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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2-Propenoic acid, 2-cyano-3-(4-methoxyphenyl)-3-phenyl-, 2-ethylhexyl ester (INCI Name: Ethylhexyl Methoxycrylene)

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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

TABLE OF CONTENTS

SUMMARY 3
CONCLUSIONS AND REGULATORY OBLIGATIONS 3
ASSESSMENT DETAILS 4
1. APPLICANT AND NOTIFICATION DETAILS 4
2. IDENTITY OF CHEMICAL 5
3. COMPOSITION 5
4. PHYSICAL AND CHEMICAL PROPERTIES 6
5. INTRODUCTION AND USE INFORMATION 6
6. HUMAN HEALTH IMPLICATIONS 7
6.1. Exposure Assessment 7
6.1.1. Occupational Exposure 7
6.1.2. Public Exposure 8
6.2. Human Health Effects Assessment 8
6.3. Human Health Risk Characterisation 10
6.3.1. Occupational Health and Safety 10
6.3.2. Public Health 11
7. ENVIRONMENTAL IMPLICATIONS 11
7.1. Environmental Exposure & Fate Assessment 11
7.1.1. Environmental Exposure 11
7.1.2. Environmental Fate 11
7.1.3. Predicted Environmental Concentration (PEC) 12
7.2. Environmental Effects Assessment 12
7.2.1. Predicted No-Effect Concentration 13
7.3. Environmental Risk Assessment 13
APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES 14
APPENDIX B: TOXICOLOGICAL INVESTIGATIONS 16
B.1. Acute toxicity – oral 16
B.2. Acute toxicity – dermal 16
B.3. Irritation – skin (*in vitro*) 17
B.4. Irritation – eye (*in vitro*) 17
B.5. Irritation – eye (*in vitro*) 18
B.6. Irritation – eye 18
B.7. Skin sensitisation – mouse local lymph node assay (LLNA) 19
B.8. Skin sensitisation – human volunteers 19
B.9. Repeat dose toxicity 20
B.10. Repeat dose toxicity 21
B.11. Genotoxicity – bacteria 22
B.12. Genotoxicity – bacteria 23
B.13. Genotoxicity – *in vitro* 23
B.14. Genotoxicity – *in vitro* 25
B.15. Genotoxicity – *in vivo* 26
B.16. Phototoxicity – Human Volunteers 26
APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS 28
C.1. Environmental Fate 28
C.1.1. Ready biodegradability 28
C.2. Ecotoxicological Investigations 28
C.2.1. Acute toxicity to fish 28
C.2.2. Chronic toxicity to aquatic invertebrates 29
C.2.3. Algal growth inhibition test 31
C.2.4. Inhibition of microbial activity 32
BIBLIOGRAPHY 33

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
STD/1444	Ingredients Plus Pty Ltd	2-Propenoic acid, 2-cyano-3-(4-methoxyphenyl)-3-phenyl-, 2-ethylhexyl ester (INCI Name: Ethylhexyl Methoxycrylene)	ND*	≤ 10 tonnes per annum	Ingredient for skin care 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 for the 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
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
On the basis of 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 safe work practices to minimise occupational exposure during handling of the neat notified chemical:
  - Avoid eye contact

  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 for the 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.

Disposal

- The notified chemical should be disposed of to landfill.

Storage

- The following precautions should be taken by the workers regarding storage of the notified chemical:
  - Avoid contact with oxidising materials

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical 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 chemical is intended to exceed 5% concentration in skin care products;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from an ingredient in skin care 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 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
Ingredients Plus Pty Ltd (ABN: 25 112 469 619)
Unit 8, 9 – 11 South Street
RYDALMERE NSW 2116

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: other names, analytical data, degree of purity, import volume, identity of manufacturer/recipients and analogue details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: hydrolysis as a function of pH, absorption/desorption, dissociation constant, flammability, acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, skin irritation, repeated dose toxicity, genotoxic damage *in vivo*, and acute toxicity to fish.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
TGA (2010)

NOTIFICATION IN OTHER COUNTRIES
EU (REACH 2009)
Canada (2013)

2. IDENTITY OF CHEMICAL

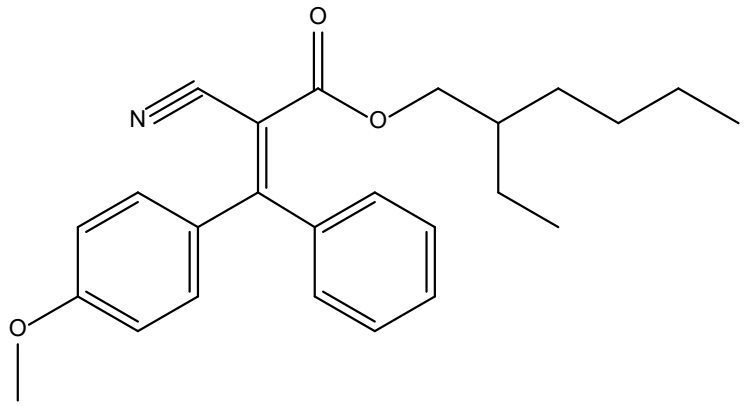
MARKETING NAME(S)
SolaStay® S1

CAS NUMBER
947753-66-4

CHEMICAL NAME
2-Propenoic acid, 2-cyano-3-(4-methoxyphenyl)-3-phenyl-, 2-ethylhexyl ester

MOLECULAR FORMULA
$C_{25}H_{29}NO_3$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
391.51 Da

ANALYTICAL DATA
Reference NMR, IR, GC-MS and UV-Vis spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 98%

HAZARDOUS IMPURITIES
None identified

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Light amber viscous liquid

Property	Value	Data Source/Justification
Pouring Point	1.85 ± 3 °C	Measured
Boiling Point	372.85 – 420.85 °C at 102.61 kPa	Measured
Density	1,080 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.5 × 10^{-11} kPa at 25 °C	Measured
Water Solubility	≤ 3.91 × 10^{-5} g/L at 20 °C; ≤ 1.8 × 10^{-6} g/L at 21 °C.	Measured, flask method, OECD 105; Measured, in daphnia toxicity study.
Hydrolysis as a Function of pH	Not determined	The notified chemical contains hydrolysable functionalities. However, significant hydrolysis is not expected under environmental conditions due to its low water solubility.
Partition Coefficient (n-octanol/water)	log Pow = 6.05- 6.32	Measured
Adsorption/Desorption	Not determined	The notified chemical is expected to partition to soil, sediment and sludge from water based on its low water solubility
Dissociation Constant	Not determined	Not expected to be ionised at environmental pH range of 4-9
Flash Point	251 ± 2 °C at 101.3 kPa	Measured
Flammability	Not determined	Estimated to be low based on the high flash point and autoignition temperature
Autoignition Temperature	> 400 °C	Measured
Explosive Properties	Not explosive	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not oxidising	Contains no functional groups that would imply oxidative properties
Photostability	Stable	Measured

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

Reactivity
The notified chemical is intended to be used as an ultraviolet (UV) stabiliser for formulations of skin care products. It is expected to be stable under normal conditions of use.

The SDS of the notified chemical states that contact with oxidising materials should be avoided.

Photostability
Forty milligram (40 mg) of a solution containing the notified chemical at 3% concentration was plated and dried on a quartz plate. The dried material was irradiated under 288 nm UV light at a dose of 40 W/m^2 for 5 hours followed by HPLC analysis. The results showed that the notified chemical was stable after exposure to 200 Watt hours/m^2 UV light.

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 for the 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. It will be imported as raw material (> 98% purity) for reformulation and as a component of finished skin care products at ≤ 5% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1 – 3	3 – 10	3 – 10	3 – 10	3 – 10

PORT OF ENTRY
Sydney, via sea

TRANSPORTATION AND PACKAGING
Notified chemical as raw material
The neat notified chemical (> 98% purity) will be imported in 19 L (5 gallon) steel pails or 209 L (55 gallon) steel drums. It will be transported by road or rail from the port of entry to the storage warehouse, and then distributed to formulation sites in original imported packages.

Finished consumer products
The reformulated finished skin care products containing ≤ 5% notified chemical will be filled into consumer size packaging (e.g. plastic tubes and bottles), packed into cardboard shipping cartons and transported by road for storage and distribution nationwide for retail sale.

Consumer size packaged finished skin care products containing the notified chemical at ≤ 5% concentration will also be directly imported from overseas and distributed nationwide in original imported packages for retail sale.

USE
The notified chemical will be used as a component of leave-on skin care products (including face creams, body lotions and cosmetic sunscreen lotions) at ≤ 5% concentration.

OPERATION DESCRIPTION
The notified chemical will be imported into Australia as part of skin care products (≤ 5% concentration), which will be sold to end-users in the same form in which they are imported.

The notified chemical will also be imported in the neat form for reformulation into skin care products.

Reformulation
Reformulation will vary depending on the intended product and reformulation site. Typically, the raw material will be weighed, transferred to a mixing vessel, and undergo a blending process using automated and closed vessels. Quality control testing will be implemented during the process. The final finished product will be transferred to a storage tank, from which an automated system will be used to fill consumer packaging containers of various types and sizes. The containers will then be sealed and packaged into cardboard transport cartons, themselves packed into shippers before being transported by road to warehouses or directly to retail outlets.

End-use
The finished skin care products containing the notified chemical at ≤ 5% concentration will be used by consumers and professionals (such as workers in beauty salons). Application of products could be spray, by hand or through the use of 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 warehouse workers	4	12
Blending workers	8	12
Chemists	3	12
Beauty salon workers	Unspecified	Unspecified

EXPOSURE DETAILS

Reformulation
Transport workers and store staff may come into contact with the neat notified chemical only in the event of an accidental rupture of containers.

During reformulation, exposure to the neat notified chemical may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The principal route of exposure would be dermal, while ocular and inhalation exposure is also possible. Exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety glasses and impervious gloves.

End-use
Exposure to the notified chemical (at ≤ 5% concentration) in end-use products may occur in professionals where the services provided involve the application of skin care products to clients (e.g. workers in beauty salons). Such professionals may use 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 (at ≤ 5% concentration) through the use of the skin care products. The principal route of exposure will be dermal, while oral, ocular and inhalation exposure (when sprayed) is also possible.

Data on typical use patterns of skin care product categories in which the notified chemical may be used are shown in the following table (SCCS, 2012). For the purposes of the exposure assessment, Australian use patterns for various product categories were assumed to be similar to those in Europe. However, considering the seasonal variations for use of sunscreens, an application of 6.0 g/day was assumed to be realistic for a cosmetic sunscreen lotion. An average adult bodyweight of 60 kg was used for the exposure estimation (SCCS, 2012). Dermal absorption of 100% was assumed for calculation purposes.

Product type	**Amount** (g/day)	**C** (%)	**Daily systemic exposure** (mg/kg bw/day)
Face cream	1.54	5	1.28
Cosmetic sunscreen lotion	6.00	5	5.00
Body lotion	7.82	5	6.52
Total	**15.36**	**5**	**12.80**

C – Concentration of the notified chemical in the product
Daily systemic exposure = Amount × C × dermal absorption/body weight

In a worst case scenario based on the assumption that a person uses daily all the products listed above simultaneously, a combined internal dose of 12.8 mg/kg bw/day for the notified chemical may be estimated.

Unintentional oral, ocular and inhalation exposure to the notified chemical at up to 5% concentration may also occur when the skin care products are applied close to the mouth and eyes, or applied by spray. However, under normal conditions of use the level of exposure via these routes is expected to be low.

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the notified chemical and an acceptable analogue of the notified chemical (Analogue 1) are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*	*Tested Substance*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity	Analogue 1
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity	Analogue 1
Skin irritation (*in vitro*) – human epidermis model	non-irritating	Analogue 1
Eye irritation (*in vitro*) – HET-CAM	non-irritating	Notified chemical (10%)
Eye irritation (*in vitro*) – human epithelium model	non-irritating	Analogue 1

Endpoint	*Result and Assessment Conclusion*	*Tested Substance*
Rabbit, eye irritation	slightly irritating	Analogue 1
Mouse, skin sensitisation – local lymph node assay (LLNA)	no evidence of sensitisation	Analogue 1
Human, skin sensitisation – RIPT	no evidence of sensitisation	Notified chemical (20%)
Rat, repeat dose oral toxicity – 14 days (range-finding)	NOAEL = 1,000 mg/kg bw/day	Analogue 1
Rat, repeat dose oral toxicity combined with reproduction / developmental toxicity screening	NOAEL = 1,000 mg/kg bw/day for systemic toxicity NOAEL = 1,000 mg/kg bw/day for reproductive toxicity	Analogue 1
Mutagenicity – bacterial reverse mutation	non mutagenic	Notified chemical
Genotoxicity – *in vitro* mammalian chromosome aberration test (human lymphocytes)	equivocal	Notified chemical
Genotoxicity – *in vitro* mammalian chromosome aberration test (mouse lymphoma)	non genotoxic	Notified chemical
Genotoxicity – *in vivo* mouse micronucleus test	non genotoxic	Analogue 1
Human, phototoxicity	non phototoxic	Notified chemical

Toxicokinetics, metabolism and distribution
The notified chemical will be used as a component of skin care products, hence dermal absorption will be the main route of exposure to the notified chemical. Given the high log Pow value (6.05-6.32) and low water solubility, dermal absorption of the notified chemical is expected to be low. This is supported by a dermal absorption of less than 5% observed for an acceptable analogue of the notified chemical (Analogue 2) (Exempt Information, 1997 and 2005).

The notified chemical is an ester of ethylhexanol and the ethylhexanol moiety may have potential of being released as a result of hydrolysis. Hepatotoxicity to ethylhexanol exposure has been observed in the rat and the mouse (Keller *et al*., 1990). The rate of hydrolysis of the notified chemical is unknown; however, data from environmental studies of analogue chemicals indicate that the notified chemical is stable and not readily hydrolysed (Exempt Information, 2006). In addition, HYDROWIN (V2.00) predicts that the notified chemical has a half-life of 20.6 years at pH 8 and 206.2 years at pH 7 respectively, which supports that the notified chemical is unlikely to hydrolyse on the skin after application of the skin care products.

Acute toxicity
Acute toxicity studies on the notified chemical were not provided. However, Analogue 1 was found to be of low acute oral and dermal toxicity in rats.

Based on the data obtained on Analogue 1, the notified chemical is expected to be of low toxicity via the oral or dermal routes.

Irritation and sensitisation
Skin irritation
No study on the notified chemical was provided for skin irritation. However, Analogue 1 was found to be non-irritating in an *in vitro* skin irritation test using the reconstituted human epidermis model.

Based on the data obtained on Analogue 1, the notified chemical is not expected to be irritating to the skin.

Eye irritation
An *in vitro* eye irritation study on the notified chemical using the HET-CAM model showed that the notified chemical at a concentration of 10% did not cause an irritation response. This is supported by a study on Analogue 1 using the reconstituted human corneal epithelium model that revealed no evidence of an irritation response. However, a study conducted in rabbits demonstrated slight irritating properties for Analogue 1.

Based on the information available, the notified chemical may be slightly irritating to eyes.

Skin sensitisation
A human repeated insult patch test using the notified chemical at 20% concentration did not reveal any signs of irritation. Furthermore, a mouse local lymph node assay (LLNA) conducted on Analogue 1 did not show evidence of skin sensitisation.

Based on the information available, the notified chemical is not considered to be a skin sensitiser.

Repeated dose toxicity and reproduction/developmental toxicity
A 14-day repeated dose oral dose range-finding study and a combined repeated dose oral toxicity study with reproduction/developmental toxicity were conducted on Analogue 1. In both studies no treatment related adverse effects were noted.

Based on the highest dose tested, a NOAEL was established at 1,000 mg/kg bw/day for systemic toxicity and reproduction/developmental toxicity for Analogue 1.

Mutagenicity/Genotoxicity
The notified chemical was negative in two bacterial reverse mutation assays and negative in a chromosomal aberration L5178Y TK +/- mouse lymphoma assay. However, a positive response was observed in an *in vitro* chromosome aberration test in human lymphocytes. In this study, the notified chemical induced a marked toxicologically significant increase in the frequency of aberrations in the absence of metabolic activation at the highest dose only (3,910 µg/mL) after a 4 hour exposure. There was no evidence of a true dose-response effect and the response was mainly due to break-type aberrations. The response was not observed in the experiment with a 24 hour exposure. The study author concluded that the observation was not due to a true clastogenic response but was the result of apoptosis causing DNA fragmentation. This is supported by the negative response observed in the mouse lymphoma assay. Furthermore, Analogue 1 was negative in an *in vivo* mouse micronucleus study. Therefore, based on the weight of evidence, the notified chemical is not expected to be genotoxic.

Phototoxicity
A study on phototoxicity of the notified chemical in 21 human volunteers was provided. Under the conditions of the study, the notified chemical did not induce visible skin reactions indicative of a phototoxic response.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the 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 is expected to be of low hazard presenting only as a slight eye irritant.

Workers most at risk of eye irritation will be those handling the neat notified chemical during reformulation processes. However the risk is expected to be minimised by use of closed systems and PPE including eye protection. Eye irritation effects are not expected from use of the skin care products containing the notified chemical at ≤ 5% concentration.

Workers in beauty salons may have dermal exposure to the notified chemical at ≤ 5% concentration, similar to public use. Therefore, the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experienced by members of the public who use such products on a regular basis. For details of the public health risk assessment see section 6.3.2.

Overall, when used in the proposed manner, the risk of the notified chemical to the health of workers is not considered to be unreasonable.

6.3.2. Public Health

The notified chemical is expected to be of low hazard presenting only as a slight eye irritant. Considering that the notified chemical is intended for use at low concentrations (≤ 5%), the potential risk of eye irritation effects is not expected.

The repeated dose toxicity potential of the notified chemical was estimated by calculation of the margin of exposure (MOE) using the worst case exposure scenario from use of multiple products of 12.8 mg/kg bw/day (see Section 6.1.2) and the NOAEL of 1,000 mg/kg bw/day, which was established in the combined repeated dose oral toxicity study with the reproduction/developmental toxicity screening test performed on the analogue chemical. The margin of exposure (MOE) was estimated to be 78.1 for a person using daily all types of products containing the notified chemical. A MOE greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences. The MOE calculated for the notified chemical is an over estimation as it is highly unlikely that all products containing the notified chemical will be used together. Furthermore, dermal aborption of the chemical itself is expected to be low (see Section 6.2: Toxicokinetics, metabolism and distribution). Therefore, in light of the conservative exposure scenario considered and based on the information available, the risk to the public associated with the use of the notified chemical at up to 5% concentration in skin care products 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 be imported into Australia as a component in finished skin care products or as raw material for blending into end-use skin care products. The blending will occur in a closed system and therefore release of the notified chemical from this activity is expected to be very low. Spills during reformulation or repacking processes are expected to be contained with absorbent material and be disposed of to landfill. Waste water produced from equipment cleaning is likely to be flushed to sewers.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used for leave on skin care products including creams, lotions, sprays, sticks and gels. It is anticipated that the majority of the notified chemical will be eventually washed off the skin and enter sewers, where it will be directed to various waste water treatment facilities.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical remaining in empty containers are likely to be disposed of to landfill along with the containers or be washed to sewers when the containers are rinsed with water.

7.1.2. Environmental Fate

The notified chemical may persist in the environment. The notified chemical contains functional groups that have the potential for hydrolysis. However, due to its limited water solubility, significant hydrolysis is not expected under environmental conditions. A biodegradation study indicated that the notified chemical was not readily biodegradable (4% biodegradability over 28 days). For the details of the environmental fate study please refer to Appendix C.

The majority of the notified chemical is expected to be released to sewers after its use as skin care products. During waste water treatment processes in sewage treatment plants (STPs), the notified chemical is expected to be partially removed from waste water to sludge due to its low water solubility. SimpleTreat (European Commission, 2003) estimates that, at most, 15% of the notified chemical will remain in effluent following STP processes, with 85% partitioning to sludge. Notified chemical that partitions to sludge will be removed with the sludge for disposal of to landfill or used in soil remediation. In sludge, landfill and soil, the notified chemical is not expected to mobile nor bioavailable based on its low water solubility. Notified chemical remaining in the effluent from STP may be released to surface waters. Notified chemical released to surface waters is expected to partition and/or adsorb to suspended solids or organic matter and disperse. Hence, it is not anticipated to be significantly bioavailable to aquatic organisms.

Although the notified chemical is likely to bioaccumulate based on its high partition coefficient and low molecular weight, the bioaccumulation may be negligible due to its limited bioavailability. In landfill, soil and

water, the notified chemical is expected to degrade via abiotic and biotic pathways to form water, oxides of carbon, and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

Based on the reported use in skin care products, it is assumed that 100% of the notified chemical will be released to sewers on a nationwide basis over 365 days per year. It is also assumed that 85% of the notified chemical will be removed during STP processes by partitioning to sludge. The Predicted Environmental Concentration (PEC) has been calculated and summarised in the table below.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	10,000	kg/year
Proportion expected to be released to sewer	100 %	
Annual quantity of chemical released to sewer	10,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	27.40	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	85%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	0.91	µg/L
PEC - Ocean:	0.09	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 51.5 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1,500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.343 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 1.72 mg/kg and 3.43 mg/kg, respectively.

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 0.91 µg/L may potentially result in a soil concentration of approximately 6.06 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 30.3 µg/kg and 60.6 µg/kg, respectively.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical and a close analogue chemical are summarised in the table below. Details of these studies can be found in Appendix C. The fish toxicity study was conducted on Analogue 1. However, Analogue 1 is less soluble than the notified chemical and therefore, expected to have reduced bioavailability. Therefore, the data should be treated with caution.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity*	LC50 (96 h) > 100% v/v saturated solution	Not harmful to fish up to the limit of solubility
Daphnia Toxicity	EC50 (21 d) > 0.016 mg/L NOEC (21 d) = 0.0048 mg/L	Not harmful to aquatic invertebrates up to the limit of solubility
Algal Toxicity	E_rC50 (72 h) > 0.011 mg/L NOE_rC (72 h) = 0.011 mg/L	Not harmful to algae up to the limit of solubility
Inhibition of Bacterial Respiration	EC50 (3 h) > 1000 mg/L	Not expected to inhibit bacterial respiration

* Analogue data

The No-Observed Effect Concentration (NOEC) for daphnia was determined to be less than the water solubility of 0.039 mg/L, meaning the notified chemical may have toxic effect on aquatic organisms. However, the toxic effects of the notified chemical on *Daphnia magna* reproduction may be artifactual due to the use of auxiliary solvent in the test. The notifier provided an addendum to the study in which the solubility of the test substance in the solvent-free test medium was determined to be 0.0018 mg/L. The solvent free solubility test was deemed to be acceptable to predict the environmental effect of the notified chemical as it is more representative of the notified chemical in the environment. Therefore, the solubility of 0.0018 mg/L is considered to be the water solubility limit of the notified chemical and the notified chemical is considered not harmful to daphnia up to its water solubility limit.

The algae toxicity endpoints are also above the water solubility of the notified chemical. Based on the daphnia and algal toxicity, the notified chemical is not considered to be harmful to aquatic life up to the limit of its water solubility under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) and therefore is not formally classified for acute and long term hazard.

7.2.1. Predicted No-Effect Concentration

A Predicted No-Effect Concentration (PNEC) was not calculated since the results from ecotoxicological investigations indicate that the notified chemical is not harmful to aquatic organisms up to its limit of solubility in water.

7.3. Environmental Risk Assessment

The risk quotient Q (= PEC/PNEC) was not calculated as the PNEC was not calculated. Although the majority of the notified chemical will be released to sewers, based on its use pattern, a significant portion of the notified chemical is expected to be removed during wastewater treatment by sorption to sewage sludge. Moreover, no toxic effects to aquatic organisms were observed up to the limit of solubility of the notified chemical in the submitted ecotoxicity studies. Based on the high partition coefficient, the notified chemical has potential to bioaccumulate. However, the notified chemical is expected to have low bioavailability potential due to its limited water solubility. Therefore, the notified chemical is not expected to pose an unreasonable risk to the environment based on the assessed use pattern.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pure Point 1.85 ± 3 °C (275 ± 3 K)

Method	EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	The test substance does not freeze but becomes more viscous on cooling.
Test Facility	Harlan (2009a)

Boiling Point 372.85 – 420.85 °C (646 to 694 ± 1 K) at 102.61 kPa

Method	EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	Differential scanning calorimetry was used to determine the boiling point.
Test Facility	Harlan (2009a)

Density 1,080 kg/m^3 at 20 °C

Method	EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Pycnometer method was used to determine the density.
Test Facility	Harlan (2009a)

Vapour Pressure 2.5×10^{-11} kPa at 25 °C

Method	EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	The result was the mean of 7 measurements.
Test Facility	Harlan (2009b)

Water Solubility $\leq 3.9 \times 10^{-5}$ g/L at 20 °C ± 0.5 °C;
$\leq 1.8 \times 10^{-6}$ g/L at 21 °C.

Method	EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method. The water solubility of the test substance was determined to be $< 1 \times 10^{-2}$ g/L in the preliminary test, indicating that column elution method may be a suitable method for its water solubility determination. However, due to the association of the test substance with the stationary phase, water solubility of the test substance was determined using flask method. The concentration of test material in the sample solutions was determined by high performance liquid chromatography (HPLC). The pH of each solution was measured to be 5.4-5.6. Two peaks for the test substance were observed in the HPLC graph, which was considered to be due to the *cis*- and *trans*- isomers of the notified chemical. In the daphnia toxicity study, the water solubility of the notified chemical in the test medium was determined to be $\leq 1.8 \times 10^{-6}$ g/L at pH 8.0. This value was accepted as water solubility to predict the environmental effect of the notified chemical for the purpose of risk assessment.
Test Facility	Harlan (2009a)

Partition Coefficient (n-octanol/water) log Pow = 6.05 – 6.32

Method	EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method. The test substance contains an alkenic double bond, and therefore two peaks were observed in the HPLC graph for its *cis*- and *trans*- isomers. The partition coefficient was determined to be log Pow = 6.05 (43% peak area) and log Pow = 6.32 (57% peak area) for these two peaks.
Test Facility	Harlan (2009a)

Flash Point 251 ± 2 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup equilibrium method was used to determine the flash point.
Test Facility	Harlan (2009b)

Autoignition Temperature > 400 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	The test substance was tested up to 400 °C and no autoignition was observed.
Test Facility	Harlan (2009b)

Photostability Stable

Method	Photostability Testing of New Active Substances and Medicinal Products (TGA 1996)
Remarks	Forty milligram (40 mg) of a solution containing the notified chemical at 3% concentration was plated and dried on a quartz plate. The dried material was irradiated under 288 nm UV at a dose of 40 W/m^2 for 5 hours followed by HPLC analysis. The results showed that the notified chemical was stable after exposure to 200 Watt hours/m^2 UV light.
Test Facility	HallStar (2013)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 420 Acute Oral Toxicity - Fixed Dose Method.
EC Directive 92/69/EEC B.1 bis Acute Toxicity (Oral) Fixed Dose Method

Species/Strain: Rat/HsdRccHan®™:WIST®™
Vehicle: Arachis oil BP
Remarks - Method: No significant protocol deviation

RESULTS

Sighting Study

Dose mg/kg bw	*Administered*	*Evident Toxicity*	*Mortality*
2000	1 F	0/1	0/1
300	1 F	0/1	0/1

Signs of Toxicity: No signs of systemic toxicity were noted.
Effects in Organs: No abnormalities were noted at necropsy.

Main Study

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	4 F	2000	0/4

Discriminating Dose: 2000 mg/kg bw
Signs of Toxicity: No signs of systemic toxicity were noted.
Effects in Organs: No abnormalities were noted at necropsy.
Remarks - Results: LD50 > 2,000 mg/kg bw

CONCLUSION: The test substance is of low toxicity via the oral route.

TEST FACILITY: Harlan (2009e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test.
EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal) – Limit Test.

Species/Strain: Rat/HsdRccHan®™:WIST®™
Vehicle: None. The test substance was administered undiluted.
Type of dressing: Semi-occlusive.
Remarks - Method: No significant protocol deviation.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 M	2,000	0/5
2	5 F	2,000	0/5

LD50: > 2,000 mg/kg bw
Signs of Toxicity - Local: No signs of dermal irritation were noted.
Signs of Toxicity - Systemic: No signs of systemic toxicity were noted.
Effects in Organs: No abnormalities were noted at necropsy.
Remarks - Results: Two female rats showed no gain in bodyweight or bodyweight loss during

the first week but recovered during the second week. One female rat showed expected gain in bodyweight during the first week but bodyweight loss during the second week.

CONCLUSION The test substance is of low toxicity via the dermal route.

TEST FACILITY Harlan (2010c)

B.3. Irritation – skin (*in vitro*)

TEST SUBSTANCE Analogue 1

METHOD Similar to OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method

Vehicle None. The test substance was administered undiluted.

Remarks - Method EPISKIN™ commercial model kit was used for the test. Following topical exposure to the test substance (10 µl, 15 min), the cell viability of the reconstituted human epidermal keratinocytes was determined using colorimetric MTT reduction assay. Post-exposure incubation period was set as 42 hours.

Negative control used was Dulbecco's phosphate buffered saline (PBS) with Ca^{2+} and Mg^{2+}. Positive control used was 5% w/v sodium dodecyl sulphate (SDS).

RESULTS

Test material	*Mean OD_{540} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.800	100.0	5.8
Test substance	0.847	105.8	3.9
Positive control	0.075	9.4	0.9

OD = optical density; SD = standard deviation

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

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

TEST FACILITY Harlan (2009f)

B.4. Irritation – eye (*in vitro*)

TEST SUBSTANCE Notified chemical

METHOD HET-CAM test (Kemper and Luepke, 1986)

Vehicle Not reported

Remarks - Method The test method was a modification of that described by Kemper and Luepke (1986). Zero point three millilitres (0.3 mL) of the test substance (10% in vehicle) and positive control substances (50% baby shampoo and 50% shampoo) were administered to quadruplet CAMs for each group. After twenty seconds the test or reference substance was rinsed from each CAM with 5 mL of physiological saline. Observations were made immediately prior to administration and at 0.5, 2 and 5 minute time points after the exposure.

RESULTS

Test material	*Mean total score*	*Standard deviation of total score*	*Results*
Test substance (10%)	2.00	1.15	Non-irritant

Test material	*Mean total score*	*Standard deviation of total score*	*Results*
Baby shampoo (50%)	11.00	2.00	Moderate irritant
Shampoo (50%)	21.00	3.56	Severe irritant

Remarks - Results

CONCLUSION The notified chemical at a concentration of 10% was not considered as an irritant to the eye under the conditions of the test.

TEST FACILITY Consumer Product Testing Co. (2007a)

B.5. Irritation – eye (*in vitro*)

TEST SUBSTANCE Analogue 1

METHOD Determination of Ocular Irritation Potential Using the SkinEthic Reconstituted Human Corneal Epithelium Model

Vehicle None. The test substance was applied undiluted.

Remarks - Method Following exposure to the test substance (30 µl, 10 min), the viability of the reconstituted human corneal epithelium was determined using colorimetric MTT reduction assay.

Negative control used was Solution A supplied with the test kit. Positive control used was 1% w/v sodium dodecyl sulphate (SDS).

RESULTS

Test material	*Mean OD_{540} of duplicate tissues*	*Relative mean viability (%)*
Negative control	0.972	100.0
Test substance	0.925	95.2
Positive control	0.730	75.1

OD = optical density

Remarks - Results The test substance did not directly reduce MTT. The positive control did not reach positive threshold (< 60% viability). Tissue histopathology was not performed on tested model.

CONCLUSION The test substance was considered to be non-irritating to the eye under the conditions of the test.

TEST FACILITY Harlan (2010e)

B.6. Irritation – eye

TEST SUBSTANCE Analogue 1

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).

Species/Strain Rabbit/New Zealand White

Number of Animals 2

Observation Period 72 hours

Remarks - Method Two male rabbits were used in the study.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: redness	0.3	0.3	2	< 48 h	0
Conjunctiva: chemosis	0.3	0.3	2	< 48 h	0

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: discharge	0	0.3	2	< 48 h	0
Corneal opacity	0	0	0	N/A	0
Iridial inflammation	0	0	0	N/A	0

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

Remarks - Results: Yellow coloured staining of the fur was noted around treated eyes throughout the study. No corneal or iridial effects were noted, but moderate conjunctival irritation was observed for treated eyes.

CONCLUSION: The test substance is slightly irritating to the eye.

TEST FACILITY: Harlan (2010d)

B.7. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Commission Regulation 440/2008, B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain: Mouse/CBA/CaOlaHsd

Vehicle: Acetone/olive oil 4:1

Remarks - Method: No significant protocol deviation. Preliminary screening test was conducted using 25 µl/day of the undiluted test substance for 3 days to observe the systemic toxicity and excessive local irritation.

Historical control data using 15% α-hexylcinnamaldehyde in acetone/olive oil 4:1 showed that the test strain of mouse produced positive response to the positive control substance.

RESULTS

Concentration (% v/v)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1,719.31	1.00
25	2,584.34	1.50
50	2,190.48	1.27
100 (no vehicle)	1,244.01	0.72
*Positive Control**		
15 (α-hexylcinnamaldehyde)	-	3.12

* Historical data

Remarks - Results: Preliminary screening test did not show systemic toxicity or excessive local irritation for the test substance.

CONCLUSION: There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.

TEST FACILITY: Harlan (2010f)

B.8. Skin sensitisation – human volunteers

TEST SUBSTANCE: Notified chemical (20% in corn oil)

METHOD: Repeated insult patch test with challenge

Study Design: Induction Procedure: 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 after 24 h of each application and the test sites were evaluated prior to each re-application. Rest Period: approximately 2 weeks Challenge Procedure: A challenge patch was applied to a naïve site adjacent to the original induction site. The patch was removed after 24 h and the site was evaluated 24 h and 72 h post-application.
Study Group	42 F, 14 M; age range 16-76 years; 50 subjects completed the test
Vehicle	Corn oil
Remarks - Method	Occluded. The test substance was spread on a 1.9 cm × 1.9 cm absorbent pad portion of an adhesive dressing.
RESULTS	
Remarks - Results	Six (6) subjects discontinued participation for non-test substance related reasons. There was no evidence of irritation during the study in any test subject.
CONCLUSION	The notified chemical at a concentration of 20% was not considered as irritating or sensitising under the conditions of the test.
TEST FACILITY	Consumer Product Testing Co. (2007b)

B.9. Repeat dose toxicity

TEST SUBSTANCE	Analogue 1 (purity 99%)
METHOD	Repeated dose oral (gavage) range-finding toxicity (14 days)
Species/Strain	Rat/Wistar Han™:HsdHan™:WIST
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 14 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Arachis oil BP
Remarks - Method	Dose levels tested: 250, 500 and 1,000 mg/kg bw/day

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	6 (3 M/3 F)	0	0/6
Low dose	6 (3 M/3 F)	250	0/6
Mid dose	6 (3 M/3 F)	500	0/6
High dose	6 (3 M/3 F)	1,000	0/6

Mortality and Time to Death

No unscheduled death of the animals was noted during the study.

Clinical Observations

No significantly observable signs of toxicity were detected. Sporadic increase of salivation after dosing was noted in 250 and 500 mg/kg bw/day dose groups and was not considered to represent a systemic effect. Two females in 250 mg/kg bw/day group showed generalised fur loss from Day 11 and were not considered as treatment related clinical signs.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No clinical chemistry, haematology and urinalysis examinations were conducted in this study.

Effects in Organs

No treatment related macroscopic abnormalities were noted at the necropsy. One male in 1,000 mg/kg bw/day

group was found to have hydronephrosis in the right kidney but was not considered as treatment related.

Remarks – Results
The oral administration of the test substance to the rats by gavage for a period of 14 days at levels of 250, 500 and 1,000 mg/kg bw/day did not reveal significant toxicological effects.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1,000 mg/kg bw/day in this study, based on the highest dose tested.

TEST FACILITY Harlan (2010g)

B.10. Repeat dose toxicity

TEST SUBSTANCE Analogue 1 (purity 99%)

METHOD OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.

Species/Strain	Wistar:HsdHan™:WIST
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 42 days (Males); until Day 5 post-partum (Females) Dose regimen: 7 days per week
Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviation

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	20 (10 M/10 F)	0	0/20
Low dose	20 (10 M/10 F)	100	0/20
Mid dose	20 (10 M/10 F)	300	0/20
High dose	20 (10 M/10 F)	1,000	0/20

Mortality and Time to Death
No unscheduled death of the animals was noted.

Clinical Observations
No significant clinical signs of toxicity were noted during the treatment period. Increased salivation after dosing was noted for all animals in the 300 and 1,000 mg/kg bw/day groups and for 1 male in the 100 mg/kg bw/day group. Instances of staining around the mouth were also observed for animals in the 300 and 1,000 mg/kg bw/day groups and instances of wet fur were noted for two females in the 1000 mg/kg bw/day group.

Noisy respiration was noted for 1 male in the 1,000 mg/kg bw/day group during the week 4 assessment.

Females in the 1,000 mg/kg bw/day group showed significant reduction in body weight gain associated with the reduction of dietary intake during the first week. Body weight gain and food consumption was recovered thereafter for these females.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Haematology – Males in the 300 and 1,000 mg/kg bw/day groups showed significant reduction in mean cell haemoglobin concentration. Males in the 100 mg/kg bw/day group showed increase in neutrophil counts. However, these findings were considered not to represent adverse effects of the treatment.

Blood chemistry – Males in the 300 and 1,000 mg/kg bw/day groups showed significant increase in blood calcium level and reduction of inorganic phosphorus level. Creatinine levels were significantly increased in all animals treated with the test substance. However, these findings were within the normal expected ranges and not considered to represent adverse effects of the treatment.

Effects in Organs

Increase of liver weight for females in the 1,000 mg/kg bw/day group was observed and associated with hepatocyte hypertrophy. For both males and females in the 1,000 mg/kg bw/day group, increase of follicular cell hypertrophy in the thyroid gland was also noted. However, in the absence of degenerative or inflammatory changes, these findings were considered to be caused by adaptive responses.

Reproduction/Developmental Toxicity

One male and female pair in the 100 mg/kg bw/day group showed evidence of mating but failed to achieve a pregnancy. No significant differences between the test groups and the control group were noted for gestation length, litter response, litter size, litter viability, and offspring development.

Remarks – Results

The treatment related effects observed in the study were not considered to represent systemic toxicity of the test substance.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for systemic toxicity of the test substance was established as 1,000 mg/kg bw/day in this study, based on the highest dose tested.

The NOAEL for reproductive toxicity of the test substance was established as 1,000 mg/kg bw/day in this study, based on the highest dose tested.

TEST FACILITY Harlan (2011b)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical (purity 99.72%)

METHOD

	OECD TG 471 Bacterial Reverse Mutation Test. EC Commission Regulation 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure – range-finding Pre incubation procedure – main test
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98 and TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	Microsomal Enzyme Fraction (S9) from phenobarbitone/β-naphthoflavone induced livers of male rats
Concentration Range in Main Test	a) With metabolic activation: 50, 150, 500, 1500, and 5000 µg/plate b) Without metabolic activation: 50, 150, 500, 1500, and 5000 µg/plate
Vehicle	Dimethyl sulphoxide (DMSO)
Remarks - Method	Preliminary toxicity test was conducted using TA100 (*S. typhimurium*) and WP2uvrA$^-$ (*E. coli*) for concentrations of 0, 0.15, 0.5, 1.5, 5 15, 50, 150, 500, 1500 and 5000 µg/plate.

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 Preliminary toxicity test showed that the test substance was non-toxic to TA100 and WP2uvrA$^-$. At concentration of 5,000 µg/plate, precipitation and film formation of the test substance were noted.

No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose of the test substance, either with or without metabolic activation.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies 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 Harlan (2009g)

B.12. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 471 Bacterial Reverse Mutation Test (Non-GLP Protocol M07-5024, Consumer Product Testing Co, 2007).

Species/Strain	*S. typhimurium*: TA97a, TA98, TA100, TA102 and TA1535
Metabolic Activation System	Microsomal enzyme fraction (S9) from Aroclor™ 1254 induced rat livers
Concentration Range in Main Test	a) With metabolic activation: 5, 50, 100, 500, 1000, and 5000 µg/plate b) Without metabolic activation: 5, 50, 100, 500, 1000, and 5000 µg/plate
Vehicle	Dimethyl sulphoxide (DMSO)
Remarks - Method	ICR 191 acridin, daunomycin, sodium azide, mitomycin C and 2-aminoanthracene (with S9) were used as positive controls. Plate incorporation procedure was used for the test.

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 The test substance was not cytotoxic to bacterial strains.

No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose of the test substance, either with or without metabolic activation.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies 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 Consumer Product Testing Co. (2007c)

B.13. Genotoxicity – *in vitro*

TEST SUBSTANCE Notified chemical (purity 99.1%)

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test.

	EC Commission Regulation 440/2008 B.10 Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test.
Species	Human cell line
Cell Type	Lymphocytes
Metabolic Activation System	Microsomal enzyme fraction (S9) from phenobarbitone/β-naphthoflavone induced rat livers
Vehicle	Dimethyl sulphoxide (DMSO)
Remarks - Method	No significant protocol deviation.
	Positive controls: With metabolic activation – mitomycin C (0.4 µg/mL in test 1 and 0.2 µg/mL in test 2) Without metabolic activation – cyclophosphamide (5 µg/mL in test 1 and 4 µg/mL in test 2)

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	122.19, 244.38, 488.75*, 977.5*, 1955*, 3910*	4	24
Test 2	7.64, 15.28, 30.56*, 61.13*, 91.7*, 122.25	24	24
Present			
Test 1 (2% S9)	122.19, 244.38, 488.75*, 977.5*, 1955*, 3910*	4	24
Test 2 (1% S9)	61.13, 122.25*, 244.5*, 489*, 733.5*, 978	4	24

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 1,955	≥ 1,955	≥ 122.19	Positive
Test 2	≥ 61.09	≥ 61.13	≥ 61.09	Negative
Present				
Test 1	≥ 977.5	≥ 977.5	≥ 122.19	Negative
Test 2	-	≥ 978	≥ 61.13	Negative

Remarks - Results

Positive controls showed expected significant increases in the frequency of cells with chromosome aberrations.

The test substance induced some evidence of toxicity in the exposure groups with most of the effect observed in the 24 h exposure group at concentrations ≥ 122.19 µg/mL. A clear plateau effect was noted in the 24 h exposure group.

A clear statistically significant increase in the frequency of structural chromosome aberrations was observed in cells exposed to 3910 µg/mL (highest dose) of the test substance with 4 hour exposure without metabolic activation. The chromosome aberrations noted were predominantly break type. Increase in frequency of chromosome aberrations was not confirmed when the cells were exposed to the test substance at concentrations up to 122.19 µg/mL for 24 hours without metabolic activation.

No marked toxicologically significant increase in the frequency of cells with aberrations was observed in the presence of metabolic activation in either of two experiments.

CONCLUSION

There was inadequate evidence to indicate that the notified chemical is not clastogenic at high concentration to human lymphocytes treated *in vitro*

under the conditions of the test.

TEST FACILITY Harlan (2011a)

B.14. Genotoxicity – *in vitro*

TEST SUBSTANCE Notified chemical (purity 99.1%)

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test.
EC Directive 2000/32/EC B.17 Mutagenicity - *In vitro* Mammalian Cell Gene Mutation Test.

Species/Strain Mouse
Cell Type/Cell Line Lymphoma/L5178Y TK+/-
Metabolic Activation System Microsomal enzyme fraction (S9) from phenobarbitone/β-naphthoflavone induced rat livers
Vehicle Dimethyl sulphoxide (DMSO)
Remarks - Method No significant protocol deviations.

Positive controls:
Without metabolic activation – ethylmethanesulphonate (EMS) (400 µg/mL in Test 1 and 150 µg/mL in Test 2)
With metabolic activation – cyclophosphamide (CP, 2 µg/mL in both Test 1 and Test 2)

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	30.55, 61.09, 122.19, 244.38, 488.75, 977.5, 1955, 3910	4 h	2 d	10-14 d
Test 2	0.5, 1, 2, 4, 8, 16, 32, 64	24 h	2 d	10-14 d
Present				
Test 1	30.55, 61.09, 122.19, 244.38, 488.75, 977.5, 1955, 3910	4 h	2 d	10-14 d
Test 2	16, 32, 64, 128, 256, 512, 1024, 1536	4 h	2 d	10-14 d

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 977.5	≥ 977.5	≥ 122.19	Negative
Test 2	≥ 15.27	≥ 16	≥ 64	Negative
Present				
Test 1	≥ 977.5	≥ 977.5	≥ 122.19	Negative
Test 2		≥ 1024	≥ 64	Negative

Remarks - Results In Test 1 in the presence of metabolic activation, a small statistically significant increase in mutation rate was observed at a dose level 977.5 µg/mL that approached the level of acceptable toxicity. However, the increase was not part of any linear trend response and the mutant frequency would have been considered acceptable for vehicle controls. Therefore the study authors concluded the response observed was not of toxicological significance. No significant increases in mutation frequency were observed in the absence of metabolic activation.

There were no significant increases in mutation rates observed in Test 2 with or without metabolic activation.

CONCLUSION The notified chemical was considered not clastogenic to mouse lymphoma L5178Y cells treated *in vitro* under the conditions of the test.

TEST FACILITY Harlan (2011c)

B.15. Genotoxicity – *in vivo*

TEST SUBSTANCE Analogue 1 (purity 98.5%)

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain Mouse/Albino HSD: ICR (CD-1®)
Route of Administration Intraperitoneal injection
Vehicle Arachis oil
Remarks - Method No significant protocol deviations.

Positive controls - cyclophosphamide (CP), orally dosed

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	7 M	0	24
II (low dose)	7 M	500	24
III (mid dose)	7 M	1,000	24
IV (high dose 1)	7 M	2,000	24
V (high dose 2)	7 M	2,000	48
V (positive control, CP)	5 M	50	24

RESULTS
Doses Producing Toxicity None
Genotoxic Effects No significant decreases in the PCE/NCE ratio were observed at any dose level. No evidence of significant increases in the incidence of micronucleated polychromatic erythrocytes was noted for the test substance.
Remarks - Results

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* mammalian erythrocyte micronucleus test.

TEST FACILITY Harlan (2013)

B.16. Phototoxicity – Human Volunteers

TEST SUBSTANCE Notified chemical

METHOD Phototoxicity response in human subjects
Remarks - Method Twenty one (21) volunteers, 2 males and 19 females aged between 19 and 60, were selected for participation.

Test sites: 3 areas of skin at the lower back (between scapulae and beltline, lateral to midline) were selected. Two sites were treated with the test substance with one irradiated using the light source. The third site remained untreated but was irradiated.

Light source: A Xenon Arc Solar Simulator (150 W) was used to produce UVA (290 – 320 nm) and UVB (320 – 400 nm). A Schott WG 345 filter was then used to block UVB to allow delivery of only UVA.

Treatment: Approximately 200 µL of the test substance was added to a 1.9 cm × 1.9 cm gauze portion of an adhesive dressing. The adhesive

	dressing was then applied to the treatment site to form an occluded patch. The patches were removed after 24 h and the appropriate sites were irradiated with 0.5 MED (Minimal Erythemal Dose) UVB followed by 20 joules of UVA. Test and control sites were examined at 48 h and 72 h after the irradiation.
RESULTS	
Remarks - Results	Among 21 volunteers, none of the test sites and control sites produced visible skin reaction under the conditions of the study.
CONCLUSION	The notified chemical did not induce a response indicative of a phototoxic reaction under the conditions of the study.
TEST FACILITY	Consumer Product Testing Co. (2008)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
- Inoculum: Activated sludge
- Exposure Period: 29 days
- Auxiliary Solvent: Acetone
- Analytical Monitoring: Carbon dioxide and dissolved organic carbon (DOC) analysis
- Remarks - Method: The test substance is poorly soluble in water. Hence, it was dissolved in acetone to prepare the solvent stock solution. To increase the dispersibility of the test substance in the test medium and to increase the surface area of the test material exposed to the test organisms, an aliquot of the solvent stock solution was dispersed onto a filter paper. The solvent was allowed to evaporate to dryness prior to addition of the filer paper to inoculated culture media.

  The test was conducted in accordance with the test guideline above without significant deviation from the protocol reported. Good Laboratory Practice (GLP) was followed.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
6	9	6	56
14	2	14	66
21	9	21	66
28	0	28	74
29	4	29	87

- Remarks - Results: All validity criteria for the test were satisfied.

  The toxicity control attained 50% degradation after 14 days, indicating the test substance was not toxic to the micro-organisms in the sewage treatment sludge used in the test.

CONCLUSION: The notified chemical is not readily biodegradable.

TEST FACILITY: Harlan (2009c)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 203 Fish, Acute Toxicity Test – Semi-static.
- Species: Rainbow trout *(Oncorhynchus mykiss)*
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 140 mg $CaCO_3$/L
- Analytical Monitoring: HPLC
- Remarks – Method: Following a preliminary range-finding test, two groups of fish (7 fish per group) were exposed to an aqueous solution of test substance at a single

concentration of 100% v/v saturated solution. The 100% v/v saturated solution was prepared by dissolving excess amount of the test substance in water, followed with stirring for 24 hours. The undissolved test substance was removed by filtration.

The test media were renewed daily. The number of mortalities and any sub-lethal effects of exposure in each test and control were monitored 3 hours after the start of exposure and then daily throughout the test.

The test was conducted in accordance with the test guideline above without significant deviation from the protocol reported. The GLP was followed.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	-	7	0	0	0	0
Saturated solution (100% v/v)	< LOQ* - 0.000564	14	0	0	0	0

* LOQ: the limit of quantitation (0.00030 mg/L)

LC50	> 100% v/v saturated solution at 96 hours.
NOEC	100% v/v saturated solution at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied.

The measured concentration for the test substance at 0, 24 and 96 hours ranged from less than the limit of quantitation of the analytical method to 0.000564 mg/L. The test substance has a lower water solubility than the notified chemical.

There were no sub-lethal effects on fish was observed for the test preparations during the test. The results indicated the test substance, Analogue 1, is not harmful to fish at its saturated concentration. However, the analogue chemical is expected to be less bioavailable than the notified chemical as Analogue 1 has the lower water solubility. Therefore, the toxic effects of the notified chemical on fish may be underestimated based on the endpoints obtained for the analogue.

CONCLUSION The analogue is not harmful to fish up to the limit of its water solubility.

TEST FACILITY Harlan (2011)

C.2.2. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 211 *Daphnia magna*. Reproduction Test – Semi-static.

Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	Tetrahydrofuran
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	The test substance is poorly soluble in water. Therefore, it was dissolved in tetrahydrofuran to prepare the solvent stock solution. An aliquot of the solvent stock solution was dispersed in water to give a 1.0 mg/L stock dispersion. This stock dispersion was centrifuged at 40,000 G-force for 30 minutes to give a nominal concentration of 0.01 mg/L. The other lower test concentrations were prepared by serial dilution of 0.01 mg/L with water. The concentration and stability of the test substance in the test preparations were verified by HPLC analysis.

Ten vessels contained the solvent control (100 µl tetrahydrofuran per litre) and 10 vessels contained the test substance with each vessel containing a neonate (≤ 24 h old) daphnid. Test solutions were renewed daily. The numbers of live and dead adult daphnids and young daphnids (live rand dead) were determined daily. The daphnids were fed daily with an algal suspension.

RESULTS

Nominal loading retested, daphnid survival and cumulative mean number of offspring released, mean total body length of daphnids over the test period of 21 days

Nominal concentration (mg/L)	Time-weighted mean measured concentration (mg/l)	Mean percentage of adult survival	Cumulative number of offspring produced per female
Solvent control	-	100	61
0.0001	0.0001*	100	64
0.00032	0.0032*	100	57
0.001	0.0021	100	55
0.0032	0.0048	90	54
0.01	0.016	100	42

*Concentrations are nominal concentrations as analytical results did not allow for the calculation of time-weighted mean measured concentrations.

EC50 (immobilisation)	> 0.016 mg/L at 21 days (based on time-weighted mean measured concentration)
NOEC (immobilisation)	0.0048 mg/L at 21 days (based on time-weighted mean measured concentration)
Remarks - Results	No significant deviations to protocol were reported and all validity criteria for the test were satisfied.

The test substance was determined to be stable in the test medium over 48 hour. However, analysis of the fresh media for the highest three test concentrations gave the measured concentrations ranging from 0.00139-0.0378 mg/L. Analysis of the old media (24 hours old) showed the measured concentration ranging from 0.00042 to 0.0182 mg/L. This decline in measured concentration was considered to be due to possible bioaccumulation to the test organisms and/or adsorption of the test substance to test vessel. Therefore, the results were reported based on the time-weighted mean measured concentrations apart from the two lowest test concentrations.

Due to poor solubility of the test substance, the measured concentrations for the two lowest concentrations were variable. It was considered unsuitable for calculation of time-weighted mean measure concentrations. These two concentrations were therefore, reported as nominal concentrations. This was not considered to affect the test results as these two concentrations are below the No Observed Effect Concentration (NOEC).

The daphnids in all the test concentrations were observed to be the same size and colour as those in the controls over the duration of the test. The filial daphnids produced by all the test groups were in the same general condition as the young produced in the control. A single mortality was observed at the test concentration of 0.0048 mg/L on Day 10. However, there was no significant mortalities difference in the parental generation and there was no significant difference in live young daphnids produced per adult when compared with the solvent control. Therefore, the reported NOEC = 0.0048 mg/L is considered justifiable.

The results from daphnia reproduction test were questionable due to the poor water solubility of the test substance. The toxic effects of the test

substance on *Daphnia magna* reproduction may be artifactual due to the use of auxiliary solvent. Therefore, the notifier provided an Addendum to the *Daphnia magna* reproduction test in which a second study was conducted to investigate the solubility of the test substance in solvent free test medium. The test substance was dissolved in the test medium using a slow stir method of preparation. The solubility of the test substance in the solvent-free test medium was determined to be 0.0018 mg/L, which is below the EC50 of 0.016 mg/L and the NOEC of 0.0048 mg/L, respectively. Therefore, the notifier concluded that the test substance has no toxic effect on daphnia up to the limit of water solubility.

For the purpose of this assessment, the solvent free solubility test was deemed to be acceptable as it is more representative of the notified chemical in the environment. Therefore, The solubility of 0.0018 mg/L is considered to be the water solubility limit of the notified chemical and the test substance is considered not harmful to daphnia up to its water solubility limit.

CONCLUSION The notified chemical is not toxic to aquatic invertebrates up to the limit of its water solubility.

TEST FACILITY Harlan (2010a)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: Solvent control, 0.009 mg/L Actual: < limit of quantitation, 0.011 mg/L (based on the geometric mean measured concentrations)
Auxiliary Solvent	Tetrahydrofuran
Water Hardness	Not available
Analytical Monitoring	HPLC
Remarks - Method	The test substance is poorly soluble in water. Therefore, it was dissolved in tetrahydrofuran to prepare the solvent stock solution. An aliquot of the solvent stock solution was dispersed in water to give a 1.0 mg/L stock dispersion. This stock dispersion was centrifuged at 40,000 G-force for 30 minutes to give a nominal concentration of 0.009 mg/L, the highest attainable test concentration. An aliquot of the 0.009 mg/L stock solution was inoculated with algal suspension. The concentration and stability of the test substance in the test preparations were verified by HPLC analysis at 0 and 72 hours. Six flasks were used for each test, the control, the solvent control (100 µl tetrahydrofuran/L) and the test substance. Based on the preliminary test, a definitive test was conducted in accordance with the test guidelines above and in compliance with GLP standards and principles.

RESULTS

*Biomass		*Growth	
E_bC50 (mg/L at 72 h)	NOE_bC (mg/L at 72 h)	E_rC50 (mg/L at 72 h)	NOE_rC (mg/L at 72 h)
> 0.011	0.011	> 0.011	0.011

*The endpoints were based on the geometric mean measured concentrations

Remarks - Results No significant deviations to protocol were reported and all validity criteria for the test were satisfied.

Following the preliminary range-finding test, green algae was exposed to an aqueous solution of the test substance at a nominal concentration of 0.009 mg/L (six replicates) under constant illumination and shaking at 24 ± 1 °C. Samples of the algal population were removed daily and cell concentrations determined for each control and treated group.

Due to the limited solubility of the test substance in the test medium, the highest attainable test concentration was determined to be 0.011 mg/L based on geometric mean measured concentrations.

CONCLUSION The notified chemical is not harmful to algae up to the limit of its solubility.

TEST FACILITY Harlan (2009d)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
- Inoculum: Activated sludge
- Exposure Period: 3 hours
- Concentration Range: Nominal: Control, 10, 32, 100, 320 and 1,000 mg/L; Actual: Not determined
- Remarks – Method: Amounts of test substance (5, 16, 50, 160 and 500 mg) were each separately dispersed into 250 mL of water, followed with ultrasonication for 15 minutes and stirring for 24 hours. Synthetic sewage, activated sludge and water were added to a final volume to give the nominal concentrations of 10, 32, 100, 320 and 1,000 mg/L.

  The test was conducted in accordance with the test guidelines above and in compliance with GLP standards and principles.

RESULTS
- IC50: > 1,000 mg/L at 3 hours
- NOEC: 1,000 mg/L at 3 hours
- Remarks – Results: In some instances, initial and final dissolved oxygen concentrations were below those recommended in the test guideline. This was considered to have no adverse effect on the results of the study given that in all cases the oxygen consumption rate was determined over the linear portion of the oxygen consumption trace.

  Variation in respiration rates of controls 1 and 2 was ± 2%, within 15% range. The EC50 (3 hour contact) for 3,5-dichlorophenol, the reference test material, was determined to be 8.3 mg/L which is within the range of 5-30 mg/L. Therefore, the results for the study are considered valid.

CONCLUSION The notified chemical is not expected to inhibit microbial respiration in the test sludge.

TEST FACILITY Harlan (2010b)

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