Phenol, 2-chloro-6-(ethylamino)-4-nitro-: Human health tier II assessment

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CAS Number: 131657-78-8

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.

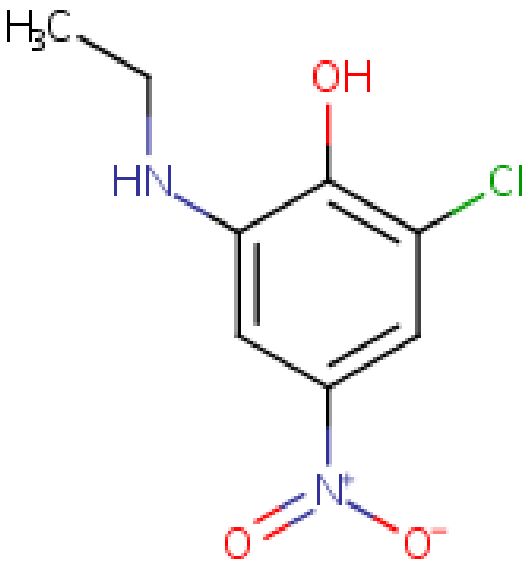
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	2-Chloro-6-(ethylamino)-4-nitrophenol
Structural Formula	
Molecular Formula	C8H9ClN2O3
Molecular Weight (g/mol)	216.62
Appearance and Odour (where available)	Reddish-orange powder.
SMILES	c1(Cl)c(O)c(NCC)cc(N(=O)=O)c1

Import, Manufacture and Use

Australian

The chemical is present 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 as an ingredient in both permanent and semi-permanent hair dyes.

International

The chemical has reported international cosmetic use as an ingredient in permanent and semi-permanent hair dyes, as identified through the following:

- the European Commission Cosmetic Ingredients and Substances (CosIng) database;
- the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and
- the US Environmental Protection Agency's (US EPA) Aggregated Computer Toxicology Resource (ACToR).

Restrictions

Australian

No known restrictions have been identified.

International

Use of the chemical in cosmetics in the European Union (EU) is subject to the restrictions described in EU Cosmetics Regulation 344/2013 (as an amendment to the listing under Annex III of Regulation 1223/2009). This chemical may be used at maximum concentrations of 3.0 % in ready-for-use preparations of oxidising (permanent) and non-oxidising (semi-permanent) colouring agents for hair dyeing. Additionally, after mixing under oxidative conditions (i.e. with hydrogen peroxide) the maximum concentration applied to hair must not exceed 1.5 % for both permanent and semi-permanent application types (CosIng).

The chemical is also listed on:

- the Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex III—Part 1, with the same use restrictions as described above for the EU (Galleria Chemica); and
- the New Zealand Cosmetic Products Group Standard—Schedule 5—Table 1: Components cosmetic products must not contain except subject to restrictions and conditions laid down. While a maximum concentration (of 3.0 %) only appears to apply to ready for use preparations of non-oxidising (semi-permanent) colouring agents for hair dyeing, the maximum concentration applied to hair must not exceed 1.5 % for both permanent and semi-permanent application types (Galleria Chemica; NZ EPA).

Existing Work Health and Safety Controls

Hazard Classification

The chemical is classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

- Xn; R22 (acute toxicity)
- Xi; R43 (sensitisation)

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

Toxicokinetics

In toxicokinetic studies in rats, the chemical has been shown to be absorped rapidly following ingestion, with blood levels peaking 35 minutes after exposure, and a reported blood half-life of one hour (SCCP, 2007). The majority (77 %) of the chemical was excreted through the urine and faeces within 24 hours of administration. While detectable residue levels of the chemical in tissue and organs were low (less than 0.03 %), the highest levels were detected in the kidneys, liver, adrenals and thyroid.

Following a 30-minute dermal exposure to the chemical, very little (less than 1 %) was reported to be absorbed, of which 90 % was excreted through the urine and faeces within 24 hours of administration (SCCP, 2007). Residue levels in most organs and tissues were below the detection limit. However, the highest levels were reported in kidneys and thyroid.

Acute Toxicity

Oral

The chemical is classified as hazardous with the risk phrase ‘Harmful if swallowed’ (Xn; R22) in the HSIS (Safe Work Australia). The available data (median lethal dose — LD50 — of 1728 mg/kg bw) support this classification (SCCP, 2007; REACH). Reported signs of toxicity included reduced activity, reduced muscle tone, raised fur and changes in motor activity.

Dermal

The chemical has low acute dermal toxicity based on results from animal tests following dermal exposure. The LD50 in rats is >2000 mg/kg bw (SCCP, 2007). Observed sub-lethal effects included diarrhoea in a number of animals and chromodacryorrhoea (appearance of red tears due to porphyrin-pigmented secretion from a gland behind the eye), which commonly occurs during periods of stress or illness.

Inhalation

No data are available for the chemical.

Corrosion / Irritation

Skin Irritation

The chemical produced no skin irritation in studies that were performed in accordance with the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 404 (SCCP, 2007).

New Zealand White rabbits were exposed to the chemical in powder or solution form for four hours under occlusive conditions on abraded or intact skin. No signs of irritation were reported during the entire study observation period (72 hours).

Eye Irritation

The chemical was reported to irritate the eyes when tested according to OECD TG 405 (SCCP, 2007).

The chemical was applied at a dose of 0.1 g into the conjunctival sac of six New Zealand White rabbits (applied to one eye only). Of the six animals, three had their treated eye rinsed with water four seconds after the chemical was applied; the remaining three animals did not have their eye rinsed. The animals were observed for 72 hours.

Although only limited scoring information was provided, at 24 hours after application of the chemical, ocular effects were more severe in the group that did not have their eye rinsed (reported as slight iridial effects—score of one in 2/3 animals, and moderate conjunctival effects—score of 2–3 in all three animals). While the majority of effects were reversible within 72 hours after application, slight redness (score of one) was still visible in the eye of one rabbit from the non-rinsing group at the end of the study period.

While there is insufficient information provided to recommend hazard classification, the chemical is considered to be irritating to the eye.

Sensitisation

Skin Sensitisation

The chemical is classified as hazardous with the risk phrase ‘May cause sensitisation by skin contact’ (R43) in the HSIS (Safe Work Australia). The positive results, reported in a local lymph node assay (LLNA), support this classification (SCCP, 2007).

In an LLNA conducted according to OECD TG 429, the skin sensitising potential of the chemical was tested in mice (five animals/dose group) at concentrations ranging from 0.5–10 % using a dimethylsulfoxide (DMSO) vehicle, and at 0.5–2.5 % using an acetone/water/olive oil vehicle (mix ratio of 2:2:1).

The estimated concentration needed to produce a three-fold increase in lymphocyte proliferation (EC3) value of 2.79 % was determined based on the concentrations used with the DSMO vehicle; a stimulation index greater than three was not observed at the lower concentrations used with the acetone/water/olive oil vehicle.

Repeated Dose Toxicity

Oral

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

In a 90-day repeated dose toxicity study following OECD TG 408, Wistar rats (15 animals/sex/dose group) were administered the chemical by oral gavage at 10, 30 or 90 mg/kg bw/day. A no observed effect level (NOEL) of 10 mg/kg bw/day was reported (SCCP, 2007). Effects observed at higher doses included significantly increased liver weights in male rats, a dose-related increase in biochemical parameters (bilirubin and uric acid levels)—although not statistically significant and reported as still within normal range, and an increase in reticulocytes (immature red blood cells) and leukocytes (white blood cells) in the high dose group females (also still within normal range). Gross and histopathological examinations of organs and tissues of the high-dose group animals were conducted, with no treatment-related abnormalities reported.

Dermal

No data are available for the chemical.

Inhalation

No data are available for the chemical.

Genotoxicity

Based on the weight of evidence from the available, well-conducted, in vitro and in vivo genotoxicity studies, the chemical is not considered to be genotoxic. Although in vitro genotoxicity tests indicate mixed results, the two in vivo tests conducted were negative.

In vitro

The chemical gave positive results in one bacterial point mutation assay (Ames test) and negative results in another. Both Ames tests were conducted according to OECD TG 417 using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with or without metabolic activation. In the study that gave positive results, test concentrations of 0.96–1000 ug/plate were used. It was reported that clear positive effects were observed without metabolic activation (specifically in strains TA98, TA100 and TA1538), while marginal effects were observed in the presence of metabolic activation (SCCP, 2007). In the study that gave negative results, test concentrations of 3–5000 ug/plate were used.

Several other non-guideline Ames tests using *S. typhimurium* are reported to have been conducted (SCCP, 2007). In studies that gave positive results, effects were mostly reported in strains TA98, TA100 and TA100 without metabolic activation; other studies gave negative results under all conditions.

In two in vitro assays using mammalian cells, positive results were only observed following metabolic activation (SCCP, 2007). The chemical was genotoxic (mutagenic and clastogenic) in a mouse lymphoma cell line L5178Y thymidine kinase (tk) locus gene mutation test conducted according to OECD TG 476 and genotoxic (clastogenic and aneugenic) in a micronucleus test on human peripheral blood lymphocytes conducted according to OECD TG 487.

In vivo

The chemical did not induce genotoxic effects in two in vivo studies in mice and rats (SCCP, 2007).

In a micronucleus test in mice, the chemical did not induce genotoxic effects (clastogenicity) at doses of 437.5–1750 mg/kg bw. Clinical signs of toxicity were observed in animals at all doses, with lethality reported in the highest dose group.

The chemical also did not induce genotoxic effects (DNA damage) in cells isolated from the liver and bladder of rats exposed to the chemical at 250 mg/kg bw in a comet assay.

Carcinogenicity

No animal toxicity data are available on the carcinogenicity of the chemical. Based on the available genotoxicity data and mechanistic information, the chemical is not considered to be carcinogenic.

Similarly 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. If a prediction is out of the applicability domain of the model, it indicates that there is greater uncertainty about the reliability of the results derived from the model. Thus, QSAR model predictions for this chemical will not be included in the weight of evidence analysis of the carcinogenic potential of the chemical.

On structural analysis, the chemical is considered to be a nitroarene, or nitroaniline derivative, containing a secondary aromatic amine. Protected secondary amines can be dealkylated to form primary amines, which can then be metabolically activated to reactive electrophiles as a primary step in a carcinogenic mechanism of action. This usually involves the activation of N-hydroxylamine metabolites with 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). While there were mixed Ames test results for the chemical, positive results were most notable in strains of *S. typhimurium* TA98 and TA100 (see **Genotoxicity**) that had not been subjected to metabolic activation. The positive results were likely to be 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 have shown that *para*-substituted nitroaniline 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 to the benzene ring in an *meta*-position to the amine, which could disrupt the activation of the N-hydroxylamine metabolites. Therefore, compared with other nitroanilines, the chemical has a lower likelihood of being a carcinogen.

Reproductive and Developmental Toxicity

No reliable data examining the effect of the chemical on fertility are available. However, one study examining developmental toxicity in rats is available, which reported an NOAEL of 90 mg/kg bw/day (highest dose tested) for both maternal and developmental toxicity (SCCP, 2007).

In a developmental toxicity study conducted according to the OECD TG 414, the chemical was administered to pregnant female rats by oral gavage at 10, 30 or 90 mg/kg bw/day, daily on days 5–15 of gestation. On day 20 of gestation, the animals were euthanised for examination. There were no reported signs of maternal toxicity, treatment-related embryotoxicity or teratogenicity at any of the dose levels.

Risk Characterisation

Critical Health Effects

The critical health effect for risk characterisation is the local effect of skin sensitisation. The chemical can also cause harmful systemic effects following a single exposure through ingestion, and has the potential to cause eye irritation.

Public Risk Characterisation

Considering that the chemical is reported to be used in hair dye products in Australia (NICNAS, 2007), the main route of public exposure is expected to be through the skin. The EU, New Zealand and the ASEAN have restricted the use of the chemical in cosmetics. Currently, there are no restrictions in Australia for using this chemical in cosmetic products. In the absence of any regulatory controls, the characterised critical local health effects have the potential to pose an unreasonable risk under the identified uses. The risk could be mitigated by implementing concentration limits and restricting uses to limit dermal exposure.

Occupational Risk Characterisation

During product formulation, dermal exposure may 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 local health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise dermal 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.

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 cosmetics be managed through changes to poisons scheduling.

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 characteristion, it is recommended that the chemical should be included in the *Poisons Standard* (the *Standard for the Uniform Scheduling of Medicines and Poisons*) with an appropriate concentration cut-off (exemption) for hair dye use.

Consideration should be given to the following:

- the chemical is a skin sensitiser;
- overseas restrictions for use of the chemical in hair dyes where the maximum concentration allowed in ready for use preparations as colouring agents for hair dyeing (both oxidising and non-oxidising hair dye products) is 3.0 % and the maximum concentration applied to hair must not exceed 1.5 % after mixing under oxidative conditions; and
- the risk could be controlled by including warning statements on labels for hair dye formulations containing the chemical at any concentration.

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 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)
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 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:

- 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 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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