Phenol, 2-amino-6-chloro-4-nitro- and its hydrochloride: Human health tier II assessment

27 November 2014

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Phenol, 2-amino-6-chloro-4-nitro-	6358-09-4
Phenol, 2-amino-6-chloro-4-nitro-, monohydrochloride	62625-14-3

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

Grouping Rationale

The chemical, phenol, 2-amino-6-chloro-4-nitro-, monohydrochloride (CAS No. 62625-14-3) is a salt resulting from phenol, 2-amino-6-chloro-4-nitro- (CAS No. 6358-09-4; referred to as the parent base in this report) reacting with one molecule of hydrochloric acid. Therefore, these two chemicals are considered together in this assessment report. The speciation of these chemicals in biological fluids will be dependent on pH, but independent of the original form.

Import, Manufacture and Use

Australian

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

The parent base has reported cosmetic use in permanent and semi-permanent hair dye preparations. The salt has reported cosmetic use in semi-permanent hair dye preparations.

International

The following international uses have been identified through European Union Registration, Evaluation and Authorisation of Chemicals (EU REACH) dossiers; Galleria Chemica; the European Commission Cosmetic Ingredients and substances (CosIng) database; United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Directory; and eChemPortal: the US Environmental Protection Agency's Aggregated Computational Toxicology Resource (ACToR) and the US National Library of Medicine's Hazardous Substances Data Bank (HSDB).

Both chemicals have reported cosmetic use in hair dye preparations.

They are listed as safe for use in hair dyes at concentrations up to 2 % (CIR, 1997). The parent base is considered safe for use at a maximum of 2 % concentration in semi-permanent and oxidative hair dye formulations (SCCP, 2006).

Restrictions

Australian

No known restrictions have been identified.

International

Both chemicals are listed on the following (Galleria Chemica).

EU Cosmetics Regulation 1223/2009 Annex III, part 1—List of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down. The restrictions include the following:

- for use as a hair dye substance in either oxidative or non-oxidative hair dye products;
- a maximum concentration of 2 % in ready-for-use preparations; and
- after mixing under oxidative conditions, the maximum concentration applied to hair must not exceed 2 %.

The parent base is listed on the following (Galleria Chemica):

- New Zealand Cosmetic Products Group Standard (2006)—Schedule 5: Components cosmetic products must not contain except subject to the restrictions and conditions laid down. These restrictions and conditions are similar to the ones indicated above.

Existing Worker Health and Safety Controls

Hazard Classification

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

Exposure Standards

Australian

There are no specific exposure standards for these two chemicals.

International

There are no specific exposure standards identified for these two chemicals (Galleria Chemica).

Health Hazard Information

The safety of both the parent base and hydrochloride salt was assessed using the toxicological data available on the parent base (CIR, 2007). Where data are unavailable for the parent compound (phenol, 2-amino-6-chloro-4-nitro-), data available for the salt (phenol, 2-amino-6-chloro-4-nitro-, monohydrochloride) are considered relevant for the hazard assessment due to the structural similarity of the two chemicals. However, the hydrochloride salt may have different properties with respect to local effects.

Toxicokinetics

In rats, the parent base is rapidly absorbed when administered orally and excreted within 72 hours. The primary route of excretion was reported to be renal (70 %) (SCCP, 2006).

The data indicate the parent base is easily removed from the skin of rats after a 30-minute exposure by rinsing with water (95.4–98.7 % of applied dose was found in the washing water) (SCCP, 2006).

It is the reactive metabolites which are thought to be related to both the mutagenic and carcinogenic activity of nitro aromatic compounds (Estrada et al., 2003).

Acute Toxicity

Oral

Based on the data available for the parent base, both chemicals are considered to have low acute oral toxicity.

The median lethal dose (LD50) in rats is >2000 mg/kg bw for the parent base (SCCP, 2006).

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Limited data are available for the parent base and no data are available for the salt. The parent base at 0.5 % dilution is not expected to be irritating to the skin.

In a skin irritation study (OECD Test Guideline (TG) 404), a 0.5 % dilution of the parent base in propylene glycol (0.5 mL, pH = 6.0) was applied to the scarified and intact skin of New Zealand White rabbits (n = 6) for four hours. Skin reactions such as erythema and oedema were observed at 72 hours post administration. The test substance was not a skin irritant (SCCP, 2006). No irritation scores were available.

Eye Irritation

Limited data are available for the parent base and no data are available for the salt. The parent base at 2 % dilution is not expected to be irritating to the eyes.

In an eye irritation study (OECD TG 405), a 2 % dilution of the parent base in propylene glycol was instilled (2 g) into the eyes of six New Zealand White rabbits. The test substance was not irritating to the eyes of rabbits (SCCP, 2006).

Sensitisation

Skin Sensitisation

Based on the data available for the parent base, both chemicals are considered to be skin sensitisers. A hazard classification is therefore warranted.

Data are available for the parent base. In a local lymph node assay (LLNA) (OECD TG 429), groups of female CBA mice were topically treated with 25 µL of the chemical at 0, 0.5, 5 and 10 % concentrations (using two vehicles: DMSO and acetone/water/olive oil), once a day for three consecutive days. The effective concentration needed to produce a three-fold increase in lymphocyte proliferation (EC3) was calculated as 6.85 % with dimethyl sulfoxide (DMSO) and 0.68 % with acetone/water/olive oil. The EC3 of 0.68 % might be an overestimate as there was no clear dose response below the 10 % concentration. The chemical was a skin sensitiser (SCCP, 2006).

Another LLNA study (OECD TG 429) calculated the EC3 as 2.2 % (Gerberick et al., 2005).

Repeated Dose Toxicity

Oral

Based on the data available for the parent base, both chemicals are not considered to cause serious damage to health from repeated oral exposure.

Data are available for the parent base. In a 90-day study (OECD TG 408), four groups of Wistar rats (15/sex) were administered the parent chemical by oral gavage at doses of 0, 10, 30 or 90 mg/kg bw/day. Decreased body weights and increased kidney weights were observed in the 90 mg/kg bw/day group (no details reported). Urine discolouration was observed in the animals at 30 and 90 mg/kg bw/day groups. There were no mortalities and no other effects related to treatment were reported. The no observed adverse effect level (NOAEL) was reported as 30 mg/kg bw/day (SCCP, 2006; CIR, 2007).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the available data, the chemicals are not considered to be genotoxic.

The following in vitro genotoxicity studies are available for the parent chemical (SCCP, 2006):

- Ames assay (OECD TG 471) with five strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537) with varying test doses to a maximum dose of 5000 µg/plate—the chemical did not induce mutations in any of the strains tested;
- a gene mutation test (OECD TG 476) with L5178Y mouse lymphoma cells indicated the chemical to be non-mutagenic; and
- in a micronucleus assay (OECD TG 487), high concentrations (1400 and 1600 µg/mL) of the chemical were shown to have the potential to induce micronuclei in human peripheral blood lymphocytes.

The hydrochloride salt produced some positive results in an Ames assay with four strains of *S. typhimurium* with doses up to 2000 µg/plate. Strains TA97, TA98 and TA100 showed positive results and the strain TA135 gave negative results, both with or without metabolic activation (CIR, 1997).

The following in vivo genotoxicity studies with the parent base produced negative results (SCCP, 2006):

- in a micronucleus assay (OECD TG 474), groups of 10 mice (NMRI) received a single intraperitoneal (i.p) injection of the chemical at 18.75, 37.5 or 75 mg/kg bw. The chemical was shown to be non-mutagenic up to the maximum tolerated dose of 75 mg/kg bw; and
- in another micronucleus assay (similar to OECD TG 474), groups of 10 NMRI mice that received the chemical as a single dose by oral gavage at 5, 15 or 150 mg/kg bw, showed negative results for mutagenicity. Severe signs of toxicity were noted at the highest dose.

Carcinogenicity

No animal toxicity data are available on the carcinogenicity of the parent base and the salt. Based on the available genotoxicity data and information available from Quantitative Structure Activity Relationship (QSAR) modelling, the chemicals are not considered to be carcinogenic.

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). However, the presence of two or more electron-withdrawing substituents inhibits metabolic activation through destabilising the nitrenium ion and reducing the mutagenic effect of the aromatic amines (Serafimova et al., 2011). This is the case with the parent base and the salt that contain the electron-withdrawing substituents -Cl and $-NO_2$ and, therefore, compared with other aromatic amines, these chemicals have a lower likelihood of being carcinogens.

Experimental genotoxicity data from animal studies (see above) showed that the parent base and the salt are not considered to be genotoxic. QSAR modelling using OASIS–TIMES resulted in negative results for genotoxicity, although it should be noted that the chemical structures of the parent base and the salt were out of the applicability domain of the models, indicating greater uncertainty about the reliability of the models for the chemicals.

Reproductive and Developmental Toxicity

No reproductive toxicity data are available. Based on the data available for the parent base, both chemicals are not considered to have developmental toxicity.

In a prenatal developmental toxicity study (OECD TG 414), 20 pregnant female rats (Wistar) were administered the parent base (by gavage) at 0, 10, 30 or 90 mg/kg bw/day on gestation days 5–15. At the highest dose, no teratogenic or foetotoxic effects were observed. The NOAEL for developmental toxicity was reported as 90 mg/kg bw/day. The NOAEL for maternal toxicity was 30 mg/kg bw/day based on reduced food consumption and body weight gain at the highest dose (SCCP, 2006).

Risk Characterisation

Critical Health Effects

The critical health effect identified for risk characterisation is skin sensitisation (local effect). While data are not available for acute or repeated dose dermal and inhalation toxicity, these exposure routes are not considered relevant to the main use of the chemicals.

Public Risk Characterisation

Both chemicals are reported to be used in semi-permanent hair dye preparations and the parent base is also reported to be used in permanent hair dye preparations in Australia (NICNAS, 2007).

New Zealand and the European Union have restricted the use of these chemicals in hair dye preparations to a maximum of 2 % concentration when applied directly to the hair.

If these chemicals are included in cosmetic products containing N-nitrosating agents, carcinogenic N-nitrosamine compounds could be formed (SCCS, 2012).

Currently, there are no restrictions in Australia on using these chemicals in hair dyes. The skin sensitisation risk could be mitigated by implementing concentration limits for use in hair dyes.

Occupational Risk Characterisation

Given the critical local health effects (skin sensitisation), the chemicals may pose an unreasonable risk to workers unless adequate control measures to minimise dermal exposure to the chemicals are implemented. The chemicals 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 potential use of these chemicals in hair dye preparations be managed through changes to the Poisons Standard, and risks for workplace health and safety be managed through changes to classification and labelling.

Assessment of these chemicals 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 chemicals should be 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.

Consideration should be given to the following:

- the chemicals are moderate to strong skin sensitisers;
- there is limited data on skin and eye irritation;
- lack of data on acute or repeated dose dermal and inhalation toxicity; and

- overseas restrictions for use of these chemicals in hair dyes. The maximum concentration allowed on hair is 2 % (SCCP, 2006). This concentration may be based on the lowest EC3 value calculated (0.68 %) for skin sensitisation of the parent base (see **Skin sensitisation**).

Work Health and Safety

The chemicals are 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]
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 instruction on the label.

Advice for industry

Control measures

Control measures to minimise the risk from dermal exposure to the chemicals 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 which may 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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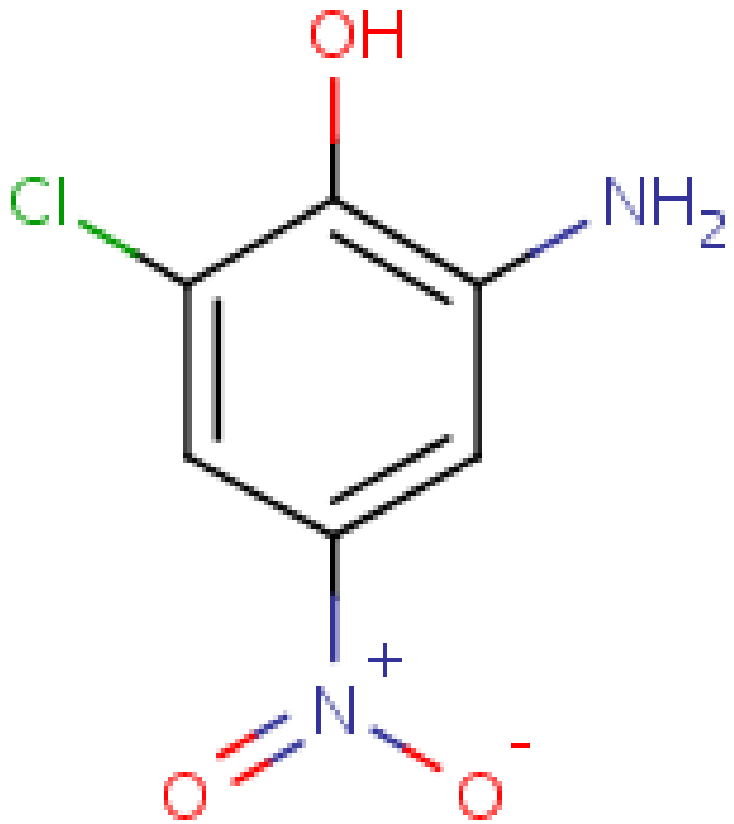
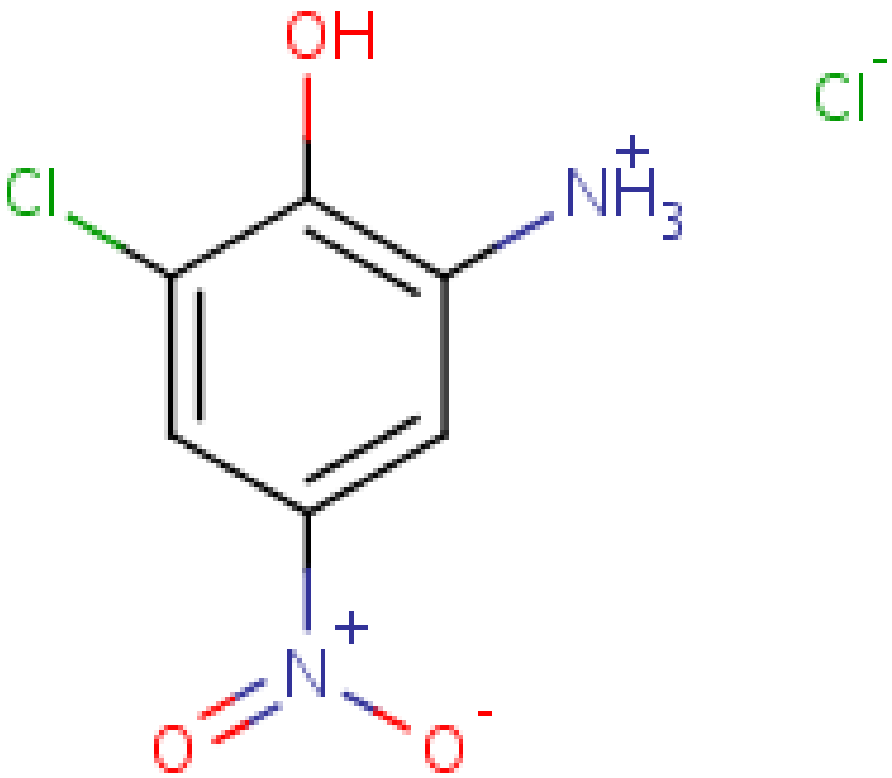
Last Update 27 November 2014

Chemical Identities

Chemical Name in the Inventory and Synonyms	**Phenol, 2-amino-6-chloro-4-nitro-** 2-amino-6-chloro-4-nitrophenol chloro-2-hydroxy-5-nitroaniline 4-nitro-6-chloro-2-aminophenol 6-chloro-4-nitro-2-aminophenol 2-amino-4-nitro-6-chlorophenol
CAS Number	6358-09-4
Structural Formula	

	
Molecular Formula	C6H5ClN2O3
Molecular Weight	188.57

Chemical Name in the Inventory and Synonyms	**Phenol, 2-amino-6-chloro-4-nitro-, monohydrochloride** 6-chloro-4-nitro-2-aminophenol, hydrochloride 2-amino-6-chloro-4-nitrophenol hydrochloride
CAS Number	62625-14-3
Structural Formula	

	
Molecular Formula	C6H5ClN2O3.ClH
Molecular Weight	225.03

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