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

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CAS Number: 89-63-4

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.

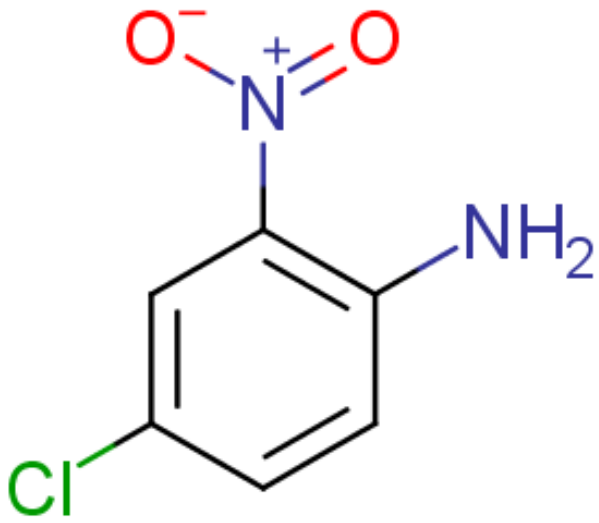
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	4-chloro-2-nitroaniline p-chloro-o-nitroaniline aniline, 4-chloro-2-nitro- C.I. 37040 C.I. azoic diazo component 9
Structural Formula	
Molecular Formula	C6H5ClN2O2
Molecular Weight (g/mol)	172.57
Appearance and Odour (where available)	Orange crystals with non-specific odour.
SMILES	c1(N)c(N(=O)=O)cc(Cl)cc1

Import, Manufacture and Use

Australian

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

International

The following international uses have been identified through Galleria Chemica; the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; US Environmental Protection Agency's Aggregated Computer Toxicology Resource (ACToR); the Substances and Preparations in Nordic countries (SPIN) database; the United States (US) National Toxicology Program (NTP); and the OECD High Production Volume chemical program (HPV).

The chemical has reported site-limited use as an intermediate in manufacturing:

- dyes and pigments (diazo component in C.I. Pigment Yellow 3 and C.I. Pigment Red 6); and
- other chemicals.

Restrictions

Australian

No known restrictions have been identified.

The chemical is not listed in the *Poisons standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP, 2019). However, it can fall under the scope of the following group entry in Schedule 5: 'AMINES for use as curing agents for epoxy resins except when separately specified in the Schedules' (SUSMP, 2019).

Schedule 5 chemicals are described as 'Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label (SUSMP, 2019). Schedule 5 chemicals are labelled with 'Caution'.

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 in rats using radiolabelled 4-chloro-2-nitroaniline, the chemical was rapidly absorbed, distributed, metabolised and excreted following oral or intravenous (IV) administration. Within 15 minutes of administration, the chemical was found in blood, liver, muscle, adipose tissue and skin. Higher concentrations were observed in kidneys and bladder associated with excretion. More than 95 % of the total radioactivity was cleared from the body within 1 hour and the clearance reached near completion in 3 days. The chemical was excreted in the form of 8 metabolites with only a trace of parent compound in urine and none in bile and faeces. Approximately 70 % of the total dose was excreted in urine as a sulfate conjugate of a single metabolite of the chemical. At the dose range of 0.13–13.60 mg/kg bw used in the study, no evidence of persistence for the chemical or its metabolites was observed in tissues tested. The rapid metabolism and excretion of the chemical suggested low likelihood of bioaccumulation in rats. The known carcinogen and potential metabolite, 4-chloro-1,2-phenylenediamine was not detected in rats tissues or excretia (Chopade and Matthews, 1983). The chemical and its metabolites do not trigger increased methaemoglobin levels, which are commonly associated with exposure to aniline and some substituted anilines (EPA, 1988).

The potential toxicity of the chemical is expected to be associated with the intermediates and products formed during the reduction of the nitro group. The metabolism of 4-chloro-2-nitroaniline by nitroreductase via well-established nitrobenzene reduction pathways yields nitroso-, hydroxylamine and amine derivatives. These metabolites, including 4-chloro-1,2-phenylenediamine, have the potential to interact with biomolecules and nucleic acids and may cause systemic toxicity and mutagenic effects.

Acute Toxicity

Oral

Based on the reported median lethal doses (LD50) in experimental animals, the chemical is expected to have moderate acute oral toxicity, warranting hazard classification (see **Recommendation** section).

The reported LD50 values from non-guideline studies are 400 mg/kg bw in rats, 800 mg/kg bw in mice and 3000 mg/kg bw in guinea pigs (Galleria Chemica; HSBD). In another non-guideline study, the reported LD50 was >5000 mg/kg bw in female rats. Reported signs of toxicity included yellow skin, orange urine, bristled coat, ptosis, passive and irregular breathing (REACH).

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Based on the available data, the chemical is not a skin irritant in rabbits; therefore, hazard classification is not warranted.

In a non-guideline skin irritation study conducted similarly to OECD Test Guideline (TG) 404 (application period 24 hours instead of 4 hours deviating reading times), 500 mg of the chemical mixed with 0.35 mL physiological saline was applied to 6 Himalayan albino rabbits under occlusion to intact or abraded skin for 24 hours. The skin was observed after 24, 48 and 72 hours after treatment and the mean primary dermal irritation index was <0.5 (REACH). The chemical was not a skin irritant under the test conditions.

Eye Irritation

Based on the available data, the chemical is considered to be mildly irritating to the eyes of rabbits. The effects are not sufficient to warrant hazard classification.

In a non-guideline study, 100 mg of the chemical mixed with 3 drops of physiological saline was applied to one eye of 6 Himalayan albino rabbits while the other eye served as the control. The eyes were washed with physiological saline after 24 hours and the effects were observed for 72 hours. The maximum mean irritation score of 5 (out of 110) was reported 1 hour after application. Yellow staining of the sclera and part of cornea was observed immediately after application, but was fully reversible after 48 hours. No other irritation scores were reported (REACH).

Sensitisation

Skin Sensitisation

No human or animal data are available.

The chemical has no structural alerts for skin sensitisation using the knowledge based expert system Deductive Estimation of Risk from Existing Knowledge (DEREK) Nexus 2.2 (Lhasa Limited). OECD quantitative structure-activity relationship (QSAR) gives a protein binding alert as ‘nitroaniline derivatives’ based on Direct Peptide Reactivity Assay (DPRA) (QSAR Toolbox 4.2). However, it is insufficient to conclude on the skin sensitisation potential of the chemical.

Repeated Dose Toxicity

Oral

Limited data are available.

In 14-day and 13-week toxicity studies in Sprague Dawley (SD) rats and Swiss mice (n=10/group), the chemical was administered by oral gavage at concentrations of 0, 50 or 800 mg/kg bw/day for rats and 0, 75 or 1200 mg/kg bw/day for mice (NTP, 1988). No other information is available for this study. No toxicity technical report on the outcome of the study was published by NTP. Based on the insufficient information available, it is not possible to reach a conclusion regarding the repeated dose toxicity of the chemical.

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

The weight of evidence from in vitro genotoxicity studies suggests that the chemical may be genotoxic in vitro. Although the genotoxic potential cannot be fully described, the chemical is not expected to be genotoxic or carcinogenic based on the Structure-Activity-Relationship (SAR) profiles (NICNAS). Furthermore, there is insufficient in vivo data available to warrant classification.

In vitro

The following tests reported mostly positive results for genotoxicity:

- positive with metabolic activation (S9) and negative without metabolic activation in a bacterial reverse mutation assay (OECD TG 471) in *Salmonella typhimurium* strains TA98 and 100; and negative in TA1535 and 1537 with and without metabolic activation at concentrations of 3.3–1000 µg/plate (NTP, 1983);
- positive with metabolic activation and negative without metabolic activation in another bacterial reverse mutation assay in *S. typhimurium* strains TA98 and 1538; and negative in TA100, 1535, 1537 and *Escherichia coli* WP2 uvrA with and without metabolic activation at concentrations of 4–2500 µg/plate (REACH);
- positive in a mammalian gene mutation assay in the thymidine kinase (tk) locus in L5178Y mouse lymphoma at concentrations above 90 µg/mL without metabolic activation and at concentrations above 1 µg/mL with metabolic activation (NTP study ID 971791, no year available);
- positive with metabolic activation and negative without metabolic activation in a chromosome aberration (CA) assay in Chinese hamster ovary (CHO) cells at concentrations of 151–351 µg/mL (NTP, 1987);
- positive in a sister chromatid exchange (SCE) assay in CHO cells with and without metabolic activation at concentrations of 18–351 µg/mL (NTP, 1987); and
- positive in an unscheduled DNA synthesis assay in rat hepatocytes at concentrations of 0.2 or 2 mM (HSDB, 1991).

In vivo

The chemical was found to be non-mutagenic in an in vivo micronucleus assay (OECD TG 474) in male and female SPF 71 mice (n=5/sex) receiving an oral dose of the chemical at 2200 mg/kg bw (REACH). No mortality was observed. Symptoms of toxicity included reduced spontaneous activity, uncoordinated gait and coloured urine. There was no significant increase in micronucleated polychromatic erythrocytes.

In silico (QSAR)

The chemical presents alerts for mutagenicity based on the molecular structure as profiled by the OECD QSAR Toolbox v4.2. As a nitroaniline derivative, the chemical has potential to interact with DNA, causing toxic and mutagenic effects. The process of reducing the nitro group of the chemical to nitroso-, hydroxylamine and amine produces reactive radical species, which can cause oxidative damage to DNA. Further metabolic activation of the hydroxylamine intermediate (such as phase II conjugation) can lead to the formation of highly toxic and reactive nitrenium ions.

Carcinogenicity

Limited data are available.

The chemical was inactive in an in vitro chemical-induced transformation assay of BALB/c-3T3 cells at concentrations of 0.22–0.87 mM (Matthews et al, 1993). However, it is insufficient to conclude on the carcinogenic potential of the chemical based on the available data.

Reproductive and Developmental Toxicity

Based on the limited information available, the chemical is not expected to cause reproductive toxicity. There are no data for developmental toxicity.

In a sperm morphology vaginal cytology (SMVCE) study, the chemical had no effects on reproductive parameters in male rats; however, it was reported to interfere with the relative frequency of estrous cycle in female rats at concentrations of 600 or 1200 mg/kg bw/day (EPA, 1988). No further information is available.

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic long-term effects (mutagenicity) and systemic acute effects (acute toxicity from oral exposure).

Public Risk Characterisation

Given the international uses identified for the chemical, it is unlikely that the public will be exposed to the chemical.

International data indicate that the chemical is solely used as an intermediate in the synthesis of dyes, pigments and other chemicals. Therefore, the public may be exposed to the chemical as a result of its' release from these dyes. The presence of multiple activating and deactivating groups may negate the genotoxic potential of the chemical. The risk to the public from the exposure from dyes and pigments may be considered in a further evaluation of these dyes (NICNAS). The use of the chemical in Australia is unknown.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical 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 HCIS (Safe Work Australia) (refer to **Recommendation** section).

NICNAS Recommendation

Assessment of the chemical is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

The need for regulatory controls to limit exposure to the chemical as a result of release from dyes manufactured from the chemical may be considered as part of an evaluation of any relevant dyes.

Regulatory Control

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 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;
- 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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