Phenol, 4-amino-3-nitro-: Human health tier II assessment

24 April 2015

CAS Number: 610-81-1

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.

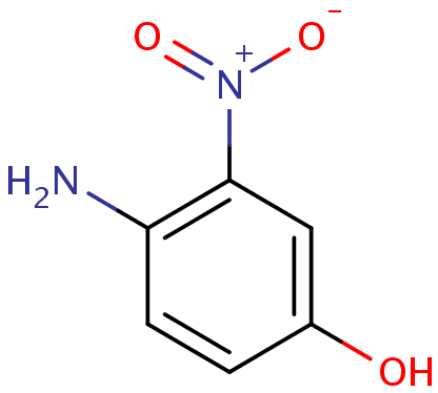
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	3-nitro-4-aminophenol 1-hydroxy-3-nitro-4-aminobenzene 2-nitro-4-hydroxyaniline
Structural Formula	
Molecular Formula	C6H6N2O3
Molecular Weight (g/mol)	154.12
Appearance and Odour (where available)	Dark red powder
SMILES	c1(N)c(N(=O)=O)cc(O)cc1

Import, Manufacture and Use

Australian

The chemical is on the 'List of chemicals used as dyes in permanent and semi-permanent hair dyes in Australia' (NICNAS, 2007).

The chemical has reported cosmetic use in permanent and semi-permanent hair dye preparations.

International

The following international uses have been identified through Galleria Chemica; the European Commission Cosmetic Ingredients and Substances (CosIng) database; United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and eChemPortal: the Organisation for Economic Co-operation and Development High Production Volume chemical program (OECD HPV), and the US Environmental Protection Agency's Aggregated Computer Toxicology Resource—ACToR.

The chemical has reported cosmetic use in hair dye preparations.

The chemical is listed as safe for use in hair dyes at concentrations up to a maximum 3.0 % concentration and up to 1.5 % when in combination with hydrogen peroxide (CosIng).

The chemical is used in non-oxidative hair dye formulations up to a 1.0 % concentration (SCCP, 2007).

Restrictions

Australian

No known restrictions have been identified.

International

The chemical is listed on the following (Galleria Chemica):

- European Union (EU) Cosmetic Directive 76/768/EEC Annex III: List of Substances which cosmetic products must not contain except subject to the restrictions and conditions laid down;
- New Zealand Cosmetic Products Group Standard—Schedule 5—Table 1: Components cosmetic products must not contain except subject to restrictions and conditions laid down;
- The Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex III—Part 2 List of substances provisionally allowed.

Existing Work Health and Safety Controls

Hazard Classification

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

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

Acute Toxicity

Oral

The chemical has moderate acute oral toxicity in rats, warranting hazard classification.

The median lethal dose (LD50) in Sprague Dawley (SD) rats after a single oral gavage dose (OECD Test Guideline (TG) 401) was 500 –1000 mg/kg bw. Observed sublethal effects included lethargy, shortness of breath, tonic–clonic convulsions, changes in motor activity, hypersalivation and orange colouration of the urogenital area (SCCP, 2007).

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Only limited data are available. The chemical is not irritating to the skin at a 6 % concentration.

In a study conducted according to the OECD TG 404, the chemical at a 6 % concentration did not produce any skin irritation in New Zealand White rabbits (SCCP, 2007).

Eye Irritation

The chemical is an eye irritant, warranting hazard classification.

The chemical was applied undiluted (100 mg) to one eye each of three New Zealand albino rabbits (OECD TG 405). The eyes were not rinsed after application and were examined at one, 24 and 72 hours, and seven days post application. Signs of irritation such as redness of the conjunctivae, partial corneal opacity, discharge and chemosis were observed when examined at one and 24 hours (irritation scores not available). Iris congestion and partial corneal opacity were reversible within 72 hours, with conjunctival reactions reversible within one week. The chemical was considered to be irritating to the eyes of rabbits (SCCP, 2007).

The chemical at a 6 % concentration was not irritating to the eyes of New Zealand albino rabbits when tested according to the OECD TG 405 (SCCP, 2007).

Sensitisation

Skin Sensitisation

The chemical is considered to be a strong skin sensitiser, warranting hazard classification.

In a local lymph node assay (LLNA) test (OECD TG 429), groups of female CBA/J mice were topically treated with 25 μL of the chemical (in an acetone/olive oil mixture) at 0.05, 0.1, 0.5, 1 or 2.5 % concentrations, once daily for three days. The lympho-proliferation response, determined by the incorporation of (^{3}H)-methyl thymidine exceeded the threshold of three (stimulation index (SI) >3) at concentrations >0.5 %. The estimated concentration needed to produce three-fold increase in lymphocyte proliferation (EC3) was calculated to be 0.2 %, indicating the chemical as a strong skin sensitiser (SCCP, 2007).

Repeated Dose Toxicity

Oral

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

In a 28-day oral gavage study (OECD TG 407), Crl:CD-(SD)BR rats were administered the chemical at doses of 0, 100, 250 and 600 mg/kg bw/day. A no observed adverse effect level (NOAEL) of 250 mg/kg bw/day was established based on two mortalities, scabbing, perinasal staining, mild convulsions and significantly decreased body weight in males at the highest dose (SCCP, 2007).

In a 90-day oral gavage study (OECG TG 408), SD rats were administered the chemical at doses of 0, 10, 50 and 250 mg/kg bw/day. The NOAEL was reported as 250 mg/kg bw/day. Based on increased liver weights (+15 %, relative weight) at 250 mg/kg bw/day (compared with the control group), the no observed effect level (NOEL) was reported to be 50 mg/kg bw/day (SCCP, 2007; Burnett et al., 2009).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the negative results from the in vivo genotoxicity data, the chemical is not considered to be genotoxic. However, an SCCP (2007) report stated that the data available are insufficient to exclude potential gene mutation.

The chemical gave the following mixed results in several in vitro genotoxicity assays (SCCP, 2007; Burnett et al., 2009):

- negative results in a bacterial gene mutation assay (OECD TG 471) with four strains of *Salmonella typhimurium* (TA100, TA1535, TA1537 and TA1538) with or without metabolic activation, but positive in strain TA98 with or without metabolic activation;
- it induced chromosome aberrations in a chromosomal aberration assay (OECD TG 473) in human lymphocytes at concentrations of 1.02 and 1.28 mM, with metabolic activation, but was not mutagenic without metabolic activation;
- it induced micronuclei in a micronucleus test (draft OECD TG) in human lymphocytes at concentrations of 985.6, 1232 or 1540 μg/mL, with metabolic activation, but was not mutagenic without metabolic activation; and
- negative results in a hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutation assay (OECD TG 476) using L51787 mouse lymphoma cells at concentrations up to 50 or 500 μg/mL, with and without metabolic activation, respectively.

Two in vivo genotoxicity assays with the chemical showed negative results:

- in a micronucleus assay (OECD TG 474), no statistically significant increases of micronucleated polychromatic erythrocytes (MPE) were observed in Crl:CD (SD)BR rats that received the chemical once by oral gavage doses of 500, 1000 and 2000 mg/kg bw. The chemical was found to be cytotoxic to the bone marrow at 2000 mg/kg bw in male rats (SCCP, 2007); and
- in a micronucleus assay, male Swiss mice that received the chemical once by intraperitoneal injection at doses of 0–300 mg/kg bw showed no increased incidence of MPE (Burnett et al., 2009).

Carcinogenicity

No animal toxicity data are available on the carcinogenicity of the chemical. Based on the available genotoxicity data, the mechanistic reaction, and mitigating factors of the chemical structure, this chemical is not considered to be carcinogenic.

Similar to the experimental genotoxicity data (see **Genotoxicity**), the Quantitative Structure Activity Relationship (QSAR) modelling using OASIS–TIMES (Optimized Approach based on Structural Indices Set–Tissue MEtabolism Simulator) predicted positive results for in vitro genotoxicity and negative results for in vivo genotoxicity. However, the chemical was out of the applicability domain of the models used for these predictions, indicating greater uncertainty about the reliability of the results.

Nitroaniline derivatives undergo heterolytic mechanisms and are 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 eventual 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.

The stability of the nitrenium ions is correlated with mutagenicity, for example in an Ames test with metabolic activation (Benigni & Bossa, 2011). In an Ames test, the chemical was negative in four strains of *S. typhimurium* with or without metabolic activation, but was positive in the TA98 strain (see **Genotoxicity**). This was to be expected due to the presence of the nitro group in the parent structure. However, the stability of the nitrenium ions depends on the type of substituents and the isomeric position of the nitro group. Studies showed that *para*-substituted nitrobenzene derivatives are more mutagenic compared with *ortho*- or *meta*-isomers (Vance & Levin, 1984; Shimizu & Yano, 1986; Assman et al., 1997). The chemical has the nitro group attached in an *ortho*-position to the amine, which could disrupt the activation of the N-hydroxylamine metabolites. Therefore, compared with other nitroaniline derivatives, the chemical has a lower likelihood of being a carcinogen.

Reproductive and Developmental Toxicity

The chemical is not considered to have reproductive or developmental toxicity.

Two developmental toxicity (OECD TG 414) studies in rats have been conducted using the chemical (SCCP, 2007). Four groups of 24 pregnant SD rats were orally dosed with the chemical at 0, 100, 250 and 700 mg/kg bw/day on gestation days (GD) 6–15.

Two females in the highest dose group died during the study. The females in this group showed significantly reduced body weights. A dose-related increase in the number of foetuses exhibiting skeletal variations (of uni- or bilateral vestigial 14^{th} rib) was observed in the 250 mg/kg bw/day dose. No maternal toxicity was observed at 250 mg/kg bw/day and the NOEL for embryo/foetotoxicity was reported to be 100 mg/kg bw/day (SCCP, 2007).

Pregnant SD rats were orally dosed with the chemical at 0, 5, 20 and 400 mg/kg bw/day on GD 6–19. No mortalities were reported and clinical signs were limited to orange coloured urine at all doses. Increased incidence of a short supernumerary rib was reported at the 400 mg/kg bw/day dose, although the authors noted that the increase in this skeletal variant was not statistically significant and was within the historical control range. As there were no maternal or developmental toxicity effects up to the highest dose tested (apart from the variations that were considered to be within the normal range), the NOAEL for maternal and developmental toxicity was reported to be 400 mg/kg bw/day (SCCP, 2007).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic acute effects (acute toxicity by the oral exposure) and local effects (skin sensitisation). The chemical can also cause eye irritation.

Data are lacking for acute or repeated dose dermal and inhalation toxicity.

Public Risk Characterisation

The chemical is reported to be used in permanent hair dye preparations in Australia.

Many countries, including New Zealand, and the EU have restricted the use of this chemical in cosmetics. The EU has imposed restrictions and conditions for the use of this chemical in cosmetics (hair dye preparations). The Cosmetic Ingredient Review (CIR) expert panel concluded that this chemical is safe for use in hair dyes at concentrations up to 3 %, or 1.5 % with hydrogen peroxide (CosIng).

If this chemical is included in cosmetic products containing N-nitrosating agents, formulation of carcinogenic N-nitrosamine compounds is possible (SCCS, 2012).

Currently, there are no restrictions in Australia on using this chemical in cosmetics or hair dye products. The risks could be mitigated by implementing concentration limits for use in hair dyes to address the risk of skin sensitisation and the lack of data on carcinogenicity.

Occupational Risk Characterisation

Given the critical health effects (acute toxicity, eye irritation, skin sensitisation and lack of data for some health end points), the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise exposure to the chemical 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 appropriate controls.

NICNAS Recommendation

Further risk management is required. Sufficient information is available to recommend that risks to public health and safety from the potential use of the chemical in hair dye products be managed through changes to poisons scheduling, and risks for workplace health and safety be managed through changes to classification and labelling.

Assessment of the chemical is considered to be sufficient provided that risk management recommendations are implemented and all requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or

territory.

Regulatory Control

Public Health

Given the risk characterisation, it is recommended that the chemical is included in Schedule 6 of the Poisons Standard (the *Standard for the Uniform Scheduling of Medicines and Poisons*—SUSMP) with an appropriate concentration cut-off (exemption) for hair dye use. Use in products intended to be used in contact with the eyes should also be considered.

Consideration should be given to the following:

- the chemical is a strong skin sensitiser;
- the chemical is used in permanent hair dye preparations in Australia;
- a lack of data on carcinogenicity;
- the overseas restrictions for use of this chemical in cosmetics; and
- the LLNA data showing clear evidence of sensitisation at concentrations lower that those permitted under the EU cosmetics regulation (CosIng).

Work Health and Safety

The chemical is recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical hazards and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Harmful if swallowed (Xn; R22)	Harmful if swallowed - Cat. 4 (H302)
Irritation / Corrosivity	Irritating to eyes (Xi; R36)	Causes serious eye irritation - Cat. 2A (H319)
Sensitisation	May cause sensitisation by skin contact (Xi; R43)	May cause an allergic skin reaction - Cat. 1 (H317)

[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, ocular and dermal 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 can minimise the risk include, but are not limited to:

- 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;
- 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 assist with meeting 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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