Phenol, 2-amino-3-nitro-: Human health tier II assessment

03 July 2015

CAS Number: 603-85-0

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

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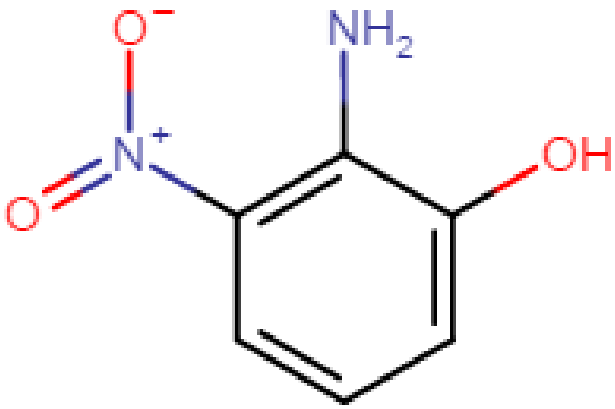
Acronyms & Abbreviations

Chemical Identity

Synonyms	1-hydroxy-2-amino-3-nitrobenzene 2-amino-3-nitrophenol 3-nitro-2-aminophenol
Structural Formula	
Molecular Formula	C6H6N2O3
Molecular Weight (g/mol)	154.124
Appearance and Odour (where available)	Reddish-brown powder
SMILES	c1(O)c(N)c(N(=O)=O)ccc1

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 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; and the United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) dictionary.

The chemical has reported cosmetic use in hair dyes.

The maximum use concentration in semi-permanent hair dyes is 3 %, and 1.5 % with hydrogen peroxide in permanent hair dyes (SCCS, 2000).

The chemical has reported site-limited use as an intermediate.

Restrictions

Australian

No known restrictions have been identified.

International

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

- EU Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products;
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain; and
- Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex II (Part 1)—List of substances which must not form part of the composition of cosmetic products.

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

Toxicokinetics

The chemical can be absorbed orally and dermally. In a 24-hour in vitro human skin penetration study, a formulation containing 1.03 % of the chemical showed 0.26 % penetration through the skin samples with hair, and 0.46 % penetration through the skin samples without hair (Burnett et al., 2009).

Acute Toxicity

Oral

The chemical has low acute oral toxicity in animal tests.

In an acute oral toxicity study, groups of Sprague Dawley (SD) rats (n = 5/sex/group) were admistered (gavage) the chemical at doses of 1000, 2000, or 3000 mg/kg bw and observed for 14 days. Mortality of 10 %, 30 % and 20 % occurred in each dose group respectively. Observed sublethal effects included sedation, dyspnoea, tonic-clonic convulsions, ataxia, and hypersalivation. Survivors recovered from these effects between days three and five. No macroscopic abnormalities were observed at the end of the study. The acute median lethal dose (LD50) was reported to be >2000 mg/kg bw (Burnett et al., 2009).

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Based on the available data, the chemical is not expected to be a skin irritant.

The chemical (0.5 g) was applied (non-occlusively) to the right flank of female New Zealand White rabbits (n = 3) for four hours. The applied site was evaluated at 1, 24, 47 and 72 hours post-exposure. Slight erythema was observed in 1/3 animals at five

hours post-exposure but was not observed at 24 hours post-exposure (Burnett et al., 2009).

Eye Irritation

The chemical is a slight eye irritant.

In an eye irritation study, female New Zealand White rabbits (n = 3) were instilled with 0.1 g of the chemical in one eye of each animal. At one hour post-treatment, slight redness in the conjunctivae was observed in all animals, and this intensified in 2/3 animals at 24 hours. Other effects observed were folded iris and translucent corneas, but these effects were fully reversible within 48 hours. The chemical was reported to be a slight eye irritant (Burnett et al., 2009).

Sensitisation

Skin Sensitisation

The available data on the chemical are inconclusive to determine whether the chemical has potential to cause skin sensitisation.

In a skin sensitisation study using the Magnusson and Kligman method (not conforming with OECD TG 406 according to the SCCS, 2000), albino guinea pigs were exposed to 2 g of the chemical. The skin reactions could not be appropriately observed due to the colouring properties of the chemical. Therefore, cosmetic products containing this chemical were recommended to have a warning label 'risk of sensitisation' (SCCS, 2000).

No protein binding alerts were identified for the chemical or its derivatives, 2-amino-5-nitrophenol and 2-amino-4-nitrophenol (which was a skin sensitiser based on a local lymph node assay (LLNA) with a predicted EC3 (effective concentration needed to produce a three-fold increase in lymphocyte proliferation) of 2.81 % (REACH cited in NICNASa)) using the OECD QSAR Toolbox version 3.2.

The position of the nitro group for aromatic nitro compounds may influence the toxicity of these compounds. Mutagenicity studies have shown different results in *ortho*- or *meta*- substituted nitrobenzene derivatives compared with *para*- substituted derivatives (Assman et al., 1997). Therefore, although a positive result was obtained for 2-amino-4-nitrophenol for skin sensitisation, it is difficult to determine the applicability of this result to 2-amino-3-nitrophenol due to the different position of the substituents.

Repeated Dose Toxicity

Oral

Based on the data available, the chemical is not considered to cause serious damage to health from repeated oral exposure.

In a repeated dose oral toxicity study, groups of SD rats (n = 10/sex/dose) were administered (gavage) the chemical at doses of 100, 300 or 1000 mg/kg bw/day for 28 days. One death occurred during week one of the study in the 300 mg/kg dose group but was not considered treatment-related. Moderately increased liver and spleen weights (haemosiderin-laden macrophages) in both sexes and decreased body weight gain in males were observed at the highest dose (Burnett et al., 2009).

In another repeated dose oral toxicity study, SD rats (n = 10/sex/dose) were administered the chemical at doses of 50, 200, or 800 mg/kg bw/day for 13 weeks. Ptyalism (excessive secretion of saliva) and loud breathing were observed at 800 mg/kg bw/day. Males in the highest dose had slightly greater absolute and relative kidney weights, with grey-green discolouration. The other observed effects at the highest dose were tubular nephrosis (both sexes), acidophilic globules in the cortical tubular epithelium of the kidneys (males), slight regenerative anaemia and greater liver and spleen weights (both sexes). At 200 mg/kg bw/day, females showed slightly lower glucose levels and high cholesterol and total bilirubin levels; and males had slightly higher protein levels and lower albumin/globulin ratios. The NOAEL was determined to be 50 mg/kg bw/day (Burnett et al., 2009).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the negative results observed in two in vivo genotoxicity studies, the chemical is not considered to be genotoxic. However, the chemical induced mutagenic activity in bacterial and mammalian cells, in vitro.

The chemical gave mostly positive results in a number of in vitro genotoxicity assays (Burnett et al., 2009):

- in an Ames test, four test strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 100 and TA 98) showed significant increases in the number of revertants, with or without metabolic activation, indicating mutagenic activity at doses 125, 250, 500, 1000, and 1500 mg/plate;
- in another Ames test (*S. typhimurium* strain TA 1535 and *Escherichia coli* strain WP2uvrA treated at 125, 250, 500, 1000 or 1500 mg/plate; *S. typhimurium* strains TA1537 and TA100 treated at 31.25, 62.5, 125, 250 or 500 mg/plate; and *S. typhimurium* strain TA98 treated at 7.8, 125, 15.625, 31.25, 62.5, or 125 mg/plate), all four strains of *S. typhimurium* showed mutagenic activity, with or without metabolic activation, but no mutagenic activity was observed in the *E.coli* strain WP2uvrA up to 1500 mg per plate, with or without metabolic activation;
- one other Ames test reported negative results in *S. typhimurium* strains TA1537, TA1538, TA98, TA1535, TA100, with doses up to 1000 mg/plate in DMSO (details not available);
- positive results in L5178Y mouse lymphoma cell forward mutation assay in the absence of metabolic activation at doses 75-525 μg/mL, but negative with metabolic activation; and
- induced morphological transformations in Syrian hamster embryo cells at doses up to 15.9 μg/mL in DMSO.

The chemical gave negative results for two in vivo genotoxicity assays (Burnett et al., 2009):

- in a micronucleus test, CD-1 mice (n = 15/sex/dose) received a single, intraperitoneal (i.p.) injection of the chemical at 0, 125, 250, or 500 mg/kg bw (in 0.5% carboxymethylcellulose). Bone marrow samples extracted at 24, 48 and 72 hours after treatment showed no micronucleated polychromatic erythrocytes and chromosomal or spindle damage; and
- the chemical did not induce DNA damage in the organ cells (e.g. hepatocytes) of male SD rat when treated orally (gavage) at doses up to 2000 mg/kg bw/day, for two days.

Carcinogenicity

No animal toxicity data are available on the carcinogenicity of the chemical. Based on the available genotoxicity data, this chemical is considered to have some potential for carcinogenicity. However, the information available is insufficient to warrant hazard classification.

Primary aromatic amines undergo metabolism to reactive electrophiles as an initial step in the carcinogenic mechanism of action. This usually involves N-hydroxylation of the aromatic amines to an N-hydroxylamine and eventual formation of the pro-carcinogenic nitrenium ions. The highly reactive nitrenium ions covalently bind to DNA, provided that they are sufficiently stabilised so as not to undergo further reactions.

The stability of the nitrenium ions is correlated with mutagenicity, for example in the Ames test, with metabolic activation (Benigni & Bossa, 2011). The Ames test results were mostly positive for the chemical (see **Genotoxicity**), indicating a potential for carcinogenicity.

Expert rules, based on the chemical structure and reaction mechanism for carcinogenicity, can be used to help to determine the carcinogenic potential of a chemical. However, for this chemical, there are no existing expert rules to identify with greater certainty whether it is carcinogenic or not. Therefore, the available genotoxicity studies are used in the overall weight of evidence analysis to ascertain the carcinogenicity of the chemical.

Reproductive and Developmental Toxicity

Based on the available information, the chemical is not considered to have specific reproductive or developmental toxicity.

In a combined reproductive and developmental toxicity study, groups of pregnant SD rats (n = 25/sex/group) were administered the chemical at doses of 100, 300, or 1000 mg/kg bw/day during gestation days (GD) 6-15 and were euthanised on day 20. Lemon or orange-coloured urine was observed in all dams from days 7-16. No maternal deaths occurred. At the highest dose, the dams displayed lower body weights (days 6-9) and food consumption (days 6-12), compared with controls. The foetuses did not display any external abnormalities or skeletal variations. Scattered cases of ventricular septal defects were observed in two foetuses at 100 mg/kg bw/day and in one at 300 mg/kg bw/day. Another two foetuses (300 mg/kg bw/day) displayed bilateral dilation of cerebral ventricles but these defects were not considered to be dose-related. The maternal and developmental NOAELs were established as 300 and 1000 mg/kg bw/day, respectively in this study (Burnett et al., 2009).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation cannot be identified due to lack of conclusive data on some important health end points, such as skin sensitisation and carcinogenicity.

No animal carcinogenicity data are available for the chemical. The Ames test results were mostly positive for the chemical (see **Genotoxicity**), indicating a potential for carcinogenicity via a nitrenium ion mechanism, as observed for a number of aromatic amines (NICNASb). Although the chemical was not recommended for classification, carcinogenicity could not be excluded as a critical health effect.

Public Risk Characterisation

The chemical was reported to be used in semi-permanent hair dyes in Australia in 2007. The international uses indicate the maximum use concentration in semi-permanent hair dyes as 3 % (and 1.5 % with hydrogen peroxide) in permanent hair dyes (SCCS, 2000).

Many countries including New Zealand and the European Union have prohibited the use of this chemical in cosmetics. Currently, there are no restrictions on using this chemical in Australia.

If this chemical is included in products containing N-nitrosating agents, carcinogenic nitrosamine compounds could be formed (SCCS, 2012).

Considering the use in hair dyes, the main route of public exposure to the chemical is expected to be through the skin. If the chemical has potential for skin sensitisation and carcinogenicity, it may pose an unreasonable risk under the identified use.

Occupational Risk Characterisation

During product formulation, exposure of workers to the chemical may occur, particularly where manual or open processes are used. Worker exposure to the chemical at lower concentrations may 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.

The data available are not conclusive to classify the chemical as a hazardous substance.

NICNAS Recommendation

The chemical was reported to be used in semi-permanent hair dyes in Australia in 2007. However, there are international restrictions on using this chemical in cosmetics (including in hair dyes). Also, hazard data for important health end points of the chemical are lacking or inconclusive.

The chemical is recommended for Tier III assessment to determine whether it is still used in semi-permanent hair dyes in Australia. If the chemical is used in hair dyes in Australia, further regulatory controls may be required to manage the potential risks.

Regulatory Control

Work Health and Safety

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.

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 chemicals has not been undertaken as part of this assessment.

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