File No: LTD/1879

June 2016

**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)**

# PUBLIC REPORT

## L-Ascorbic acid, 3-O-ethyl- (INCI Name: 3-O-ethyl ascorbic acid)

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment.

This Public Report is available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:


| | |
|---|---|
| Street Address: | Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA. |
| Postal Address: | GPO Box 58, SYDNEY NSW 2001, AUSTRALIA. |
| TEL: | + 61 2 8577 8800 |
| FAX: | + 61 2 8577 8888 |
| Website: | www.nicnas.gov.au |


**Director**
**NICNAS**

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# SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

| ASSESSMENT REFERENCE | APPLICANT(S) | CHEMICAL OR TRADE NAME | HAZARDOUS CHEMICAL | INTRODUCTION VOLUME | USE |
|---|---|---|---|---|---|
| LTD/1879 | L'Oreal Australia Pty Ltd and Ceechem Australia Pty Ltd | L-Ascorbic acid, 3-O-ethyl- (INCI Name: 3-O-ethyl ascorbic acid) | ND* | ≤ 1 tonne per annum | Component of cosmetic products |

*ND = not determined

# CONCLUSIONS AND REGULATORY OBLIGATIONS

**Hazard classification**
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

**Human health risk assessment**
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

**Environmental risk assessment**
Based on the assumed low hazard and the assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

**Recommendations**

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation:
  - Enclosed, automated processes, where possible
  - Adequate general ventilation and local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during reformulation:
  - Avoid contact with skin and eyes

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation:
  - Coveralls
  - Safety glasses or goggles
  - Impervious gloves
  - Respiratory protection if necessary

Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Public Health

- Use of the notified chemical in cosmetic products applied topically may result in skin depigmentation. It is recommended that these products are labelled appropriately in order to inform consumers. This recommendation is consistent with the requirement of the Australian Consumer Law that all representations made in relation to the supply of consumer goods and services must be truthful, including not omitting information that would be relevant to consumers.

- Formulators should exercise due care when using the notified chemical in cosmetic products given its potential ability to cause pro-oxidant activity and eye irritation effects.

- As the notified chemical may potentially also be present in products meeting the definition of a therapeutic good, this report will be referred to the Therapeutic Goods Administration for their consideration.

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, collection and subsequent safe disposal.

**Regulatory Obligations**

*Secondary Notification*
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
  - the importation volume exceeds one tonne per annum notified chemical;
  - the use concentration of the chemical is intended to exceed 10% in cosmetic products;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a component of cosmetic products, or is likely to change significantly;
  - the amount of chemical being introduced has increased, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;

- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

*(Material) Safety Data Sheet*
The (M)SDS of the notified chemical and products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

# ASSESSMENT DETAILS

## 1. APPLICANT AND NOTIFICATION DETAILS

APPLICANTS
L'Oreal Australia Pty Ltd (ABN: 40 004 191 673)
564 St Kilda Road
MELBOURNE VIC 3004

Ceechem Australia Pty Ltd (ABN: 61 081 398 192)
227a Belmore Road
RIVERWOOD NSW 2210

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year)

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: other names, analytical data, degree of purity, impurities and use details

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for flash point, hydrolysis as a function of pH and dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU (2015)

## 2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Et-VC™

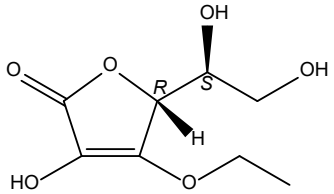
CAS NUMBER
86404-04-8

CHEMICAL NAME
L-Ascorbic acid, 3-O-ethyl-

OTHER NAME
3-O-Ethyl Ascorbic Acid (INCI Name)

MOLECULAR FORMULA
$C_8H_{12}O_6$

STRUCTURAL FORMULA




MOLECULAR WEIGHT
204.18 Da

ANALYTICAL DATA
Reference FTIR, HPLC and UV-Vis spectra were provided.

## 3. COMPOSITION

DEGREE OF PURITY
> 99%

## 4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white crystalline powder

| Property | Value | Data Source/Justification |
|---|---|---|
| Melting Point | 112 °C | Measured |
| Boiling Point | 214 ± 5 °C at 98.7 kPa | Measured |
| Density | 1,410 kg/m$^3$ at 27.2 °C | Measured |
| Vapour Pressure | 3.1 × $10^{-10}$ kPa at 25 °C | Calculated (SWISSI, 2013) |
| Water Solubility | 778 g/L at 20 °C | Measured |
| Hydrolysis as a Function of pH | Not determined | Does not contain hydrolysable functionalities |
| Partition Coefficient (n-octanol/water) | log Pow = -0.71 at 20 °C | Measured |
| Surface Tension | 69.2 mN/m | Measured |
| Adsorption/Desorption | log $K_{oc}$ = -0.447 at 20 °C | Estimated (US EPA EPI Suite™ v EPIWIN WSKOW v.I.41) |
| Dissociation Constant | Not determined | The notified chemical does not contain any functional groups that are expected to dissociate in water |
| Particle Size | Inhalable fraction (< 100 µm): 51.1%<br>Respirable fraction (< 10 µm): 7.89%<br>$d_{50}$ = 95.9 µm | Measured |
| Flash Point | > 93 °C | (M)SDS |
| Flammability (Solid) | Not highly flammable | Measured |
| Autoignition Temperature | Not determined | Solid with melting point ≤ 160 °C |
| Explosive Properties | Not explosive | Measured |
| Oxidising Properties | Not oxidising | Based on chemical structure |

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

*Reactivity*
The notified chemical is expected to be stable under normal conditions of use.

***Physical hazard classification***
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

## 5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported as a neat chemical for local formulation into end-use cosmetic products at ≤ 10% concentration. The notified chemical will also be imported as a component of finished cosmetics at ≤ 10% concentration.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

| *Year* | *1* | *2* | *3* | *4* | *5* |
|---|---|---|---|---|---|
| *Tonnes* | 1 | 1 | 1 | 1 | 1 |

PORT OF ENTRY
Melbourne and Sydney

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a neat chemical in 0.5 kg or 5 kg aluminium-plastic bags. Finished cosmetic products containing the notified chemical at ≤ 10% concentration will be imported in containers suitable for retail sale such as plastic bottles or tubes up to the size of 500 g. The notified chemical and finished cosmetic products containing the chemical will be transported by road or rail for distribution to industrial customers and retailers.

USE
The notified chemical will be used as a component of leave-on and rinse-off cosmetic products at ≤ 10% concentration. The categories of the end use products may include:

- leave-on face products including face only sunscreens;
- makeup and lipstick products;
- leave-on body products; and
- leave-on and rinse off hair products.

No aerosol spray products containing the notified chemical are proposed.

OPERATION DESCRIPTION
The notified chemical will be imported as a neat chemical for formulation of cosmetic products, or as a component of finished cosmetic products at ≤ 10% concentration that are to be sold to the public in the finished form as imported.

*Reformulation*
The procedures for incorporating the notified chemical into end-use products will vary depending on the nature of the cosmetic product being formulated, and both manual and automated steps will likely be involved. However, in general, it is expected that for the reformulation process, the notified chemical will be weighed and added to the mixing tank where it will be blended with additional additives to form the finished cosmetic products. This will be followed by automated filling of the reformulated products into containers of various sizes. The blending operations are expected to be highly automated and use closed systems and/or adequate ventilation. During the formulation process, samples of the notified chemical and the finished cosmetic products will be taken for quality control testing.

*End-use*
Finished cosmetic products containing the notified chemical at ≤ 10% concentration will be used by the public, and may also be used by professionals such as hairdressers and workers in beauty salons. Depending on the nature of the product, these will be applied by hand or by using an applicator.

# 6. HUMAN HEALTH IMPLICATIONS

## 6.1. Exposure Assessment

### 6.1.1. Occupational Exposure

CATEGORY OF WORKERS

| *Category of Worker* | *Exposure Duration (hours/day)* | *Exposure Frequency (days/year)* |
|---|---|---|
| Transport and storage workers | 4 | 12 |
| Production compounders | 8 | 12 |
| Chemists | 3 | 12 |
| Packers (for dispensing) | 8 | 12 |
| Store persons | 4 | 12 |
| Professional end users | 8 | 365 |

EXPOSURE DETAILS

*Transport and storage*

Transport and storage workers are not expected to come into contact with the notified chemical when handling the packaged notified chemical or products containing the chemical unless accidental breach of sealed packaging occurs.

*Reformulation*

During reformulation into cosmetic products, dermal, ocular and inhalation exposure of workers to the notified chemical at various concentrations up to 100% may occur. Exposure is expected to be minimised through the use of exhaust ventilation and/or automated/enclosed systems as well as through the use of personal protective equipment (PPE) such as coveralls, eye protection, impervious gloves and respiratory protection (as appropriate).

*End-use*

Exposure to the notified chemical in end-use products at ≤ 10% concentration may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. hair dressers, workers in beauty salons). The principal route of exposure will be dermal while ocular exposure may also be possible. Such professionals may use some PPE to minimise repeated exposure, but this is not expected to occur in all workplaces. However, good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or less extent than that experienced by consumers using the products containing the notified chemical.

### 6.1.2. Public Exposure

The notified chemical is proposed to be formulated in a range of leave-on or rinse-off cosmetic products. There will be widespread and repeated exposure of the public to the notified chemical through the use of the cosmetic products at ≤ 10% concentration. The principal route of exposure will be dermal while ocular exposure is also possible. Based on its low vapour pressure with no use in aerosol spray products proposed, inhalation exposure to the notified chemical is not expected.

Based on the use information available for the notified chemical, a combined internal dose of 9.827 mg/kg bw/day was estimated using data on typical use patterns for cosmetic product categories in which the notified chemical may be used (SCCS, 2012; specific use details of the notified chemical are considered as exempt information). This estimation assumed a worst case scenario and is for a person who is a simultaneous user of a selection of cosmetic products that may contain the notified chemical.

## 6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

| *Endpoint* | *Result and Assessment Conclusion* |
|---|---|
| Rat, acute oral toxicity (QSAR calculation) | estimated LD50 = 5,903.13 mg/kg bw; low toxicity |
| Skin irritation (*in vitro* reconstructed human *Epidermis* test) | non-irritating |
| Skin irritation (*in vitro* reconstructed human *Epidermis* test with penetration) | non-irritating; penetration rate = 2.13% in 8 h at 32 °C when tested at 2.06% concentration |

| *Endpoint* | *Result and Assessment Conclusion* |
|---|---|
| Human, skin irritation (patch test at 2%) | non-irritating |
| Eye irritation potential (*in vitro* NRR test at 10 %) | non-cytotoxic |
| Eye irritation (*in vitro* HET-CAM at 3%) | moderately irritating |
| Human, skin sensitisation – RIPT (100%) | no evidence of sensitisation |
| Mutagenicity – bacterial reverse mutation | non mutagenic |

*Toxicokinetics, metabolism and distribution*
No toxicokinetic data were provided on the notified chemical. The notified chemical is a derivative of L-ascorbic acid (vitamin C, CAS RN 50-81-7) with only the addition of an ethyl group to the molecule. If systemically absorbed, the notified chemical is expected to undergo metabolic pathways similar to those of vitamin C.

Based on its low molecular weight, passive diffusion of the notified chemical across the gastrointestinal tract is likely to occur. However, based on its high water solubility and low partition coefficient (log Pow = -0.71), dermal absorption may be limited. This is supported by the low dermal penetration rate (2.13% in 8 hours at 32 °C) obtained from an *in vitro* test.

L-ascorbic acid has an important relationship with the oxidation of transition metals such as iron or copper at enzyme active sites and in food. It is readily and reversibly oxidised to L-dehydroascorbic acid and both forms exist in equilibrium in the body. In alkaline solution, L-dehydroascorbic acid is hydrolysed to L-diketogulonic acid and this reaction is not reversible (CIR, 2005).

Available information indicates that, after an intraperitoneal injection of $^{14}C$-labelled ascorbic acid into rats, 19% to 29% was converted to $CO_2$ and only 0.4% was excreted as oxalic acid in the urine within 24 hours. In guinea pigs, about 48% to 63% of ingested ascorbic acid was eliminated in the urine, 0.2% to 0.43% in the faeces, and 5.5% in expired air. In guinea pigs, distributions of ascorbic acid in organs or tissues were mainly found in adrenals, lungs, and bones (CIR, 2005).

*Biochemical action*
As a close derivative of L-ascorbic acid, the notified chemical is expected to biochemically function in a similar way to vitamin C. In cosmetic formulations, vitamin C is typically known to function as an antioxidant and pH adjuster (CIR, 2005). It is also known to be used on skin for functions of photoprotection, neocollagenesis, inhibition of melanogenesis and improvement in inflammatory skin disorders (Stamford, 2012).

As a photoprotectant, L-ascorbic acid has been shown to decrease UV-B induced photooxidation on human sebum, stabilise the plasma membranes and mitochondrial membrane potential to prevent UV-A induced apoptosis (Xiao *et.al*, 2009), and suppress the elevation of intracellular peroxide after UV-B irradiation (Ochiai *et.al*, 2006). L-ascorbic acid has also shown the ability to stimulate collagen production in human fibroblasts and enhance mRNA transcription levels of collagen genes (CIR, 2005).

In inhibition of melanogenesis, L-ascorbic acid interacts with copper ions at the tyrosinase active site, acting as an anti-oxidative agent at various oxidation steps of melanin formation (Sarkar *et.al*, 2013). However, the effects of L-ascorbic acid in the skin were still not fully understood (Michels, 2011).

*Depigmentation effects*
Pigmentation in the skin is caused by enhanced melanin production or melanocyte proliferation (Maeda and Fukuda, 1991). L-ascorbic acid has been reported to inhibit the production of melanin (Parvez *et.al*, 2006) and used as an active whitening component in cosmetics that prevents melanin synthesis. The notified chemical is expected to have similar skin depigmentation effects when used topically.

Available data showed that, if skin depigmentation is caused by cytotoxicity, irreversible hypopigmentation may occur on the skin (Maeda and Fukuda, 1991). It has been reported that L-ascorbic acid is cytotoxic *in vitro* at high concentrations (Siddique *et.al*, 2009). Cytotoxic effects have also been described when L-ascorbic acid and the ascorbyl radical have undergone auto-oxidation (Eberlein-Konig *et.al,* 2005). Therefore, when used topically at high concentration, the potential for the notified chemical to cause cytotoxic effects leading to irreversible skin depigmentation cannot be ruled out.

*Potential for pro-oxidant activity*
The notified chemical is proposed to have antioxidant activity. At high concentrations or under special conditions, almost all antioxidants have pro-oxidant effects *in vitro*; however, the relevance of these effects *in*

*vivo* is not currently known (Eberlein-Konig *et.al*¸ 2005). It has been demonstrated that L-ascorbic acid had dose-dependent antioxidant or pro-oxidant effects in rats after hepatic ischemia/perfusion (Seo and Lee, 2002), and extracellular antioxidant activity and intracellular pro-oxidant activity may be simultaneously occurring (Osiecki *et. al*, 2010). Pro-oxidation activity of a chemical *in vivo* is known to generate oxidative damages to biomolecules such as proteins, DNA and lipids that may further lead to cell death or genotoxicity (Aruoma, 2003; CIR, 2005).

It has been shown that L-Ascorbic acid may contribute to pro-oxidant activity by reducing metal ions which further are capable of converting hydrogen peroxide to hydroxyl radicals through a Fenton type reaction (Duarte and Lunec, 2005). Metal ions are widely present in cosmetic products at trace concentrations that may penetrate into or through human skin to produce systemic exposure after applications (Bocca *et.al*, 2014).

In light of the incomplete knowledge regarding the pro-oxidation potential of L-ascorbic acid and ascorbic acid derivative chemicals, and the likelihood that metals will be present in cosmetics containing the notified chemical, its potential for pro-oxidation cannot be ruled out.

*Acute toxicity*
No acute toxicity study data were provided for the notified chemical.

QSAR modelling using *Toxicity Estimation Software Tool* (version 4.1, US EPA) predicted that the notified chemical is of low toxicity via the oral route (KVD/TG, 2013). This prediction is supported by the low acute oral toxicity (> 5,000 mg/kg bw in most cases) obtained for L-ascorbic acid in a range of species (OECD SIDS, 1994; CIR, 2005).

*Irritation and sensitisation*
Test results of the notified chemical using the *in vitro* reconstructed human *Epidermis* model suggested that the chemical is non-irritating to skin. This is supported by test results on human volunteers using patches of the notified chemical at 2% concentration. The notified chemical did show skin irritation effects in the patch test.

In an *in vitro* eye irritation study, the notified chemical at 10% concentration was not cytotoxic to fibroblast cells of rabbit cornea. However, an *in vitro* eye irritation study using the HET-CAM model indicated that the notified chemical at 3% concentration was moderately irritating. Based on the latter study, the notified chemical is likely to be irritating to the eyes. It is also noted that the notified chemical is classified as a Category 2 eye irritant in the MSDS provided by the notifier.

In a human repeat insult patch test (HRIPT) completed on the skin of 56 volunteers, the notified chemical at 100% concentration was considered by the study authors to be non-sensitising. This result is supported by observations on L-ascorbic acid (CIR, 2005), showing non-sensitising properties via the dermal route in a study of 103 human subjects using an opaque cream at 5% concentration, and a maximisation assay on 26 human subjects using a facial treatment containing 10% L-ascorbic acid.

*Repeated dose toxicity*
No repeated dose toxicity data were provided for the notified chemical.

Information on the repeat dose toxicity of L-ascorbic acid is available (CIR, 2005) which generally indicates the absence of effects deemed toxicologically adverse at doses ≤ 1,000 mg/kg bw/day.

*Mutagenicity/Genotoxicity*
The notified chemical was not mutagenic in a bacterial reverse mutation study.

For L-ascorbic acid, the weight of evidence from assays in bacteria and mammalian cells *in vitro* indicate the absence of ability to produce gene mutations. Some positive results were noted in the *in vitro* assays for unscheduled DNA synthesis (UDS) and sister chromatid exchanges (SCE), but were considered to be due to formation of hydrogen peroxide and reactive oxygen species at very high concentrations which would not be expected to be relevant to the *in vivo* situation. Negative results were obtained *in vivo* for both clastogenicity and SCE induction. Ascorbic acid has been considered to have no significant mutagenic potential (OECD SIDS, 1994).

*Toxicity for reproduction*
No reproductive/developmental toxicity data were provided for the notified chemical.

A number of developmental studies for L-ascorbic acid have been published (OECD SIDS, 1994; CIR, 2005). All the studies showed that high doses of L-ascorbic acid (1,000 mg/kg bw/day) had no effect on development of the offspring or on breeding, pregnancy, parturition or lactation in the maternal animals.

*Observations on human exposure*
No human exposure observation information for the notified chemical was submitted.

According to *Nutrient Reference Values for Australia and New Zealand* (NRV, 2005), the recommended daily intake (RDI) for vitamin C (L-ascorbic acid) is 45 mg/day with a prudent upper level limit of 1,000 mg/day for an average adult. Gastrointestinal effects were the most common adverse effects associated with acute, high doses given over a short period of time. Other reported effects included metabolic acidosis, changes in prothrombin activity and low ingestion in pregnancy conditioning the need for higher amounts in the infant. It has also been suggested that consumption of L-ascorbic acid may increase oxalate excretion. However, studies in humans have not revealed a substantial increase in urinary oxalate stones with high intakes of L-ascorbic acid. The studies concluded that L-ascorbic acid is not associated with significant adverse effects (NRV, 2005).

***Health hazard classification***
Based on the limited available information, the notified chemical cannot be recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

### 6.3. Human Health Risk Characterisation

#### 6.3.1. Occupational Health and Safety
Based on the available information, the notified chemical has potential to cause eye irritation effects. As an antioxidant used in cosmetics, the notified chemical may present topical depigmentation effects on skin which sometimes may not be reversible. Under complex combination of topical conditions, the notified chemical may produce pro-oxidant activity leading to adverse cytotoxicity.

*Reformulation*
Dermal, ocular and inhalation exposure of workers to the notified chemical at various concentrations up to 100% may occur during formulation of cosmetics. As stated by the notifier, the use of PPE such as coveralls, eye protection, impervious gloves and respiratory protection (as appropriate), and engineering controls including automated/enclosed blending processes and local exhaust ventilation should minimise the risk for workers. Provided that the protective measures and engineering controls proposed are implemented, the use of the notified chemical is not expected to pose an unreasonable risk to workers under the occupational conditions described.

*End use*
Store persons and professional end users may come into contact with cosmetic products containing the notified chemical at ≤ 10% concentration. These products will also be available to the public. The risk to workers who regularly handle these products is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified polymer (for details of the public health risk assessment, see Section 6.3.2).

#### 6.3.2. Public Health
The notified chemical is a derivative of L-ascorbic acid. Use of ascorbic acid and its derivatives in cosmetic products has been reviewed by Cosmetic Ingredient Review Expert Panel (CIR, 1999 and 2005).

In Europe, ascorbic acid derivatives have been used as skin depigmenting agents at concentrations of 2-3% (Prakash *et.al*, 2009) and in cosmetic water/oil emulsions as antioxidants at ≤ 2% (CIR, 1999). It has been reported (CIR, 2005) that there were 431 cosmetic formulations containing L-ascorbic acid from 10 ppm to 10% from various product categories based on information from US Food and Drug Administration (US FDA). Various ascorbic acid derivatives have also been previously used in Australia at concentrations ≤ 5% (typically in the range of 0.01 to 2% as an antioxidant and ≤ 5% in specialised skin care products as skin lightening agent).

There will be widespread and repeated exposure of the public to the notified chemical through the use of the cosmetic products at proposed concentrations of ≤ 10% in individual products. The principal route of exposure

will be dermal, while ocular exposure is also possible. Inhalation exposure to the notified chemical is not expected based on proposed use scenario and its low vapour pressure.

*Eye irritation*
The notified chemical has potential to cause eye irritation. However, at the proposed use concentrations of ≤ 10% in cosmetic products, significant eye irritation effects of the notified chemical are not expected. The eye irritation risk may be further minimised by the inclusion of appropriate labelling and directions for use to warn consumers against eye contact.

*Risk of repeated exposure*
Estimation of repeated dose toxicity potential of the notified chemical using the worst case exposure scenario from the use of multiple products would result in a combined internal dose of 9.827 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 1,000 mg/kg bw/day based on the results of studies conducted on L-ascorbic acid, the margin of exposure (MoE) was estimated to be 102. A MoE value greater than or equal to 100 is generally considered acceptable to account for intra- and inter-species differences. Based on the above estimation, the notified chemical is unlikely to cause systemic or reproductive/development toxicity by normal use of the cosmetic products containing the chemical.

*Depigmentation and pro-oxidant effects*
As an antioxidant used in cosmetics, the notified chemical has the potential to cause skin depigmentation that may be irreversible. The extent of this effect depends on many factors, including the type of cosmetic product, and the type and frequency of application. Therefore it is recommended that products containing the notified chemical be labelled to warn consumers of the possibility of such unintended consequences.

Under certain complex topical use conditions, the notified chemical may also produce pro-oxidant activity. It has been recommended that formulators of the cosmetics should ensure vitamin C and its derivatives acting as antioxidants in the formulations avoid possible pro-oxidant activity (CIR, 2005).

Based on the available information, with appropriate labelling regarding risks associated with eye irritation, skin depigmentation and possible adverse effects caused by pro-oxidant activity of the chemical, the risk to the public from use of the notified chemical at ≤ 10% in cosmetics is not considered to be unreasonable.

## 7. ENVIRONMENTAL IMPLICATIONS

### 7.1. Environmental Exposure & Fate Assessment

#### 7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will not be manufactured in Australia; therefore there is no release of the notified chemical to the environment from this activity. Environmental release during importation, transport and distribution may occur as a result of accidental spills. In the event of a spill, the notified chemical is expected to be contained and collected in an inert absorbent material and disposed of in accordance with local regulations.

The notified chemical will be blended with other ingredients in automated/enclosed facilities to produce cosmetic products. Release from blending is expected to be very low. A total of up to < 1% of the import volume is estimated to be generated as waste from residues in empty containers and spills during blending. Empty containers containing the notified chemical will either be recycled or disposed of through an approved waste management facility.

RELEASE OF CHEMICAL FROM USE
The majority of the notified chemical is expected to be released to sewers across Australia as a result of its use in cosmetic products, which are washed off the hair and skin of consumers and disposed of to the sewer.

RELEASE OF CHEMICAL FROM DISPOSAL
It is expected that about 3% (30 kg) of the product containing the notified chemical will remain in end-use containers. The containers are expected be disposed of through domestic garbage disposal and will enter landfill, or be subjected to recycling processes.

### 7.1.2. Environmental Fate

Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system through its use as a component of cosmetics before potential release to surface waters nationwide. The notified chemical is not readily biodegradable (38% after 28 days) in the environment and is not expected to hydrolyse under environmental conditions. Therefore, the notified chemical has the potential to persist in the aquatic compartment. However, in surface waters the notified chemical is expected to disperse and degrade into water and oxides of carbon. For details of the environmental fate studies, please refer to Appendix C. Upon release to the aquatic environment in effluent from sewage treatment plants (STPs), the notified chemical is expected to remain in the water column due to very high water solubility, low vapour pressure and low n-octanol/water partition coefficient (log $K_{OW}$). The notified chemical is expected to leach through soil and sediments given its low adsorption/desorption coefficient (log $K_{OC}$). Given the notified chemical's low log $K_{OW}$, it is not expected to bioaccumulate.

The half-life of the notified chemical in air is calculated to be 1.749 hours based on reactions with hydroxyl radicals (AOPWIN v1.92; US EPA, 2011). Therefore, in the event of release to atmosphere, the notified chemical is not expected to persist in the atmospheric compartment.

The majority of the notified chemical will be released to sewer after use. A small proportion of notified chemical may be applied to land when effluent is used for irrigation, or disposed of to landfill as waste. Notified chemical residues in landfill and soils are expected to leach through soil and sediments based on its low soil adsorption coefficient. In the aquatic and soil compartments, the notified chemical is expected to degrade through biotic and abiotic processes to form water and oxides of carbon.

### 7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. Based on the reported uses in cosmetic products, it is conservatively assumed that 100% of the notified chemical will be released to sewer on a nationwide basis over 365 days per year. It is also assumed that under a worst-case scenario there is no removal of the notified chemical during STP processes.

| Predicted Environmental Concentration (PEC) for the Aquatic Compartment | | |
|---|---|---|
| Total Annual Import/Manufactured Volume | 1,000 | kg/year |
| Proportion expected to be released to sewer | 100% | |
| Annual quantity of chemical released to sewer | 1,000 | kg/year |
| Days per year where release occurs | 365 | days/year |
| Daily chemical release: | 2.74 | kg/day |
| Water use | 200.0 | L/person/day |
| Population of Australia (Millions) | 22.613 | million |
| Removal within STP | 0% | |
| Daily effluent production: | 4,523 | ML |
| Dilution Factor - River | 1.0 | |
| Dilution Factor - Ocean | 10.0 | |
| PEC - River: | 0.61 | µg/L |
| PEC - Ocean: | 0.06 | µg/L |

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m$^2$/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1,500 kg/m$^3$). Using these assumptions, irrigation with a concentration of 0.6 µg/L may potentially result in a soil concentration of approximately 4.03 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years may be approximately 20.19 µg/kg and 40.39 µg/kg, respectively.

## 7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

| *Endpoint* | *Result* | *Assessment Conclusion* |
|---|---|---|
| Daphnia Toxicity | EC50 (48 h) > 100 mg/L | Not harmful to aquatic invertebrates |

| *Endpoint* | *Result* | *Assessment Conclusion* |
|---|---|---|
| Algal Toxicity | $E_rC50$ (72 h) > 100 mg/L | Not harmful to algae |

Based on the endpoints for toxicity of the notified chemical to aquatic organisms, the notified chemical is not considered to be harmful to aquatic organisms under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). Therefore, the notified chemical is not formally classified under the GHS. Based on its measured acute toxicity, lack of ready biodegradability and expected low bioaccumulation potential, the notified chemical is not formally classified under the GHS for the chronic hazard.

#### 7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has not been calculated for the notified chemical as no significant adverse effects were observed in any of the ecotoxicity tests submitted.

### 7.3. Environmental Risk Assessment

The majority of the notified chemical will be disposed of to the sewer, based on its use as a component in cosmetic products. The notified chemical has a low potential for bioaccumulation and is not expected to be harmful to aquatic organisms. Therefore, on the basis of the assessed use pattern in cosmetic products, the notified chemical is not expected to pose an unreasonable risk to the environment.

## APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

**Melting Point** 112 °C

| | |
|---|---|
| Method | OECD TG 102 Melting Point/Melting Range<br>EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature |
| Remarks | Capillary method |
| Test Facility | Exempt Information (2013a) |

**Boiling Point** 214 ± 5 °C at 98.7 kPa

| | |
|---|---|
| Method | OECD TG 103 Boiling Point<br>EC Council Regulation No 440/2008 A.2 Boiling Temperature |
| Remarks | Siwoloboff test method. At boiling point the test substance turned from clear to brown indicating a sign of decomposition. |
| Test Facility | Exempt Information (2013a) |

**Density** 1,410 kg/$m^3$ at 27.2 °C

| | |
|---|---|
| Method | OECD TG 109 Density of Liquids and Solids<br>EC Council Regulation No 440/2008 A.3 Relative Density |
| Remarks | Determined with a helium pycnometer |
| Test Facility | Exempt Information (2013a) |

**Vapour Pressure** $3.1 \times 10^{-10}$ kPa at 25 °C

| | |
|---|---|
| Method | OECD TG 104 Vapour Pressure<br>EC Council Regulation No 440/2008 A.4 Vapour Pressure |
| Remarks | Calculated with EPI Suite 4.10 using measured melting point and boiling point. Modified grain method. |
| Test Facility | Exempt Information (2013a) |

**Water Solubility** 778 g/L at 20 °C

| | |
|---|---|
| Method | OECD TG 105 Water Solubility.<br>EC Council Regulation No 440/2008 A.6 Water Solubility. |
| Remarks | Flask Method |
| Test Facility | Exempt Information (2013a) |

**Partition Coefficient (n-octanol/water)** log Pow = - 0.71 at 20 °C

| | |
|---|---|
| Method | OECD TG 117 Partition Coefficient (n-octanol/water).<br>EC Council Regulation No 440/2008 A.8 Partition Coefficient. |
| Remarks | Flask Method |
| Test Facility | Exempt Information (2013a) |

**Surface Tension** 69.2 mN/m at 20 °C

| | |
|---|---|
| Method | OECD TG 115 Surface Tension of Aqueous Solutions.<br>EC Council Regulation No 440/2008 A.5 Surface Tension. |
| Remarks | Concentration: 1 g/L |
| Test Facility | Exempt Information (2013a) |

**Particle Size** $d_{50}$ = 95.9 µm

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

| *Range (µm)* | *Mass (%)* |
|---|---|
| < 10 | 7.89 |

| *Range (μm)* | *Mass (%)* |
|---|---|
| < 50 | 36.0 |
| < 100 | 51.1 |

Remarks Laser diffraction method was used.
Test Facility Exempt Information (2013a)

**Flammability – Solids** Not highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks Propagation time for combustion over 200 mm of a mould powder train of 250 (L) × 20 (W) × 10 (H) mm was > 4 minutes.
Test Facility Exempt Information (2013a)

**Explosive Properties** Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks Thermal stability was determined by DSC (Differential Scanning Calorimetry). The exothermic decomposition energy was determined to be 518 kJ/kg between 30 °C and 400 °C which is slightly above the limit of 500 kJ/kg for exclusion under the guidelines. However, the notified chemical is not expected to be explosive based on the chemical structure.
Test Facility Exempt Information (2013a)

**Oxidizing Properties** Not oxidising

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids).
Remarks Based on chemical structure
Test Facility Exempt Information (2013a)

# APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

## B.1. Acute toxicity – oral (QSAR calculation)

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | QSAR (Toxicity Estimation Software Tool, version 4.1, US EPA) |
| Species | Rat |
| Remarks - Method | The notified chemical is within the applicability domain of the training data set. |
| RESULTS | |
| LD50 | 5903.13 mg/kg bw |
| Remarks - Results | Validation of the prediction resulted in a correlation ($R^2$) value of 0.626 and a coverage of 0.984 for the consensus. Further weight of evidence was required to support the prediction. |
| CONCLUSION | The notified chemical was predicted to be of low toxicity via the oral route. |
| TEST FACILITY | Exempt Information (2013b) |

## B.2. Irritation – skin (*in vitro* reconstructed human *Epidermis* test)

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method<br>EC Commission Regulation No 761/2009 B.46. *In vitro* Skin Irritation - Reconstructed Human *Epidermis* Test Method |
| Vehicle | None, skin model was moistened with phosphate buffered saline. |
| Remarks - Method | No significant deviation of the protocol was noted. 25 mg of the test substance was applied directly to the skin model with a surface area of 0.6 $cm^2$. 5% SDS was used as positive control. |

RESULTS

| *Test material* | *Mean $OD_{570}$ of triplicate tissues* | *SD of mean $OD_{570}$* | *Relative mean viability (%)* |
|---|---|---|---|
| *Negative control* | 2.271 | 0.142 | 100 |
| *Test substance* | 2.243 | 0.079 | 98.8 |
| *Positive control* | 0.086 | 0.004 | 3.8 |

OD = optical density; SD = standard deviation

| | |
|---|---|
| Remarks - Results | The relative mean viability of the test substance was > 50%, the cut-off value for a skin irritant. |
| CONCLUSION | The notified chemical was non-irritating to the skin under the conditions of the test. |
| TEST FACILITY | Exempt Information (2013c) |

## B.3. Irritation – skin (*in vitro* reconstructed human *Epidermis* test with penetration)

| | |
|---|---|
| TEST SUBSTANCE | Cosmetic product containing 2% notified chemical |
| METHOD | Similar to OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method |
| Vehicle | None, test substance applied directly |
| Remarks - Method | Penetration test<br>26.3 $mg/cm^2$ test substance (containing 0.542 $mg/cm^2$ notified chemical) |

was applied to the top of epidermis. Receptor fluids were collected every hour for 8 hours at 32 °C and analysed using HPLC/DAD for the notified chemical that reached receptor fluids.

Irritation test
MTT assay and IL-1α release quantitatively measured using $OD_{595}$ and ELISA, respectively, were utilised to determine the irritation potential of the test substance.

Positive control
Potassium hydroxide (8N).

RESULTS

Penetration

| *Time (h)* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
|---|---|---|---|---|---|---|---|---|
| *Cumulative penetration (µg/cm²)* | 0.54 | 1.96 | 3.23 | 4.80 | 6.42 | 7.85 | 9.74 | 11.47 |
| *SD of cumulative penetration (µg/cm²)* | 0.17 | 0.52 | 1.06 | 1.42 | 1.97 | 2.33 | 2.36 | 2.57 |
| *Penetration rate (%)* | 0.10 | 0.36 | 0.60 | 0.89 | 1.19 | 1.46 | 1.81 | 2.13 |

SD = standard deviation

Irritation
*Results*

| *Test material* | *Relative viability by MTT test (%)* | *IL-1α release (pg/mL)* |
|---|---|---|
| *Negative control* | 100 | < 50 |
| *Test substance* | 113.97 | < 50 |
| *Positive control* | 34.0 | < 50 |

*Interpretation Criteria*

| *Criteria* | *Interpretation* |
|---|---|
| Relative viability ≤ 50% | Irritant |
| Relative viability > 50% AND IL-1α release ≥ 50 pg/mL | Irritant |
| Relative viability > 50% AND IL-1α release < 50 pg/mL | Non-irritant |

Remarks - Results
The test substance did not directly reduce MTT in a pre-test.

The test substance was interpreted to be a non-irritant with notified chemical penetrating at 11.47 ± 2.57 µg/cm² (2.13% of the amount applied) in 8 hours at 32 °C.

CONCLUSION
The test substance was non-irritating to the skin under the conditions of the test.

TEST FACILITY
Exempt Information (undated)

**B.4. Irritation – skin (patch test)**

TEST SUBSTANCE
Notified chemical (2%)

METHOD
Semi-occlusive patch test on volunteers

Volunteers
11 (8 F/3 M) volunteers aged 19 to 63 years participated.

Vehicle
Unspecified

Application Period
48 hours

Type of Dressing
Semi-occlusive

Remarks - Method
Single application of 0.02 mL of the test substance diluted at 2% was administered on the external side of the arm and maintained for 48 hours under semi-occlusive conditions. Skin reactions including erythema, oedema, papules, vesicles and blisters were observed 30 minutes after the patches were removed.

| | |
|---|---|
| RESULTS | No significant skin reactions were recoded. |
| Remarks - Results | All volunteers completed the test. |
| CONCLUSION | The notified chemical at 2% concentration is non-irritating to the skin under the conditions of the test. |
| TEST FACILITY | Exempt Information (2009a) |

**B.5. Irritation – eye (*in vitro* NRR test)**

| | |
|---|---|
| TEST SUBSTANCE | Notifier chemical |
| METHOD | Reader *et al* (1990) Neutral Red Release (NRR) from Pre-Loaded Cells |
| Vehicle | Distilled water and 0.9% sodium chloride |
| Remarks - Method | The test substance was dissolved in distilled water at 10% and further diluted with 0.9% sodium chloride at 5, 15, 25, 35 and 50% by weight to yield 0.5, 1.5, 2.5, 3.5 and 5% final test concentrations. Diluted test substance was put in contact for 60 seconds with fibroblast cells of rabbit cornea marked by neutral red. Cytotoxicity was examined by the percentage of cell death and the IC50 (inhibition concentration of 50% cell survival) determined by measuring the neutral red retained in the cells using $OD_{540}$. Negative control used was 0.9% sodium chloride. Positive controls were 0.01%, 0.05% and 0.2% SDS in 0.9% sodium chloride. |

RESULTS

| *Dilution (%)* | *PC* | *NC* | *5* | *15* | *25* | *35* | *50* |
|---|---|---|---|---|---|---|---|
| *Mean $OD_{540}$* | 0.405 | 1.032 | 1.037 | 1.057 | 1.002 | 1.021 | 0.999 |
| *Cell death (%)* | 61 | 0 | 0 | 0 | 3 | 1 | 3 |

NC = Negative Control (0.9% sodium chloride); PC = Positive Control (0.2% SDS)

*Interpretation Criteria*

| *IC50 (% dilution)* | *Cell death at 50% dilution (%)* | *Interpretation* |
|---|---|---|
| > 50 | ≤ 20 | Negligible cytotoxicity |
| > 50 | > 20 and < 50 | Slightly cytotoxic |
| > 25 and ≤ 50 | | Moderately cytotoxic |
| ≤ 25 | | Severely cytotoxic |

| | |
|---|---|
| Remarks - Results | The IC50 for the test substance was determined to be > 50% (equivalent to 5% concentration) with a cell death rate of 3% at 50% dilution, indicating negligible level of cytotoxicity. The IC50 for the positive control substance (SDS) was determined to be 0.16%, indicating expected severe cytotoxicity. |
| CONCLUSION | The notified chemical at 10% concentration was not cytotoxic under the conditions of the test. |
| TEST FACILITY | Exempt Information (2009b) |

**B.6. Irritation – eye (*in vitro* HET-CAM)**

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical (3%) |
| METHOD | HET-CAM Test - Official Journal of the Republic France (#302, 26 December 1996) |
| Vehicle | Physiological serum |
| Remarks - Method | The notified chemical was tested at 3% dilution in 4 eggs. The treated membranes were observed for 5 minutes to record signs of hyperaemia, haemorrhage, coagulation, opacity and/or thrombus. |

*N*-Dodecylsulfobetaine (CAS RN 14933-08-5) in physiological serum was used as a negative control at 0.05% and as a positive control at 0.4% and 3.2%.

RESULTS

| *Egg No.* | *Score* | | | *Total score* |
|---|---|---|---|---|
| | *Hyperaemia* | *Haemorrhage* | *Coagulation, opacity/thrombus* | |
| 1 | 3 | 3 | 0 | 6 |
| 2 | 3 | 3 | 0 | 6 |
| 3 | 3 | 3 | 0 | 6 |
| 4 | 3 | 3 | 0 | 6 |

Remarks - Results: Negative and positive controls showed expected results.

The test substance at 3% dilution resulted in a mean score of 6, indicating moderate irritancy.

CONCLUSION: The notified chemical at 3% concentration was considered to be moderately irritating to the eye under the conditions of the test.

TEST FACILITY: Exempt Information (2009c)

**B.7. Skin sensitisation – volunteers**

TEST SUBSTANCE: Notified chemical

METHOD: Repeated insult patch test with challenge

Study Design: *Induction Procedure:* 160 mg of the test substance was applied to the back of each subject every Monday, Wednesday and Friday and replaced on every Wednesday, Friday and Monday respectively until 9 applications were reached. Skin reactions were recorded on every replacement.

*Rest Period:* 2 weeks

*Challenge Procedure:* The challenge patches were applied to both a previously unpatched site and a patched site. Skin reactions were recorded 20 minutes, 24 hours and 48 hours after patch removal.

Study Group: 46 F, 11 M; age range 19 to 69 years (24 with sensitive skin)

Vehicle: None; the patches were moistened with 160 µL of water before application.

Remarks - Method: Semi-occluded. The test substance was spread on a patch in the size of 4 $cm^2$.

RESULTS

Remarks - Results: One subject did not return for the challenge procedure due to family reasons. No skin irritation effects were observed during the induction and challenge procedures. No evidence of induced allergic contact dermatitis was recorded during the study.

CONCLUSION: The test substance was non-sensitising under the conditions of the test.

TEST FACILITY: Exempt Information (2013d)

**B.8. Genotoxicity – bacteria**

TEST SUBSTANCE: Notified chemical

| | |
|---|---|
| METHOD | OECD TG 471 Bacterial Reverse Mutation Test<br>EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria<br>Plate incorporation procedure (Test 1), repeated with pre incubation procedure (Test 2) |
| Species/Strain | *S. typhimurium*: TA1535, TA1537, TA98 and TA100<br>*E. coli*: WP2 (pKM101) |
| Metabolic Activation System | S9 fraction from Aroclor induced rat liver |
| Concentration Range in Main Test | a) With metabolic activation: 200 – 5,000 µg/plate<br>b) Without metabolic activation: 200 – 5,000 µg/plate |
| Vehicle | Distilled water |
| Remarks - Method | No significant deviation of protocol was noted. |

RESULTS

| *Metabolic Activation* | *Test Substance Concentration (µg/plate) Resulting in:* | | | |
|---|---|---|---|---|
| | *Cytotoxicity in Preliminary Test* | *Cytotoxicity in Main Test* | *Precipitation* | *Genotoxic Effect* |
| *Absent* | | | | |
| Test 1 | > 5,000 | > 5,000 | > 5,000 | Negative |
| Test 2 | > 5,000 | > 5,000 | > 5,000 | Negative |
| *Present* | | | | |
| Test 1 | > 5,000 | > 5,000 | > 5,000 | Negative |
| Test 2 | > 5,000 | > 5,000 | > 5,000 | Negative |

| | |
|---|---|
| Remarks - Results | There were no biological significant increases in the number of revertant colonies observed in any of the strains, at any of the concentrations tested, either in the presence or absence of metabolic activation. No dose response was observed in the tested bacterial strains.<br><br>The positive controls produced satisfactory responses, thus confirming the performance of the test system and the metabolic activation. |
| CONCLUSION | The notified chemical was not mutagenic to bacteria under the conditions of the test. |
| TEST FACILITY | Exempt Information (2009d) |

# APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

## C.1. Environmental Fate

### C.1.1. Ready biodegradability

| | |
|---|---|
| TEST SUBSTANCE | Notified Chemical |
| METHOD | OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test. |
| Inoculum | Activated sludge |
| Exposure Period | 28 days |
| Auxiliary Solvent | None Reported |
| Analytical Monitoring | Biochemical oxygen demand (BOD) |
| Remarks - Method | The test was conducted in accordance with the test guideline above with no significant deviation from the protocol reported. |

RESULTS

| *Test substance* | | *Sodium benzoate* | |
|---|---|---|---|
| *Day* | *% Degradation* | *Day* | *% Degradation* |
| 3 | 2.67 | 4 | 58.51 |
| 7 | 2.67 | 7 | 74.47 |
| 14 | 10.00 | 14 | 85.11 |
| 21 | 24.67 | 21 | 87.77 |
| 28 | 38.00 | 28 | 89.76 |

| | |
|---|---|
| Remarks - Results | After 28 days, the percent degradation for the notified chemical was 38.0%. The percent degradation calculated in the reference item replicate (procedure control) up to day 28 was 89.76%. In the toxicity control, more than 25% degradation was observed up to day 14. |
| CONCLUSION | The notified chemical is not readily biodegradable. |
| TEST FACILITY | Exempt Information (2014) |

## C.2. Ecotoxicological Investigations

### C.2.1. Acute toxicity to aquatic invertebrates

| | |
|---|---|
| TEST SUBSTANCE | Notified chemical |
| METHOD | OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static. |
| Species | *Daphnia magna* |
| Exposure Period | 48 hours |
| Auxiliary Solvent | None reported |
| Water Hardness | None reported |
| Analytical Monitoring | High performance liquid chromatography (HPLC) |
| Remarks - Method | The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed. |

RESULTS

| *Concentration mg/L* | *Number of D. magna* | *Number Immobilised* | |
|---|---|---|---|
| *Nominal* | | *24 h* | *48 h* |
| Control | 20 | 0 | 0 |
| 100 | 20 | 0 | 0 |

| | |
|---|---|
| LC50 | > 100 mg/L at 48 hours |

| | |
|---|---|
| NOEC | 100 mg/L at 48 hours |
| Remarks - Results | Because significant toxic response was not observed during the preliminary concentration range-finding tests, only one test concentration at 100 mg/L was used in the main study. The deviation from the nominal concentration of the measured concentration was less than 20%. |
| CONCLUSION | The notified chemical is not harmful to aquatic invertebrates |
| TEST FACILITY | Exempt Information (2013e) |

**C.2.2. Algal growth inhibition test**

| | |
|---|---|
| TEST SUBSTANCE | Notified Chemical |
| METHOD | OECD TG 201 Alga, Growth Inhibition Test.<br>Effects on Biotic Systems, Version 2, 201 Algae Growth Inhibition Test, The China Environment Press. 2013. |
| Species | *Pseudokirchneriella subcapitata* |
| Exposure Period | 72 hours |
| Concentration Range | Nominal: 100 mg/L |
| Auxiliary Solvent | None reported. |
| Water Hardness | None reported. |
| Analytical Monitoring | High performance liquid chromatography (HPLC) |
| Remarks - Method | Because significant toxic response was not observed during the preliminary concentration range-finding tests, only one test concentration at 100 mg/L was used in the main study.<br><br>The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed. |

RESULTS

| *Biomass* | | *Growth* | |
|---|---|---|---|
| *$E_yC50$ (mg/L at 72h)* | *NOEC (mg/L at 72h)* | *$E_rC50$ (mg/L at 72 h)* | *NOEC (mg/L at 72h)* |
| > 100 | 100 | > 100 | 100 |

| | |
|---|---|
| Remarks - Results | Because significant toxic response was not observed in the preliminary range-finding test at a concentration of 100 mg/L, only this concentration and one control were tested in a limit-test. |
| CONCLUSION | The notified chemical not harmful to algae. |
| TEST FACILITY | Exempt Information (2015) |

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