Ethanol, 2-[(4-amino-2-chloro-5-nitrophenyl)amino]-: Human health tier II assessment

24 April 2015

CAS Number: 50610-28-1

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.

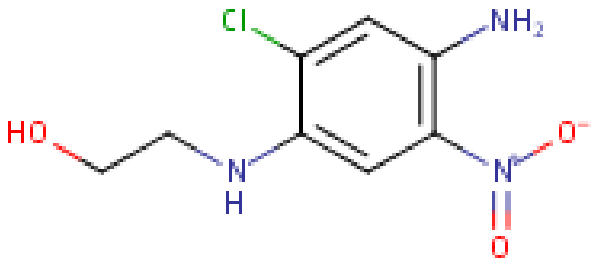
This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

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

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	2-chloro-5-nitro-N-hydroxyethyl p-phenylenediamine 2-[(4-amino-2-chloro-5-nitrophenyl)amino]ethanol 2-(4-amino-2-chloro-5-nitroanilino)ethanol Colipa No. B49 ethanol, 2-((4-amino-2-chloro-5-nitrophenyl)amino)-
Structural Formula	
Molecular Formula	C8H10ClN3O3
Molecular Weight (g/mol)	231.64
Appearance and Odour (where available)	Brown-black coloured powder
SMILES	c1(NCCO)c(Cl)cc(N)c(N(=O)=O)c1

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 the European Commission Cosmetic Ingredients and Substances (CosIng) database; reports of the European Commission Scientific Committee on Cosmetology (EC SCC); United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) dictionary; and the US Environmental Protection Agency's Aggregated Computational Toxicology Resource (ACToR).

The chemical has reported cosmetic uses:

- in hair dyes; and

- in hair tinting products and colour setting lotions (at a maximum concentration of 1 %) (EC SCC, 2000).

The maximum concentration of the chemical allowed in oxidative hair dyes was reported to be 2 % (or 1 % in combination with hydrogen peroxide) (EC SCC, 2000).

Restrictions

Australian

No known restrictions have been identified specifically for the chemical.

There is a group entry in Schedule 6 of the Poisons Standard (the *Standard for the Uniform Scheduling of Medicines and Poisons*—SUSMP) for 'Phenylenediamines and alkylated phenylenediamines not elsewhere specified in these Schedules'. 'Phenylenediamines, including alkylated and arylated derivatives, in preparations for skin colouration and dyeing of eyelashes or eyebrows **except** when included in Schedule 6' are listed in Schedule 10 (Appendix C) of the SUSMP (2015). However, these group entries do not include nitro- or chloro-substituted derivatives of phenylenediamines (SUSMP, 2015).

International

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

- EU Cosmetic Directive 76/768/EEC Annex II: List of Substances which must not form part of the composition of cosmetic products;
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain; and
- The 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 absorption of the chemical was studied in human volunteers (n = 5), who received an average quantity of 52.78 g of hair dye formulation containing 1 % of the chemical. The dye was applied to washed hair for 15 minutes and blood samples were taken at 10, 20, 30, 45 and 60 minutes, and 2, 3 and 24 hours post treatment. Urine was collected at 2, 4, 6, 8, 10, 12 and 24 hours after treatment. The chemical was not detected in the blood or urine samples (detection limits were 20 and 6 ng/mL, respectively). Based on the detection limits and average body weight of 62.5 kg, the maximum dermal absorption was calculated to be 130 mg (0.25 %) (EC SCC, 2000).

Acute Toxicity

Oral

The chemical has low acute oral toxicity.

A commercial sample containing the chemical at 91 % was administered to female CF1 mice (n = 10/dose) as a single oral gavage dose of 2000, 2500, 3000 and 3500 mg/kg bw as a 10 % suspension in 20 % gum arabic. A median lethal dose (LD50) of 2850 mg/kg bw was determined. The sublethal effects reported were reduced activity and staggering gait (EC SCC, 2000).

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Only limited data are available. The chemical at a 0.25 % concentration is not iritating to the skin.

A skin irritation study was conducted using a 0.25 % solution of a commercial sample containing the chemical at 91 %. Female guinea pigs (n = 10) received three skin paintings on shaved skin (3 x 4 cm), 20 minutes apart, for two consecutive days. The skin was washed after each painting. Using the Draize scoring system, no skin reactions were observed for up to three days after the last application (EC SCC, 2000).

Eye Irritation

Only limited data are available. The chemical at a 0.25 % concentration is slightly iritating to the eyes.

A 0.25 % solution of a commercial sample containing the chemical at 91 % was applied to the conjunctival sac of the eyes of female Pirbright guinea pigs (n = 10). Thirty minutes after application (without washing), six animals showed conjunctival redness, which persited for 1–2 hours in two animals (EC SCC, 2000). No irritation scores were available.

Sensitisation

Skin Sensitisation

Although the animal data (two limited quality non-guideline studies) indicated the chemical to be a non-skin sensitiser, predictions from Quantitative Structure Activity Relationship (QSAR) modelling indicated that the chemical was a moderate to strong skin sensitiser, warranting hazard classification.

A 0.5 % solution of the chemical (in 50 % ethanol) was administered as intracutaneous injections (0.1 mL) to the shaved skin of 15 female Pirbright guinea pigs, two times/day, six days/week for three weeks. A control group of five female guinea pigs was used. After four weeks, the animals were challenged with an intracutaneous injection (0.1 mL) containing a 0.5 % solution of the chemical (in 50 % ethanol) at dilutions of 1:10, 1:100, 1:500 and 1:1000 in Ringer's solution. Severe erythema was observed 24 hours after challenge in both treated and control animals, which reduced to slight–well-defined erythema after 48 hours. The test material was reported to have 'shined through the skin' during the challenge phase, making it difficult to evaluate. However, this effect was not mentioned during the induction phase. The chemical was reported as non-sensitising (EC SCC, 2000).

In another study, Dunkin-Hartley Pirbright guinea pigs (n = 10/sex/dose) were induced with two intradermal injections (0.05 mL each); one containing Freund's complete adjuvant (FCA) (1:1 with distilled water) and the other containing a 3 % solution of the chemical in water. The animals were then treated with a dermal application of 10 % sodium lauryl sulfate in white vaseline (unoccluded) on the following day. This was followed by (6–8 hours later) an occluded application (0.5 mL) of the chemical at 3 % in white vaseline. Forty-eight hours after the first induction, two intradermal injections (0.05 mL each) were given with 3 % of the chemical in FCA (diluted with 1:1 arachis oil). The animals were challenged 14 days later using a patch test (occluded for 24 hours) using 0.5 mL of 1, 2 or 3 % of the chemical in FCA (1:1 in arachis oil). No primary irritation or sensitisation was observed in the animals, either immediately after the challenge or 24 hours later, when compared with the control group (EC SCC, 2000). However, the EC SCC (2000) reported that the test protocol deviated from the OECD test guidelines (TG) for the Magnusson–Kligman test.

The sensitisation potency of the chemical was predicted in a study on 229 hair dye substances using a QSAR model based on the local lymph node assay (LLNA). The study predicted the chemical to be a moderate to strong skin sensitiser, with an estimated concentration needed to produce a three-fold increase in lymphocyte proliferation (EC3) value of 2.7 (Sosted, 2004).

Skin sensitisation prediction using the QSAR Toolbox v3.2 model was negative for the parent chemical—there were no protein binding alerts. However, of nine possible metabolites of the chemical, three were predicted to be skin sensitisers. Potential protein binding reactions of the metabolites were Michael additions or Schiff Base formation.

Skin sensitisation prediction using OASIS–TIMES v2.27.14 (Optimized Approach based on Structural Indices Set–TIssue MEtabolism Simulator) modelling was also negative for the parent chemical, although the model prediction was out of applicability domain, which indicates greater uncertainty about its reliability. The possible metabolites of the chemical, based on the metabolism simulators of OASIS–TIMES, were predicted to be strong skin sensitisers.

Repeated Dose Toxicity

Oral

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

Wistar rats (n=20-25/sex/dose) were administered oral gavage doses of the chemical at 0, 10, 25 or 40 mg/kg bw/day (in aqueous solution), for 90 days. A separate group at the highest dose was kept on a control diet for a four-week recovery period. From 25 mg/kg bw/day and up, a dose-related slight to moderate increase in locomotor activity in males and females, increase in serum glucose levels in males, and a slight increase in inflammatory lymphocytic infiltrations in the trachea, kidney and the uterus were observed, compared with the controls. There were no haematological effects related to the treatment. The no observed effect level (NOEL) was reported as 10 mg/kg bw/d (EC SCC, 2000).

Dermal

Only limited data are available. The available data are not sufficient to derive a conclusion on repeated dose dermal toxicity of the chemical.

In a chronic dermal toxicity study, NMRI mice (n = 75/sex/dose) were applied formulations containing the chemical at 0.5 % or 1 % by skin painting, three times a week for 18 months. No treatment related effects or increased tumour frequencies were observed, compared with the control (EC SCC, 2000).

Inhalation

No data are available.

Genotoxicity

Based on the available data, the chemical is not expected to be genotoxic.

The chemical gave mixed results in genotoxicity assays in vitro (EC SCC, 2000):

- the chemical (at 91 % purity, commercial sample) gave positive results for frameshift mutations in two *Salmonella typhimurium* strains (TA1537 and TA 1538), with or without metabolic activation. Tests in *S. typhimurium* strain TA 1535, with or without metabolic activation and *Escherichia coli* strain 343/133 without metabolic activation gave negative results. It was reported that nitroanilines often give false-positive results in *Salmonella* assays;
- negative results in a gene mutation assay using mouse lymphoma L5178Y cells, with or without metabolic activation at up to 555 µg/mL of the chemical; and
- negative results in a chromosomal aberration assay using Chinese hamster ovary cells with up to 800 µg/mL of the chemical.

The chemical gave negative results for genotoxicity in two in vivo genotoxicity assays (EC SCC, 2000):

- a micronucleus test in CFY rats that were administered two oral doses (24 hours apart) of 1600 mg/kg bw of the chemical (at 91 % purity, commercial sample) as a suspension in 0.5 % gum tragacanth; and
- an unscheduled DNA synthesis assay in hepatocytes of the Wistar rat following administration of the chemical (90 % pure) at 170, 500 or 1500 mg/kg bw.

Carcinogenicity

Only limited animal data are available. Based on the available genotoxicity data, mitigating factors relating to the mechanisms of aromatic amine carcinogenicity and due to its chemical structure, this chemical is not considered to be carcinogenic.

There was no increase in tumour frequency in NMRI mice exposed dermally to formulations containing the chemical at 0.5 % or 1 % for 18 months (EC SCC, 2000) (see **Repeat dose toxicity: Dermal**).

Although the experimental genotoxicity data mostly showed negative results for both in vitro and in vivo assays (see **Genotoxicity**), the QSAR modelling using OASIS–TIMES 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 are metabolically activated to reactive electrophiles as an initial step in carcinogenic mechanism of action. This usually involves the activation of N-hydroxylamine metabolites and their enzymatic reactions, which lead to 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). Although the chemical gave positive results in an Ames test with *Salmonella typhimurium* strains TA1537 and 1538, nitroanilines were reported to often yield false-positive results in *Salmonella* assays (see **Genotoxicity**).

Additionally, the presence of two or more electron-withdrawing substituents inhibits metabolic activation through destabilising the nitrenium ion, reducing the mutagenic effect of the aromatic amines (Serafimova et al., 2007). This chemical has two electron-withdrawing substituents, -Cl and -NO_2 and, therefore, compared with other aromatic amines, these chemicals have a lower likelihood of being carcinogenic.

Reproductive and Developmental Toxicity

Only limited data are available. The available data are not sufficient to derive a conclusion on reproductive or developmental toxicity of the chemical.

Pregnant Sprague Dawley rats (n = 25) received the chemical (dissolved in distilled water and with one or two drops of ammonia) via oral gavage dosing at 0 or 10 mg/kg bw/day during gestation day (GD) 6–15. The dams were euthanised on GD 20 for examination. No maternal toxicity or 'irreversible structural abnormalities or embryotoxic effects' were observed at 10 mg/kg bw/d. However, this dose level was too low to evaluate developmental toxicity (EC SCC, 2000).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include the potential for skin sensitisation.

Data are lacking for acute dermal and inhalation toxicity, repeated dose inhalation toxicity, and reproductive and developmental toxicity. Only limited data are available for eye and skin irritation, indicating low concentrations of the chemical could be slightly irritating to the eyes and non-irritating to the skin.

Public Risk Characterisation

The chemical is reported to be used in permanent and semi-permanent hair dye preparations in Australia (NICNAS, 2007). Many countries, including those in the European Union, have banned the use of this chemical in cosmetics. Currently, there are no restrictions in Australia on using this chemical in cosmetics.

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

In the absence of any regulatory controls, the characterised critical health effects have the potential to pose an unreasonable risk under the identified uses.

Occupational Risk Characterisation

During product formulation, exposure of workers to the chemical can occur, particularly where manual or open processes are used. These can 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 lack of data for critical health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise dermal and ocular 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 cosmetics and/or domestic products 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 characterisation, it is recommended that the chemical should be included in the *Poisons Standard* (SUSMP) for use in hair dyes and other hair products.

Consideration should be given to the following:

- the chemical and its metabolites could have moderate to strong skin sensitisation potential according to multiple QSAR models;
- a lack of data on reproductive and developmental toxicity; and
- the overseas restrictions for use of this chemical in cosmetics.

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]
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 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 which 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.

References

Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008(2004)] Third edition. Accessed at http://www.safeworkaustralia.gov.au/sites/SWA/about/Publications/Documents/258/ApprovedCriteria_Classifying_Hazardous_Substances_NOHSC1008-2004_PDF.pdf

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The Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) 2015. The Poisons Standard No: 6. Accessed at http://www.comlaw.gov.au/Details/F2015L00128/Download

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