosiderin accumulation in the spleen, Kupffer cell pigmentation in the liver, and hypercellularity in the bone marrow.

In a previously described carcinogenicity study (see **Repeated Dose Toxicity: Oral**) in Sprague Dawley rats, the treatment-related lesion was pigment accumulation observed in the liver and spleen. The study authors indicated that this effect was not related to neoplasia in rats (Nair et al (1990), as cited in NTP (1993)).

The major metabolites of the chemical were determined to be p-phenylenediamine and 2-amino-5-nitrophenol (NTP, 1993). Available carcinogenicity data for p-phenylenediamine indicate that the chemical is not carcinogenic up to dietary doses of 1250 ppm (NICNASa). The chemical 2-amino-5-nitrophenol is considered to have carcinogenic potential in male rats but the information is insufficient to classify the chemical as a carcinogen (NICNASb).

Reproductive and Developmental Toxicity

The chemical does not show specific reproductive or developmental toxicity. Any reproductive and developmental effects were only observed secondary to maternal toxicity.

In a developmental toxicity study, the chemical in corn oil was administered by gavage at doses of 25, 85, or 250 mg/kg bw/day to mated SD rats on gestation days 6 through 19. Survivors were euthanised and evaluated on day 20. At the 250 mg/kg bw/day dose, significant decrease in bodyweight and increase in spleen weights of the dams were reported. Also at this dose, increased resorptions, decreased foetal bodyweights, and terata were seen. At the 85 mg/kg bw/day dose, effects include increased dam spleen weights and foetotoxicity (Nair et al (1985), as cited in NTP (1993)).

In a reproductive toxicity study, groups of SD rats (15 males and 30 females per dose, F0) were administered 0, 0.25, 1.5 or 9 mg/kg bw/day of the chemical for 14 weeks before and during mating, gestation, and lactation. From the resulting F1 generation, random groups of 15 males and 30 females were administered the same treatment. There was a slight reduction in the rate of pregnancy observed in the high dose F0 group. No other treatment-related effects were reported in the F0 and F1 groups (Nair et al (1990), as cited in NTP (1993)).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic acute and chronic effects (acute and chronic toxicity from oral, dermal and inhalation exposure, and skin sensitisation). Additionally, the genotoxic and carcinogenic potential of the

chemical cannot be ruled out, and should be reviewed if further information becomes available.

Public Risk Characterisation

The chemical could be used as an intermediate in the manufacture of azo dyes and pigments (see **International use** section) which could be available to the public, and it may then be regenerated by reductive cleavage of the azo dyes. The chemical was indicated as a potential amine cleavage product of concern from azo dyes (Bruschweiler et al., 2014). 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 systemic long-term health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise dermal, ocular and inhalation 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 HCIS (Safe Work Australia) (see **Recommendation** section).

NICNAS Recommendation

Further risk management is required. Sufficient information is available to recommend that risks for workplace health and safety be managed through changes to classification and labelling.

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	Toxic if swallowed - Cat. 3 (H301)* Toxic in contact with skin - Cat. 3 (H311)* Toxic if inhaled - Cat. 3 (H331)*

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Sensitisation	Not Applicable	May cause an allergic skin reaction - Cat. 1 (H317)
Repeat Dose Toxicity	Not Applicable	May cause damage to organs through prolonged or repeated exposure - Cat. 2 (H373)*

[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 consumers

Products containing the chemical should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal and inhalation 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:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemical, if valid techniques are available to monitor the effect on the worker's health;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- 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 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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