# Urea, [2-[(4-nitrophenyl)amino]ethyl]-: Human health tier II assessment

**05 February 2016**

## CAS Number: 27080-42-8



## 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.

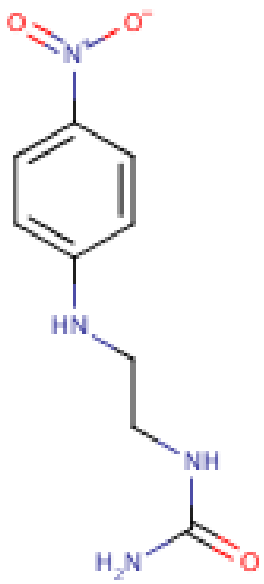
For more detail on this program please visit:www.nicnas.gov.au

**Disclaimer**



Acronyms & Abbreviations

## Chemical Identity

| | |
|---|---|
| Synonyms | 4-nitrophenyl aminoethylurea<br>amino-N-{2-[(4-nitrophenyl)amino]ethyl}amide<br>(2-(p-nitroanilino)ethyl)-urea<br>1-(2'-ureldoethyl)amino-4-nitrobenzene<br>nitrogelb |
| Structural Formula |  |
| Molecular Formula | C9H12N4O3 |
| Molecular Weight (g/mol) | 224.22 |
| Appearance and Odour (where available) | Yellow crystalline powder |
| SMILES | c1=cc(NCCNC(=O)N)=cc=c1N(=O)=O |

# 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 dyes.

## International

The following international uses have been identified through 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 various international assessments (reports from the Scientific Committee on Consumer Products (SCCP, 2007) and Scientific Committee on Consumer Safety (SCCS, 2010)).

The chemical has reported cosmetic use in oxidative and non-oxidative hair dyes.

# Restrictions

## Australian

No known restrictions have been identified.

## International

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

- European Union (EU) Cosmetics Regulation 1223/2009 Annex III—List of Substances which cosmetic products must not contain except subject to the restrictions laid down;
- New Zealand Cosmetic Products Group Standard—Schedule 5:  Components Cosmetic Products Must Not Contain Except Subject to the Restrictions and Conditions Laid Down; and
- Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex III—List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down.

All jurisdictions indicate that:

- the chemical is restricted to use in oxidative and non-oxidative hair dye products, with a maximum on-head concentration of 0.5 % in ready for use preparations, and a maximum on-head concentration of 0.25 % after mixing under oxidative conditions;
- the chemical should not be used with nitrosating agents (maximum nitrosamine content is 50 µg/kg) and should be stored in nitrite-free containers; and
- the chemical must be labelled with the mixing ratio and the following statement: ‘Hair colourants can cause severe allergic reactions. Read and follow instructions. This product is not intended for use on persons under the age of 16. Temporary “black henna” tattoos may increase your risk of allergy. Do not colour your hair if: - you have a rash on your face or sensitive, irritated and damaged scalp, - you have ever experienced any reaction after colouring your hair, - you have experienced a reaction to a temporary “black henna” tattoo in the past.’

# Existing Work Health and Safety Controls

## Hazard Classification

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

- Xi; R43 (sensitisation)

## Exposure Standards

### Australian

No specific exposure standards are available.

### International

No specific international exposure standards are available.

# Health Hazard Information

## Toxicokinetics

The chemical is a secondary amine that is susceptible to nitrosation (SCCS, 2007). It contains two easily nitrosatable nitrogens, one of which is expected to form a directly acting N-nitroso compound (NOC)—a known potent carcinogen (SCCS, 2012). No specific data are available on the metabolism of the chemical.

In two in vitro absorption studies performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 428, porcine back and flank skin (n = 6/study) was exposed to the chemical at 0.5 % in a non-oxidative hair dye preparation or 0.25 % in an oxidative hair preparation (containing 3 % hydrogen peroxide), for 60 minutes. Each skin sample was then washed twice with water, once with a shampoo formulation and twice again with water. The majority (96 ± 1 % and 97 ± 5 % for non-oxidative and oxidative preparations, respectively) of the chemical remained on the upper skin surface (epidermis). After washing, small amounts of the chemical migrated to deeper skin layers (upper dermis), acting as a potential reservoir of bioavailable chemical. The maximum skin penetration was 0.31 and 0.22 µg/cm$^2$ for non-oxidative and oxidative preparations, respectively (SCCS, 2010).

In an in vivo percutaneous absorption study, Sprague Dawley (SD) rats (n = 3/sex/treatment) were exposed to the chemical at 0.25 % in a formulation without hydrogen peroxide, 0.25 % in a formulation with hydrogen peroxide or a 0.83 % solution in dimethyl sulfoxide (DMSO)/water (1:0.69) for 30 minutes. All treatments were approximately equal in dose at 0.28–0.29 mg/cm$^2$ and were performed under anaesthesia. Skin was rinsed with approximately 100 mL of a shampoo formulation and warm water, and the treatment area covered to prevent inadvertent oral exposure. Rats were euthanised 72 hours after the exposure ceased and the amount of chemical that remained at the application site was 0.32, 0.38 and 0.13 % of the applied dose from the formulation without hydrogen peroxide, the formulation with hydrogen peroxide and the solution in DMSO/water (1:0.69), respectively. The chemical was mainly eliminated via the urine (78 %), with 58–91 % of the applied dose excreted within 24 hours. The amount of chemical distributed in the carcass was at the limit of detection for all tissues and organs examined, suggesting that bioaccumulation did not occur. Cutaneous absorption rates were 0.938, 1.097 and 0.679 µg/cm$^2$ for the formulation without hydrogen peroxide, the formulation with hydrogen peroxide and the solution in DMSO/water, respectively (SCCS, 2010).

In an in vitro study on gastrointestinal absorption, the permeability of the chemical across human intestinal epithelial cells (TC-7) was examined. Cells were exposed to the chemical on the apical side at 50 µM for 60 minutes, and the chemical measured on the apical and basolateral sides for the determination of the apparent permeability coefficient. Compared with reference standards with a high (propranolol) or low (ranitidine) permeability, the chemical 'was classified to be of medium permeability, indicating a substantial absorption from the gastro-intestinal tract' (SCCS, 2010).

## Acute Toxicity

### Oral

Based on the available data, the chemical has low acute oral toxicity.

The oral median lethal dose (LD50) is 8000 mg/kg bw and 7320 mg/kg in Wistar rats and female CF1 mice, respectively. Observed sublethal effects in mice included reduced activity and muscle spasms (SCCS, 2010).

### Dermal

Based on the available data, the chemical has low acute dermal toxicity.

The dermal LD50 is >2000 mg/kg bw in SD rats (SCCS, 2010).

### Inhalation

No data are available.

## Corrosion / Irritation

### Skin Irritation

Based on the available data, the chemical is not considered to be irritating to skin.

In a skin irritation study in New Zealand White rabbits (n = 3 males), 500 mg of the chemical moistened with water was administered (semi-occlusive patch) to clipped dorsal skin for four hours and effects evaluated at one, 24, 48 and 72 hours after the exposure ceased. Slight redness was observed in one rabbit, one hour after the patch was removed from the skin (SCCS, 2010).

### Eye Irritation

Based on the available data, the chemical is considered to be a slight eye irritant.

In an eye irritation study in New Zealand White rabbits (n = 3 males), 100 mg of the chemical was administered into the conjunctival sac of one eye of each rabbit and effects observed at one, 24, 48 and 72 hours, and at seven days, following exposure. Slight and reversible iritis was observed in two rabbits 24 hours after exposure. Slight to moderate conjunctival redness was observed in 3/3 rabbits up to 48 hours after exposure and in 2/3 rabbits up to 72 hours after exposure, but the effects were reversed by seven days (SCCS, 2010). No irritation scores were available.

## 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 results of the available animal study (local lymph node assay—LLNA) with up to 10 % concentration of the chemical do not support this classification. However, the SCCS (2013) stated that this study was insufficient. Three Quantitative Structure Activity Relationship (QSAR) model predictions indicated the chemical or its metabolites as having skin sensitisation potential. Therefore, the existing classification is supported.

In a mouse LLNA study (OECD TG 429), CBA/J mice (n = 5 females/dose) were exposed to the chemical in DMSO at 0, 0.5, 1.5, 5.0 or 10 %, once daily for three days. The estimated concentration needed to produce a three-fold increase in lymphocyte proliferation (EC3) could not be calculated because the mean stimulation indices (SI) were below the cut-off SI of 3 (1.0, 0.9, 1.0 and 0.9 for each of the test concentrations, respectively) (SCCS, 2010). This study indicates no skin sensitisation up to a concentration of 10 % of the chemical. An SCCS report (2013) stated that sensitisation testing for this chemical was insufficient, and has 'No value. Should have been tested at higher conc.'

The sensitisation potency of the chemical was examined in a study evaluating a total of 229 hair dye substances, using a QSAR model. The QSAR model prediction based on a local lymph node assay (LLNA) indicated the chemical to be a moderate to strong sensitiser, with a predicted sensitisation potency value of 2.0 compared to all chemicals evaluated in this study (range for all chemicals is 0.1–16.3, and the higher the number the stronger the predicted sensitisation potency) (Sosted et al., 2004).

Skin sensitisation prediction using the QSAR Toolbox (version 3.3) was negative for the parent chemical—there were no protein binding alerts. However, of the nine possible metabolites of the chemical, five were predicted to be skin sensitisers. Potential protein binding reactions of the metabolites were Michael additions, nucleophilic additions or Schiff base formation.

Skin sensitisation prediction using OASIS–TIMES (Optimized Approach based on Structural Indices Set–TIssue MEtabolism Simulator; version 2.27.16) modelling also gave a negative prediction for the parent chemical, although the model prediction was out of applicability domain, which indicates greater uncertainty about its reliability. Of the nine possible metabolites of the chemical, based on the metabolism simulators of OASIS–TIMES, seven were predicted to be weak skin sensitisers.

## Repeated Dose Toxicity

### Oral

Based on the available data, the chemical is not considered to cause severe effects from repeated oral exposure.

In a 90-day study (OECD TG 408) in SD rats (n = 10/sex/dose), animals were exposed to the chemical by gavage at 0, 1, 5, 25 or 125 mg/kg bw/day. A no observed adverse effect level (NOAEL) of 5 mg/kg bw/day was reported based on mild anaemia, characterised by reduced red blood cells and reduced haemoglobin concentration, observed at doses ≥25 mg/kg bw/day. This was associated with adaptive changes in the spleen (increased extramedullary haemopoiesis, indicative of haemolytic anaemia) and/or the bone marrow (decreased fatty tissue in femoral bone marrow, indicative of regeneration) (SCCS, 2010).

### Dermal

No data are available.

### Inhalation

No data are available.

## Genotoxicity

Based on the weight of evidence from the available well-conducted (mostly using OECD TG) in vitro and in vivo genotoxicity studies, the chemical is not considered to be genotoxic.

Mixed results were reported in Ames tests (OECD TG 471)—positive with *Salmonella typhimurium* strains TA100 and TA1538, and negative with *S. typhimurium* strains TA98, TA1535 and TA1537, all exposed at 1–5000 µg/plate, with or without metabolic activation (SCCS, 2010).

Negative results were observed in two other in vitro assays using the chemical (SCCS, 2010):

- a mammalian gene mutation assay (OECD TG 476) in mouse lymphoma cells (L5178Y) exposed at 22–350 µg/mL, with or without metabolic activation; and
- a chromosome aberration test in Chinese hamster ovary (CHO) cells exposed at 5–250 µg/mL, with or without metabolic activation.

Negative results were also observed in two in vivo tests using the chemical (SCCS, 2010):

- a mouse bone marrow micronucleus test (OECD TG 474) in NMRI mice (n = 6 males/dose) exposed once by an intraperitoneal (i.p.) injection at 250, 500 or 1000 mg/kg bw; and
- an unscheduled DNA synthesis test (OECD TG 486) in Wistar rats (n = 4 males/dose) exposed once by an oral gavage dose of 1000 or 2000 mg/kg bw.

### Carcinogenicity

No animal data are available for the chemical. There are insufficient data to derive a conclusion on the carcinogenicity of the chemical.

The chemical is a secondary amine that is expected to form a directly acting NOC (see **Toxicokinetics** section), and according to the SCCS (2012) 'the default assumption that all potentially generated NOC will be mutagenic/carcinogenic should be applied'. However, under basic oxidative hair dyeing conditions, 'this NOC is expected to rapidly decompose' (SCCS, 2012).

Expert rules, based on the chemical structure and reaction mechanism for carcinogenicity, can be used 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.

### Reproductive and Developmental Toxicity

No data are available on toxicity to fertility of the chemical. The chemical is not considered to cause developmental toxicity—observed foetal effects in the study below were secondary to maternal toxicity.

In a developmental toxicity study (OECD TG 414), pregnant Wistar rats (n = 25/dose) were exposed to the chemical by oral gavage at doses of 0, 50, 250 or 600 mg/kg bw/day on gestation days (GD) 6–19. In dams receiving the highest dose, there was one death on GD 12 and three animals were euthanised between GD 14–17 due to poor condition. A dam from the 250 mg/kg bw/day dose group was also euthanised due to poor condition. Body weight and food intake were reduced in dams exposed at ≥250 mg/kg bw/day. Foetal body weight was significantly reduced at ≥250 mg/kg bw/day and there was an increased incidence of reduced foetal ossification. There was no effect on embryo-foetal survival, and embryo-foetal toxicity was considered secondary to maternal toxicity (SCCS, 2010).

## Risk Characterisation

### Critical Health Effects

The identified critical health effect for risk characterisation is skin sensitisation (local effect). The available animal data using the chemical up to 10 % concentration do not support the existing classification for skin sensitisation, although multiple QSAR

predictions indicated a potential for skin sensitisation.

There are no data on the carcinogenicity of the chemical, but it is not considered to be genotoxic.

### Public Risk Characterisation

The chemical is used in permanent and semi-permanent hair dyes in Australia (NICNAS, 2007) and overseas. The EU, New Zealand and the ASEAN have restricted the use of this chemical in hair dyes. Currently, there are no restrictions on using this chemical in Australia.

Although the public could be exposed to the chemical through cosmetic use in hair dyes at low concentrations (<10 % concentration, not shown to be sensitising in animals), it is not considered to pose an unreasonable risk to public health.

### Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation 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.

Based on the available data, the hazard classification in the HSIS (Safe Work Australia) is considered appropriate.

## NICNAS Recommendation

Current risk management measures are considered adequate to protect the public and workers' health and safety, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory. No further assessment is required, unless new hazard data become available specifically on carcinogenicity.

### Regulatory Control

#### 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] |
|---|---|---|
| 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 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 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.

# References

European Commission Cosmetic Ingredients and Substances (CosIng) Database. Accessed December 2015 at http://ec.europa.eu/consumers/cosmetics/cosing/

Galleria Chemica. Accessed December 2015 at http://jr.chemwatch.net/galleria/

National Industrial Chemicals Notification and Assessment Scheme (NICNAS) 2007. List of Chemicals used as Dyes in Permanent and Semi-Permanent Hair Dyes in Australia.

Safe Work Australia (SWA). Hazardous Substances Information system (HSIS). Accessed December 2015 at http://hsis.safeworkaustralia.gov.au/HazardousSubstance

Scientific Committee on Consumer Products (SCCP) 2007. Opinion on 4-Nitrophenyl aminoethylurea. Accessed at http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_103.pdf

Scientific Committee on Consumer Safety (SCCS) 2010. Opinion on 4-Nitrophenyl aminoethylurea. Accessed at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_036.pdf

Scientific Committee on Consumer Safety (SCCS) 2012. Opinion on Nitrosamines and Secondary Amines in Cosmetic Products. Accessed at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_090.pdf

Scientific Committee on Consumer Safety (SCCS) 2013. Memorandum on hair dye Chemical Sensitisation. Accessed at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_007.pdf

Sosted H, Basketter DA, Estrada E, Johansen JD, Patlewicz GY 2004. Ranking of hair dye substances according to predicted sensitization potency: quantitative structure-activity relationships. Contact Dermatitis 51, pp. 241-254.

United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) dictionary. Accessed December 2015 at http://gov.personalcarecouncil.org/jsp/gov/GovHomePage.jsp

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