File No: LTD/1705

July 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

L-Ascorbic acid, 2,3,5,6-tetrakis(2-hexyldecanoate) (INCI name: Ascorbyl Tetraisopalmitate)

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.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also 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:

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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/1705	Ceechem Australia Pty Ltd	L-Ascorbic acid, 2,3,5,6-tetrakis(2-hexyldecanoate) (INCI name: Ascorbyl Tetraisopalmitate)	ND*	≤ 1 tonne/s per annum	Cosmetic ingredient.

*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).

The environmental hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Acute (Category 3)	H402 - Harmful to aquatic life
Chronic (Category 3)	H412 - Harmful to aquatic life with long lasting effects

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

Based on the available information, when used at ≤ 10% in cosmetic products the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio 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:
  - Enclosed, automated processes, where possible

- 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:
  - 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:
  - Coveralls, impervious gloves, safety glasses

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.

- As the notified chemical may 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 unavailable or impracticable, dispose of the 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, physical 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 concentration of the notified chemical exceeds or is intended to exceed 10% in cosmetic products;
- additional information on the repeated dose toxicity of the notified chemical becomes available.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from cosmetic ingredient, 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 provided by the notifier was 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

APPLICANT(S)
Ceechem Australia Pty Ltd (ABN: 61081398192)
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: analytical data, degree of purity, residual impurities, additives/adjuvants, import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: All physico-chemical properties, acute dermal toxicity, acute inhalation toxicity, repeated dose toxicity, *in vitro* and *in vivo* genotoxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
Japan, Korea.

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Nikkol VC-IP

CHEMICAL NAME
L-Ascorbic acid, 2,3,5,6-tetrakis(2-hexyldecanoate)

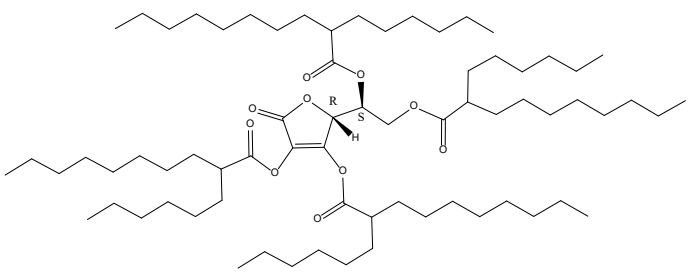
OTHER NAME(S)
Ascorbic acid tetraisopalmitate
Ascorbyl 2,3,5,6-tetrahexyldecanoic acid
Ascorbyl Tetraisopalmitate (INCI name)
IPAA

CAS NUMBER
183476-82-6

MOLECULAR FORMULA
$C_{70}H_{128}O_{10}$

MOLECULAR WEIGHT
1,129.76 Da

STRUCTURAL FORMULA



ANALYTICAL DATA
Reference HNMR and FTIR spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None.

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None.

ADDITIVES/ADJUVANTS
None.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: colourless to light yellow liquid.

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Not determined	-
Boiling Point	Not determined	-
Density	930 - 943 kg/m^3 at 20 °C	(M)SDS
Vapour Pressure	1.64 x 10^{-27} kPa at 25 °C	Calculated (US EPA MPBPVP, 2014)
Water Solubility	Not determined	Expected to be low based on the predominantly hydrophobic structure and high molecular weight of the notified chemical.
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functional groups. However, significant hydrolysis is not expected in the environmental pH range of 4 – 9.
Partition Coefficient (n-octanol/water)	Not determined	Expected to partition to n-octanol based on the expected low water solubility of the notified chemical.
Adsorption/Desorption	Not determined	Expected to partition to soil/sludge based on the expected low water solubility and high molecular weight of the notified chemical.
Dissociation Constant	Not determined	Does not contain dissociable functionality.
Flash Point	> 100 °C at 101 kPa	(M)SDS
Autoignition Temperature	Not determined	Not expected to autoignite under normal conditions of use.
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties.

DISCUSSION OF PROPERTIES
Reactivity
In stability testing, formulations containing the notified chemical had a medium stability at the optimal pH of 5.5 (with shelf lives of 6 to 12 months) (Campos *et.al*, 2012).

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 be imported as a neat chemical for local reformulation into end-use cosmetic products containing the notified chemical at proposed concentrations ≤ 30%.

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
Sydney.

IDENTITY OF MANUFACTURER/RECIPIENTS
Ceechem Australia Pty Ltd.

TRANSPORTATION AND PACKAGING
The notified chemical in its neat form will be imported in 1 and 5 kg cans and transported to the customer sites for formulation into cosmetic products. After reformulation, products containing the notified chemical will be packed in tubes or jars (ranging in size from 10 to 200 mL) and distributed to retail stores or salons. Within Australia, the notified chemical will be transported by road and/or rail.

USE
The notified chemical will be used as a cosmetic ingredient in a variety of skin care products (such as skin creams and lotions) at proposed usage concentrations of ≤ 30%.

The notifier has stated that no therapeutic claims will be made for products containing the notified chemical.

OPERATION DESCRIPTION

Reformulation
At the reformulation sites, the notified chemical will be blended into end-use cosmetic products. Procedures will vary depending on the nature of the cosmetic product being formulated. The notified chemical will form part of the oil phase of topical creams and lotions, using standard emulsification techniques. Both manual and automated steps will be involved. Manual processes could include weighing of an appropriate amount of the notified chemical into a container then transferring the chemical directly into a blending tank, with periodic sampling for quality control purposes carried out during the manufacturing process. Automated processes may include mixing stages and filling of end-use products into containers.

End-use
Finished cosmetic products containing the notified chemical (at proposed concentrations of ≤ 30%) may be used by consumers or by professionals, such as workers in beauty salons. Depending on the nature of the product, the application to skin could be by hand, or 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)*
Distribution (Transport and Storage)	4	12 - 24
Reformulation	8	12 – 24
QA	2	12 – 24
End Use (Retail workers)	8	260

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come in contact with the notified chemical either in neat form or in end-use cosmetic products, only in the event of accidental rupture of containers.

At the notifier's facility, the primary work undertaken by transport and warehouse workers will be the handling, loading and off-loading of drums containing the notified chemical at ≤ 100% concentration. Exposure of these workers will be limited to situations involving product sampling for quality control or, in the event of a discharge, clean up from a spill or leaking drum. If such an event occurs, a worker may be exposed through dermal or ocular contact. The notifier has indicated that such exposures will be minimised to the extent possible through the use of personal protective equipment (PPE).

Reformulation

During reformulation into cosmetic products, dermal and ocular exposure of workers may occur when handling the notified chemical or products containing it. Inhalation exposure is not expected unless aerosols are generated. Exposure is expected to be minimised through the use of exhaust ventilation and/or automated/enclosed systems as well as through the use of PPE, such as coveralls, safety glasses and impervious gloves.

End-use

Exposure to the notified chemical in end-use products (at proposed concentrations of ≤ 30%) may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. workers in beauty salons). The principal route of exposure will be dermal, while ocular exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, and 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 lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

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 ≤ 30% in individual products). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. The notifier has stated the products containing the notified chemical will be applied sparingly to sun-exposed areas of the face/neck, hands and arms.

The notified chemical is predicted to be formulated in a diverse range of cosmetic products (both leave-on and rinse-off types). The notifier has provided data indicating the product range that may contain the notified chemical to include creams (emollient, gel and mask), lotions, essence and toners, lipsticks, spot sticks and line filler emulsions.

Due to the predicted significant public exposure to the notified chemical in cosmetic products, consideration of the typical concentration range of both the notified chemical and similar ascorbic acid (a form of Vitamin C) derivative chemicals, used internationally and domestically in cosmetic formulations, has been made.

An array of cosmetic products containing ascorbic acid derivative chemicals has been examined in Cosmetic Ingredient Reviews (CIR) in 1999 and 2005. The 1999 Review featuring the ascorbic acid derivative chemical Ascorbyl palmitate indicated that the ingredient is used in a variety of cosmetic products (as seen in the table

below) such as eye creams, body cleansers, cologne and body oils at 0.01 to 0.2% concentration. It was noted that Ascorbyl palmitate is also used at 0.1 to 4% concentration in Japanese cosmetic preparations to inhibit the formation of melanin. Ascorbyl dipalmitate was described as not in current use, with the notation that 'were the ingredient to be used in the future, the expectation is that it would be used at concentrations comparable to others in the group' (CIR, 1999).

TABLE 5
Product formulation data for Ascorbyl Palmitate (FDA 1996)

Product category	Total no. of formulations in category	Total no. containing ingredient
Eyebrow pencil	99	3
Eyeliner	533	1
Eye shadow	588	51
Eye lotion	22	2
Other eye makeup preparations	136	17
Powders	307	2
Other fragrance preparations	195	3
Hair conditioners	715	1
Hair sprays (aerosol fixatives)	334	4
Rinses (noncoloring)	60	2
Shampoos (noncoloring)	972	1
Tonics, dressings, and other hair grooming aids	604	3
Blushers (all types)	277	50
Face powders	313	31
Foundations	355	57
Lipstick	997	215
Makeup bases	154	16
Rouges	30	2
Other makeup preparations	157	8
Cuticle softeners	26	1
Other personal cleanliness products	339	1
Shaving cream	158	5
Shaving soap	3	1
Cleansing	820	2
Face and neck (excluding shaving)	300	5
Body and hand (excluding shaving)	1012	13
Moisturizing preparations	942	24
Night	226	4
Paste masks (mud packs)	300	12
Other skin care preparations	810	15
Suntan gels, creams, and liquids	196	6
Indoor tanning preparations	67	1
Other suntan preparations	68	2
1996 total		**561**

Table: Final report of the safety assessment of Ascorbyl palmitate as used in cosmetics. International Journal of Toxicology, 18/Suppl. 3):1-26, 1999. Copyright © American College of Toxicology ISSN:1091-5818/99

The 2005 Review considered 431 cosmetic formulations containing ascorbic acid from various product categories, as reported by the American Food and Drug Administration (FDA). The following table (CIR, 2005) illustrates the product types and the respective concentration range of L-ascorbic acid. Products containing L-ascorbic acid appear to fall in the concentration range of 0.00001 to 10% active ingredient.

TABLE 4
Product formulation data

Product category (total formulations reported to FDA) (FDA 2001)	Number of formulations containing ingredient (FDA 2001)	Current concentrations of use (CTFA 2000) (%)	Historical concentrations of use (FDA 1984) (%)
		Ascorbic Acid	
Bubble baths (209)	1	—	—
Powders (272)	9	—	—
Hair conditioners (630)	15	0.001–0.05	≤1
Hair sprays (aerosol fixatives) (276)	4	—	≤0.1
Permanent waves*	—	—	≤1
Rinses (noncoloring) (41)	1	—	—
Shampoos (noncoloring) (851)	17	0.0001–0.01	≤0.1
Tonics, dressings, and other hair-grooming aids (577)	7	—	≤0.1
Wave sets*	—	—	≤1
Other hair preparations (276)	6	—	—
Hair dyes and colors (1588)	345	0.3–0.6	—
Hair tints (49)	3	—	—
Lipstick (942)	1	0.001	—
Makeup bases (136)	1	—	≤0.1
Other makeup preparations (186)	1	—	≤0.1
Mouthwashes and breath fresheners	1	—	—
Other manicuring preparations*	—	—	≤1
Mouthwashes and breath fresheners (46)	1	—	—
Bath soaps and detergents (405)	2	0.001	≤0.1
Cleansing (733)	3	0.001–5	≤0.1
Face and neck (excluding shaving) (304)	3	0.001–10	≤1
Body and hand (excluding shaving) (827)	3	0.0001–10	—
Foot powders and sprays (35)	1	0.1–5	—
Night preparations*	—	—	≤1
Paste masks*	—	—	≤5
Moisturizing (881)	2	0.001–0.05	≤1
Skin lighteners*	—	—	≤1
Other skin care preparations (715)	4	0.01	≤1
Eyebrow pencil (91)	—	0.0005	—
Eyeliner (514)	—	0.001	—
Eye lotion (18)	—	0.00001	—
Foundations (287)	—	0.1	—
Douches (5)	—	0.001	—
Shaving cream (aerosol, brushless, and lather) (139)	—	0.001	—
2001 Total for Ascorbic Acid	**431**	**0.00001–10**	**≤1–5**

Table: Final report of the safety assessment of L-Ascorbic acid (i.e., Vitamin C) as used in cosmetics. International Journal of Toxicology, 24/Suppl. 2):51-111, 2005. Copyright © American College of Toxicology ISSN:1091-5818 print / 1092-874X online DOI: 10.1080/10915810590953851

In Europe, ascorbic acid derivative chemicals have been used for over 25 years as skin depigmenting agents at concentrations of 2 to 3% (Prakash *et.al*, 2009) and in cosmetic water/oil emulsions as antioxidants at ≤ 2% (CIR, 1999).

Domestically, various ascorbic acid derivative chemicals have been previously approved for use in Australia at concentrations ≤ 5% (typically in the range of 0.01 to 2% as an antioxidant and ≤ 5% in specialised skin care products for skin lightening).

The notified chemical is currently listed on the Australian Inventory of Chemical Substances with conditions that it be used only for cosmetics, and only at concentrations ≤ 1% (AICS Chemical Gazette, 2011). The notified chemical is also available in the United States as part of a lip product at 5% concentration (AMA, 2004). While L-ascorbic acid is typically used in cosmetics at concentrations ≤ 10%, there is evidence

suggesting the notified chemical is commonly used at lower concentrations, being recommended for use in a variety of cosmetic products in the range of 0.05 to 1% concentration.

In light of the international and domestic use patterns of both the notified chemical and other ascorbic acid derivative chemicals outlined in the evidence above, a data gap and subsequent uncertainty exist on the use of such ingredients at concentrations exceeding 10% in cosmetic products.

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 A.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD_{50} > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test (3%)	no evidence of sensitisation
Human, skin sensitisation – RIPT (10%)	no evidence of sensitisation
Mutagenicity – bacterial reverse mutation	non mutagenic

Toxicokinetics, metabolism and distribution.
No toxicokinetic data were provided on the notified chemical. Passive diffusion of the notified chemical across the gastrointestinal (GI) tract and dermal absorption could occur, although based on the molecular weight and the expected physico-chemical properties of the notified chemical, the extent of absorption may be limited.

The notified chemical is a tetraester of L-ascorbic acid and isopalmitic acid, bonded at the 2, 3, 5 and 6 carbon positions of ascorbic acid (Stamford, 2012), leaving no free hydroxyl groups on the ascorbic acid ring. As with other ascorbic acid derivative chemicals, it is expected that the esters of ascorbic acid in the notified chemical are cleaved by enzymes in the skin to release the ascorbic acid. The notified chemical has been demonstrated to enhance intracellular accumulation of ascorbic acid *in vitro* in human skin fibroblasts, assumedly through esterolytic conversion to ascorbic acid (Xiao *et.al*, 2009). However, it is not known whether partial as well as full de-esterification occurs.

It is also not known whether dermal penetration of the notified chemical would lead to systemic exposure to the chemical and/or L-ascorbic acid. Limited evidence exists for the potential of ascorbic acid derivative chemicals to be systemically available. Pokorski & Marczak (2005) reported that after both intravenous administration and oral ingestion, Ascorbyl-6-palmitate has been shown to cross the blood-brain barrier, penetrating neural tissues as an intact ester molecule resisting hydrolysis in the brain, to show short-term biological effects. There is also evidence that after dermal application of Ascorbyl-6-palmitate, L-ascorbic acid is systemically distributed, with increased content detected in the liver (7-fold) and the blood (4-fold) (CIR, 1999).

Dermal absorption.
L-Ascorbic acid itself has been suggested to have very limited transcellular potency owing to its hydrophilicity, and its labile oxidative properties incurring early degradation in aqueous solutions (Stamford, 2012). A study by Pinnell *et.al* (2001) suggested L-ascorbic acid must be formulated at pH levels less than 3.5 to enter skin, as the molecule must be non-ionised for delivery across the stratum corneum barrier. However, the CIR (2005) report examining L-Ascorbic acid suggested that high cutaneous levels of this chemical may result from topical application of products containing it.

Compared to L-ascorbic acid in its free form, derivatives such as the notified chemical are said to be better able to penetrate the skin (Campos *et.al*, 2012) because of the lipophilicity created by the ester chains in such derivatives (Meeves *et.al*, 2002). In a human reconstructed skin model the notified chemical penetrated the reconstructed skin and was converted efficiently to L-ascorbic acid (Ochiai *et.al*, 2006). A review by Stamford (2012) noted that *in vivo* evidence was available for the percutaneous absorption of two ascorbic acid derivatives and *in vitro* evidence for conversion to ascorbic acid for several derivatives, including the notified chemical. The review concluded that data gaps exist in the current available evidence regarding the transdermal penetration and the conversion to active L-ascorbic acid of such ascorbic acid derivatives.

Biochemical action.
Ochiai *et.al* (2006) suggest that based on its structure the notified chemical itself has no inherent capabilities as an antioxidant, being itself inactive. No data is available on the effect of the palmitic group; however, it is not expected to play a major role in the reactivity of the notified chemical. The biochemical action of the notified chemical is expected to be that of L-ascorbic acid, and dependent on its release from the notified chemical in human tissues or cells (Ochiai *et.al*, 2006).

L-Ascorbic acid functions as an antioxidant and pH adjuster in cosmetic formulations (CIR, 2005). According to Stamford (2012), it has also been used for its other effects on the skin such as photoprotection, neocollagenesis, inhibition of melanogenesis and an improvement in some inflammatory skin disorders.

The likely mechanisms by which L-ascorbic acid exerts functional benefits have been described in literature. As a photoprotectant, L-ascorbic acid has been shown to decrease UV-B induced photooxidation on human sebum, stabilise the plasma membranes and mitochondria 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 been shown to stimulate collagen production in human fibroblasts and enhanced mRNA transcription levels of type I and III collagen genes (CIR, 2005). In the inhibition of melanogenesis, released L-ascorbic acid interacts with copper ions at the tyrosinase active site, acting as a reductive and antioxidant agent at various oxidative steps of melanin formation (affecting tyrosine and l-DOPA) (Sarkar *et.al*, 2013). However, it is also acknowledged in the scientific literature that the effects of L-ascorbic acid in the skin are not well understood (Michels, 2011), with more mechanistic and human *in vivo* studies warranted to establish its beneficial claims (Naidu, 2003).

Consequently, the mechanisms by which the notified chemical exerts functional changes (beneficial or adverse) are also not well understood. The study by Xiao *et.al* (2009) noted that although the notified chemical has been in use for some time, it remains to be analysed from the viewpoints of molecular and cellular pharmacology.

Depigmentation effects.
L-ascorbic acid has been tested extensively and is reported to inhibit the production of melanin (Parvez *et.al*, 2006). Pigmentation in the skin is caused by enhanced melanin production or melanocyte proliferation (Maeda and Fukuda, 1991). L-Ascorbic acid has been stated by Maeda and Fukuda (1991) to be an active whitening cosmetic component that may prevent melanin synthesis by its anti-enzymatic properties (reduction in tyrosinase activity), suppression of inflammation and by inhibiting the auto-oxidation of dopa and dopaquinone.

A study by Ochiai *et.al* (2006) on the effects of the notified chemical at 3% concentration on keratinocyte cultures demonstrated that suppression of melanocyte proliferation factors alleviated the hyperpigmentation effect induced by UVB. The same paper reported a clinical study where 3% notified chemical was applied to the skin of 22 volunteers for 3 weeks after exposure to UV radiation. Compared to controls, a suppression of pigmentation with significant skin lightening effects was seen, with complete age spot pigmentation removal seen after 16 weeks. Therefore, there is evidence that the notified chemical has the potential to cause skin depigmentation, presumably by the action of the released ascorbic acid component.

The notifier has stated that the notified chemical is approved as a quasi-drug active in Japan at 3%, and that it is registered in Korea as a functional skin lightening ingredient at 2% concentration. Being categorized as a "quasi-drug" in this capacity indicates that the products containing the notified chemical at these concentrations have proven efficacy in a specific claim category, in this case for skin whitening, recognized by the Japanese Ministry of Health and Welfare (Pisacane, 2009).

Acute toxicity.
The notified chemical was found to have low acute toxicity by the oral route in a study conducted on rats (refer Appendix A for details). No acute dermal or inhalation toxicity data were provided for the notified chemical.

CIR (1999) discusses an acute dermal toxicity study on rats, using the ascorbic acid derivative chemical Ascorbyl palmitate. A LD_{50} of > 3,000 mg/kg bw/day was established in this study.

Irritation.
In an acute dermal irritation study on rabbits (refer Appendix A for details), a single 4-hour, semi-occluded application of the notified chemical resulted in erythema at all treated sites, with effects evident at the 1 hour observation after patch removal. At the 24 hour observation, erythema was noted in one animal only, with no

responses recorded 48 hours after patch removal. No oedema was noted. The effects noted in this study were insufficient to warrant classification of the notified chemical as a skin irritant.

In a rabbit eye irritation study with the notified chemical (refer Appendix A for details), conjunctival irritation was noted in all treated eyes from 1 hour after treatment, persisting at the 24 hour observation in one animal. Slight reddening of the sclera was also present in one animal at the 1 hour observation. The effects noted in this study were insufficient to warrant classification of the notified chemical as an eye irritant.

Sensitisation.
A guinea pig maximisation test (using the Magnusson-Kligman method) was conducted at a low concentration of the notified chemical (3%), to determine its skin sensitisation potential (refer Appendix A for details). Under the conditions of the study, the notified chemical (at 3% induction and challenge concentrations) was found to be a non-sensitiser, with no responses noted in any animals at both the 24 and 48 hour observations after challenge patch removal.

In a human repeat insult patch test (HRIPT) completed on 100 subjects (refer Appendix A for details), the notified chemical (at 10% concentration) was determined by the study authors to not induce skin sensitisation. However, it is noted that faint, minimal erythema was evident in 4 subjects during the challenge phase (in 2 subjects at 24 hours post-patch removal, 1 subject at 24-72 hours post-patch removal and 1 subject at 48-72 hours post-patch removal), with no responses observed in these subjects during the induction phase.

No data were provided that would provide information on the skin sensitisation potential of the notified chemical at concentrations > 10%.

The CIR (2005) reported L-ascorbic acid to be a non-sensitiser 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 repeated dose toxicity of L-ascorbic acid and a derivative chemical is available from previous CIR reports, generally indicating the absence of effects deemed toxicologically adverse at doses ≤ 1,000 mg/kg bw/day. However, these chemicals are likely to have different patterns of physiological action due to individual dermal penetration ability, differing log K_{ow} and the varied presence of specific functional groups, which together limit the usefulness of such chemicals for quantitative read-across to the notified chemical.

Mutagenicity / Genotoxicity.
The notified chemical was not mutagenic in a bacterial reverse mutation study (refer Appendix A.6 for details). No further *in vitro* or *in vivo* genotoxicity data were provided for the notified chemical.

Potential for pro-oxidant action.
According to Eberlein-Konig *et.al* (2005), almost all antioxidants have pro-oxidant effects *in vitro* at high concentrations or under special conditions; however, the relevance of these effects *in vivo* is not currently known. The type, dosage and matrix of exogenous antioxidants may be determining factors that impact the balance between beneficial or deleterious effects (Bouayed and Bohn, 2010). Pro-oxidation generates oxidative damage to biomolecules such as proteins, DNA and lipids and eventually cells and tissues, which may end with cell death (Aruoma, 2003).

L-Ascorbic acid had both antioxidant and pro-oxidant effects in rats after hepatic ischemia/perfusion, depending on the dose (Seo and Lee, 2002). Osiecki *et. al* (2010) reported L-ascorbic acid to function as a pro-oxidant *in vitro*. Pro-oxidant activity with L-ascorbic acid was also observed in *in vivo* studies where intracellular markers were evaluated, suggesting that perhaps two different mechanisms (intracellular pro-oxidant activity and extracellular antioxidant activity) are simultaneously at play (Osiecki *et. al*, 2010).

L-Ascorbic acid may contribute to oxidative damage (pro-oxidant effect) by reducing metal ions (e.g. Fe^{3+} to Fe^{2+}), which in turn can convert hydrogen peroxide to hydroxyl radicals through a Fenton type reaction (Duarte and Lunec, 2005). It has been argued that this mechanism may not be relevant *in vivo* in relation to the ready availability of catalytically active free metal ions (Naidu, 2003). However, Osiecki *et.al* (2010) suggested that pro-oxidant activity of L-ascorbic acid may be the result of purposeful signalling mechanisms, rather than

simply the spillage of intracellular metal ions. Other potential toxic effects of L-ascorbic acid that are independent of metal ions have been suggested, including the induction of genotoxic structures from lipid hydroperoxides (Duarte & Lunec, 2005); and auto-oxidation of L-ascorbic acid, whereby it is transported through the glucose transporter (GLUT) into the cell and can influence gene expression (Rahal *et.al*, 2014).

The CIR (2005) evaluation of L-ascorbic acid also raises related concerns. Specifically, a study conducted combining ascorbic acid with xanthine oxidase (plus hypoxanthine as a free radical generating system) reports to have demonstrated that it can be genotoxic. The CIR concluded that when ascorbic acid acts as an antioxidant, it is not genotoxic, and attributed the small number of genotoxic results to factors such as metals or enzyme systems that convert ascorbic acid's antioxidant action to pro-oxidant action. This suggests that L-ascorbic acid and derivative chemicals may act as pro-oxidants under certain conditions, which are not completely understood.

A status report review of metals in cosmetics found that they are widely present in products at trace concentrations, may penetrate into or through human skin, and produce systemic exposure after topical application (Bocca *et.al*, 2014). The Cosmetic Ingredient Review Panel cautioned formulators to be certain that ingredients (including L-ascorbic acid) are acting as antioxidants in cosmetic formulations, because of the concern that certain metal ions may combine with these ingredients to produce pro-oxidant activity (CIR, 2005).

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.

Potential for cytotoxicity.
Cytotoxicity is the property of certain chemicals to be toxic to certain cells. Some skin lightening actives have cytotoxic properties. For example, hydroquinone can cause permanent loss of melanocytes through oxidative damage to membrane lipids, leading to irreversible loss of inherited skin colour (Gillbro *et.al*, 2011). Maeda and Fukuda (1991) stated that, if depigmentation is caused by cytotoxicity, irreversible hypopigmentation will occur somewhat in the skin or hair, as whitening cosmetics are usually used daily. After testing at 20, 40 and 80 µM concentration, Siddique *et.al* (2009) concluded that L-ascorbic acid was cytotoxic *in vitro* at higher concentrations. Cytotoxic effects have also been described when L-ascorbic acid and the ascorbyl radical have undergone auto-oxidation (Eberlein-Konig *et.al,* 2005).

In a clinical trial (Ochiai *et. al,* 2006**),** topical application of a cream containing the notified chemical at 3% concentration suppressed pigmentation after irradiation without showing a harmful effect on the treated melanocytes. Another study also reported the notified chemical to be non-cytotoxic to keratinocytes *in vitro* up to a fairly high concentration (4,500 µM) (Xiao *et.al,* 2009), although it is not known whether the *in vitro* exposure was to the chemical itself or metabolites. However, in a separate *in vitro* study, Xiao *et.al* (2006) showed that the notified chemical became less cytoprotective at concentrations above 20 µM.

Overall, the available data do not rule out the possibility that the notified chemical has cytotoxic properties.

Health 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).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation processes
Workers may experience dermal, ocular and perhaps inhalation exposure to the notified chemical (at ≤ 100% concentration) during reformulation processes. The notified chemical is considered to be slightly irritating to both the skin and eyes and based on uncertainties related to its hazard profile, particularly when repeated exposure to high concentrations is expected, caution should be exercised when handling the notified chemical during reformulation processes.

The use of enclosed, automated processes and PPE should minimise the potential for exposure. Provided that adequate control measures are in place to minimise worker exposure, the risk to workers from use of the notified chemical is not considered to be unreasonable.

End-use
Beauty care professionals will handle the notified chemical at ≤ 30% concentration. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the risk to these workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical on a regular basis (for details of the public health risk assessment, see Section 6.3.2.). However, if such workers neglect consistent use of PPE and hygiene practices, given uncertainties in the hazard profile of the notified chemical, the potential for adverse effects following repeated exposure of such workers to products containing the notified chemical at ≤ 30% concentration, in the absence of adequate controls (e.g. gloves), cannot be ruled out.

6.3.2. Public Health

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 ≤ 30% in individual products). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible.

Exposure is expected to be to both the notified chemical and the breakdown products L-ascorbic acid, isopalmitic acid and possibly intermediate breakdown products. Due to lack of *in vivo* data, significant uncertainty exists regarding the concentrations of the notified chemical and L-ascorbic acid that can be generated in the skin and/or systemically after dermal application of cosmetic products containing the notified chemical. This is especially of note in light of the notified chemical's enhanced penetration potential.

Acute effects associated with the use of cosmetics containing the notified chemical at ≤ 30% proposed concentration mainly relate to eye and skin irritation. While the effects noted in the studies provided by the notifier for these endpoints were insufficient to warrant classification, the notified chemical holds a restriction on its use in topical products intended for use in the eye (AICS Chemical Gazette, 2011). For products containing the notified chemical which are designed for application to the face, care must therefore be taken while applying them around the eyes (Telang, 2013).

Section 6.2 outlined the available data and uncertainties associated with establishing an accurate chronic hazard profile for the notified chemical. Due to these uncertainties and in light of the significant consumer exposure associated with the use of cosmetics containing the notified chemical at ≤ 30% proposed concentration, calculation of a Margin of Exposure (MoE) is deemed inappropriate in this risk assessment. It is also noted that the notified chemical is on the CIR proposed review list for 2015.

The CIR (2005) considered 431 cosmetic formulations containing L-ascorbic acid from various product categories, as reported by the FDA. As illustrated in Section 6.1.2, products containing L-ascorbic acid appear to fall in the concentration range of 0.00001 to 10% active ingredient. Furthermore, for the ascorbic acid derivative chemical Ascorbyl palmitate, CIR (1999) indicated that while the ingredient is used in a variety of cosmetic products such as eye creams, body cleansers, cologne and body oils, it ranges in concentrations between 0.01 and 0.2%. While there are no adverse incidents reported in the literature for ascorbic acid derivative chemicals in the concentration range currently reported in cosmetics (i.e. 0.00001 to 10%), there is sufficient evidence that even at ≤ 10% concentration such chemicals can have skin depigmenting effects.

On the basis of the available information and the data gaps that still exist, long term health effects *in vivo* cannot be ruled out. Therefore based on current concentrations of ascorbic acid derivative chemicals in cosmetics at ≤ 10%, concentrations above this are not supported in this risk assessment. Furthermore, when present in cosmetics at up to 10% concentration, the notified chemical will result in various degrees of skin depigmentation. 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.

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 will be no release of the notified chemical to the environment from this activity. The notified chemical will be used as a cosmetic ingredient in a variety of cosmetic skin care products (such as skin creams and lotions). 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 with an inert absorbent material and disposed of to landfill.

The notified chemical will be blended into end-use consumer products at customer sites in Australia. During reformulation processes, limited release of the notified chemical is expected from cleaning of equipment as washings are expected to be reused. A total of ≤ 2% of the import volume is estimated to be generated as waste from residues in empty containers and spills during reformulation, and is expected to be disposed of to landfill.

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 as a cosmetic ingredient in a variety of skin products, which are washed off the skin of consumers, and disposed as waste waters from domestic cleaning activities. A small percentage of the notified chemical, as residues in empty end use containers, is expected to be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

It is expected that some of the product containing the notified chemical will remain in end-use containers. The containers are expected to be disposed of through domestic garbage disposal and will enter landfill, or be subjected to recycling processes.

7.1.2. Environmental Fate

No environmental fate data were submitted for the notified chemical. Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system before potential release to surface waters on a nationwide basis. During waste water treatment processes in sewage treatment plants (STPs), a significant amount of the notified chemical is expected to be removed from waste waters, by partition to sludge, due to its expected low water solubility and high molecular weight. The notified chemical is not likely to bioaccumulate due to its high molecular weight. If released to surface waters, the notified chemical is expected to disperse and slowly degrade through biotic and abiotic processes to form water and oxides of carbon.

A proportion of the notified chemical may be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. Notified chemical residues in landfill, soil and sludge are not expected to be mobile based on its low water solubility. In landfill, soil or sludge, it is expected to eventually degrade to form water and oxides of oxygen.

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 use in cosmetic products, as a worst case scenario, it is assumed that 100% of the total import volume of the notified chemical is released to the sewer. The release is assumed to be nationwide over 365 days per year. It is conservatively assumed that 0% of the notified chemical will be removed during sewage treatment 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 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.6 µg/L may potentially result in a soil concentration of approximately 4.04 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 20.2 µg /kg and 40.4 µg/kg, respectively.

7.2. Environmental Effects Assessment

Ecotoxicological data were submitted for the notified chemical. The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of the studies can be found in Appendix B.

Endpoint	*Result*	*Assessment Conclusion*
Daphnia Toxicity	EL_{50} (48 hours) ~ 67 mg/L*	Harmful to aquatic invertebrates
Algal Toxicity	E_rL_{50} (72 hours) > 100 mg/L*	Not harmful to algae

* Filtered Water Accommodated Fraction (WAF)

Based on the ecotoxicological endpoints for the notified chemical, it is harmful to aquatic invertebrates. However, the notified chemical is not harmful to algae. Therefore, the notified chemical is considered to be harmful to aquatic organisms. Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009), the notified chemical is formally classified as Acute Category 3; Harmful to aquatic life. Based on the acute toxicity and lack of data on the ready biodegradability of the notified chemical, it is expected to be harmful to the aquatic life on long term basis. Therefore, the notified chemical has been formally classified under the GHS as Chronic Category 3; Harmful to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated and is presented in the table below. The PNEC is calculated based on the endpoint for the most sensitive species (Invertebrates) for the notified chemical. An assessment factor of 1000 has been used as acute toxicity endpoints for only two trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	67	mg/L
Assessment Factor	1000	
PNEC:	67	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.61	67	0.009
Q - Ocean:	0.06	67	0.001

The risk quotient for discharge containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations based on its reported use pattern and annual introduction volume. The notified chemical is expected to slowly degrade in the environment and it is not expected to be bioaccumulative. On the basis of the PEC/PNEC ratio, maximum annual import volume and assessed use pattern in cosmetic and domestic products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: TOXICOLOGICAL INVESTIGATIONS

A.1. Acute toxicity – oral

TEST SUBSTANCE — Notified chemical.

METHOD — OECD TG 401 Acute Oral Toxicity.
- Species/Strain: Rat/Wistar Crl:(WI)BR
- Vehicle: None.
- Remarks - Method: No significant protocol deviations. GLP Compliance.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2,000	0/10

- LD50: > 2,000 mg/kg bw
- Signs of Toxicity: No clinical signs were observed during the study period.
- Effects in Organs: No macroscopic findings were observed at necropsy in any of the test animals.
- Remarks - Results: No deaths occurred and all animals gained weight over the course of the study.

CONCLUSION — The notified chemical is of low toxicity via the oral route.

TEST FACILITY — NOTOX (1996a)

A.2. Irritation – skin

TEST SUBSTANCE — Notified chemical.

METHOD — OECD TG 404 Acute Dermal Irritation/Corrosion.
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3 M
- Vehicle: None
- Observation Period: 72 hours
- Type of Dressing: Semi-occlusive.
- Remarks - Method: No significant protocol deviations. GLP Compliance.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0.3	0.3	0	2	< 48 hours	0
Oedema	0	0	0	1	< 24 hours	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

- Remarks - Results: Well defined erythema and very slight oedema was observed in all animals at 1 hour after patch removal. Oedema resolved after this observation, however very slight erythema persisted in 2 animals up to and including the 24 hour observation.

CONCLUSION — The notified chemical is slightly irritating to the skin.

TEST FACILITY — NOTOX (1996b)

A.3. Irritation – eye

TEST SUBSTANCE	Notified chemical.
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Observation Period	72 hours
Remarks - Method	No significant protocol deviations. GLP Compliance.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0	0.7	0	1	< 72 hours	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	1	< 24 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results	All animals displayed conjunctival redness at 1 hour after treatment and this continued in 1animal (with the effect decreased to partial eversion of the lids) up to and including the 48 hour observation. 2 animals showed conjunctival discharge at the 1 hour observation only. No iridial irritation, corneal opacity or ocular corrosion was observed.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	NOTOX (1996c)

A.4. Skin sensitisation

TEST SUBSTANCE	Notified chemical (3%).
METHOD	OECD TG 406 Skin Sensitisation – Magnusson and Kligman Maximisation.
Species/Strain	Guinea pig/Dunkin Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 25% w/v in arachis oil BP topical: 100% v/v in arachis oil BP
MAIN STUDY	
Number of Animals	Test Group: 10 per sex Control Group: 5 per sex
INDUCTION PHASE	Induction Concentration: intradermal: 25% w/v in arachis oil BP topical: 100%

Signs of Irritation	After intradermal induction, very slight to well defined erythema was observed in all test and control animals (treated with 25% w/v mixture of FCA with distilled water (1:1)) at 24 hours post patch removal. Very slight to well defined erythema continued to be evident in all test animals at 48 hours post patch removal. Due to a technical error, the 48 hour observation of the control animals was not performed. After topical induction, very slight to well defined erythema, with or without very slight oedema (7/20) was noted in all test animals at 1 hour post patch removal. Very slight erythema was noted in 3/20 test animals at 24 hours after patch removal. Very slight erythema was noted in 2/10 control animals (treated with a blank patch) at the 1 hour observation, with no skin reactions noted at 24 hours post patch removal.
CHALLENGE PHASE	
1st challenge	topical: 100% and 75% v/v in arachis oil BP
Remarks - Method	The test substance was stated to be 3% of the notified chemical, however the diluent was not disclosed. The preliminary study used 8 animals. Challenge was performed on study day 21. A concurrent positive control study was not conducted.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after challenge:*	
		24 h	*48 h*
Test Group	100%	0/20	0/20
	75%	0/20	0/20
Control Group	100%	0/10	0/10
	75%	0/10	0/10

Remarks - Results	No toxic symptoms or skin reactions (after challenge) were observed in any animal. Bodyweight gains of test animals were comparable to the control animals over the study period.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Safepharm (1997)

A.5. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical (10% in silicone)
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: 20 x 20 mm Parke-Davis Hypoallergenic Readi (occlusive) patches containing 0.2 mL test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed by the applicants after 24 hours and graded after an additional 24 hours (or 48 hours for patches applied on Friday). Rest Period: 10 - 14 days Challenge Procedure: a patch was applied to a naïve site. Patches were removed by a technician and the sites graded at patch removal and 24 and 48 hours post-patch removal.
Study Group	93 F, 13 M; age range 18 - 78 years
Vehicle	Silicone
Remarks - Method	A panel of 106 healthy human subjects (devoid of any physical or dermatological conditions) was amassed. Of these, 102 (89 female and 13

male) test subjects completed the study; 4 female subjects reportedly discontinued. No explanations were given for these discontinuations in the study report.

RESULTS

Remarks - Results: 3 test subjects experienced barely perceptible/minimal faint uniform or spotty erythema, noted as observations during the induction phase of single day duration. These reactions were not considered by the study authors to be evidence of skin sensitisation. No subjects showed responses to the test substance during the challenge phase.

CONCLUSION: The notified chemical was considered by the study authors to be non-sensitising under the conditions of the test.

TEST FACILITY: AMA Laboratories (1997)

A.6. Genotoxicity – bacteria

TEST SUBSTANCE: Notified chemical.

METHOD: OECD TG 471 Bacterial Reverse Mutation Test.
OECD TG 472 Genetic Toxicology: Escherichia coli.
Reverse Assay (conducted prior to merge with 471. in 1997)
Plate incorporation procedure

Species/Strain: *S. typhimurium*: TA1535, TA1537, TA98, TA100
E. coli: WP2uvrA

Metabolic Activation System: S9 fraction from Arochlor 1254 induced rat liver

Concentration Range in Main Test: With and without metabolic activation: 1000 or 5000 µg/plate in Test 1 and 1000 µg/plate in Test 2.

Vehicle: -

Remarks - Method: No significant protocol deviations.
GLP Compliance.

A preliminary toxicity test (3-5,000 µg/plate; plate incorporation) was performed for the tester strains TA100 and WP2uvrA to determine the toxicity of the test material. The results are reported as part of Test 1.

Positive control tests were conducted in parallel to the main test using sodium azide, 9-aminoacridine, daunomycine, methylmethanesulfonate and 4-nitro-o-phenylene-diamine in the absence of S9-mix, and 2-aminoanthracene with S9-mix.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:* *Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 5,000	≥ 333	negative
Test 2	> 5,000	≥ 333	negative
Present			
Test 1	> 1,000	≥ 333	negative
Test 2	> 1,000	≥ 333	negative

Remarks - Results: No visible reduction in the growth of the bacterial background lawn was seen at any dose level, with and without metabolic activation.

No increases in the frequency of revertant colonies were recorded for any of the bacterial strains.

The positive controls produced satisfactory responses, thus confirming the

	activity of the S9-mix and the sensitivity of the bacterial strains.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	NOTOX (1996d)

APPENDIX B: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

B.1. Ecotoxicological Investigations

B.1.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not reported
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	Not reported
Remarks – Method	No significant protocol deviations. GLP Compliance. The daphnia ecotoxicity test was conducted in Water Accommodated Fractions (WAF) of the notified chemical as it has low water solubility. WAF of a nominal loading rate of 100 mg/L was prepared by stirring the test substance in water for 24 hours. The resulting mixture was a slightly hazy dispersion containing undissolved materials and an oily layer floating on the surface. The dispersion was left to stabilise for approximately 30 minutes. Then, the middle fraction was collected and filtered through a glass-filter to remove the undissolved materials. The lower test treatments were prepared by diluting the filtrate in test medium. All treatments were clear and colourless.

RESULTS

Nominal loading rate (Filtered WAF;mg/L)	*Number of D. magna*	*Cumulative % Immobilised*	
		24 hours	*48 hours*
Control	20	0	0
1	20	0	0
10	20	0	0
100	20	0	75

EL50	~ 67 mg/L at 48 hours (estimated based on the raw data)
NOEL	10 mg/L at 48 hours
Remarks – Results	All validity criteria for the test were satisfied. A statistical analysis was not reported. The 48-hour EL_{50} was provided in a range of 10 – 100 mg/L rather than a specific value.
CONCLUSION	The notified chemical is harmful to aquatic invertebrates
TEST FACILITY	NOTOX (2008a)

B.1.2. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1, 10, and 100 mg/L
Auxiliary Solvent	Not reported
Water Hardness	Not reported
Analytical Monitoring	Not reported
Remarks – Method	No significant protocol deviations.

GLP Compliance.

The algal ecotoxicity test was conducted in Water Accommodated Fractions (WAF) of the notified chemical as it has low water solubility. WAF of a nominal loading rate of 100 mg/L was prepared by stirring the test substance in water for 24 hours. The resulting mixture was a slightly hazy dispersion containing undissolved materials and an oily layer floating on the surface. The dispersion was left to stabilise for approximately 30 minutes. Then, the middle fraction was collected and filtered through a glass-filter to remove the undissolved materials. The lower test treatments were prepared by diluting the filtrate in test medium. All treatments were clear and colourless.

RESULTS

Biomass (72 hours)		*Growth (72 hours)*	
E_yL50 (mg/L)	*NOE_yL (mg/L)*	*E_rL50 (mg/L)*	*NOE_rL (mg/L)*
> 100	100	> 100	100

Remarks – Results

All validity criteria for the test were satisfied.
Statistical analysis of E_rL50 or E_yL50 was not required as the effects recorded were not significant (< 10%).
The test substance did not exhibit any effect at the highest concentration tested.

CONCLUSION

The notified chemical is not harmful to algae.

TEST FACILITY

NOTOX (2008b)

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