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December 2016

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

1-Butanone, 3-(dodecylthio)-1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-

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 and Energy.

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

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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
STD/1599	Firmenich Limited	1-Butanone, 3-(dodecylthio)-1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-	Yes	≤ 10 tonnes per annum	A fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Sensitisation, Skin (Category 1)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R43: May cause skin sensitisation by skin contact

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

Based on the available information, when used in the assessed pattern, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the reported use pattern and low expected aquatic exposure, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Sensitisation, Skin (Category 1): H317 – May cause an allergic skin reaction

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

- The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the notified chemical for listing on the SUSMP.

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES

Occupational Health and Safety

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

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

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

  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.

Disposal

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, 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 ten tonnes per annum notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed 0.2% in leave-on and rinse-off cosmetics, 0.48% in fine fragrances, 1% in household products, 1.5% in instant action air fresheners and 15% in other types of air fresheners;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a fragrance 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)
Firmenich Limited (ABN: 86 002 964 794)
73 Kenneth Road
BAGOWLAH NSW 2093

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, impurities, and use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for all physical-chemical and toxicological/ecotoxicological endpoints that were measured for two diastereomers of the notified chemical.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Canada (2014), USA (2005), South Korea (2007), Philippines (2006), Japan (2006, and 2015), Europe (2005, and 2015) and China (2007)

2. IDENTITY OF CHEMICAL

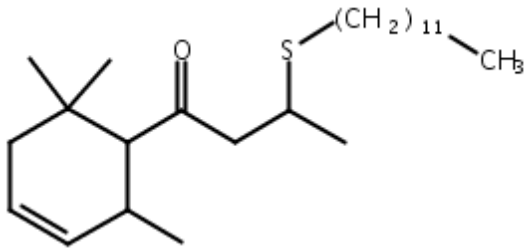
MARKETING NAME
3-(dodecylthio)-1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-1-butanone

CAS NUMBER
543724-31-8

CHEMICAL NAME
1-Butanone, 3-(dodecylthio)-1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-

MOLECULAR FORMULA
$C_{25}H_{46}OS$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
394.70 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 90%

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless to pale yellow liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	19 °C	Measured
Boiling Point	> 294 °C at 98.0 kPa	The test substance decomposed from 294 °C at 98.0 kPa prior to boiling
Density	921 kg/m^3 at 20 °C	Measured
Vapour Pressure	7.5×10^{-9} kPa at 25 °C	Measured
Water Solubility	$\leq 9.3 \times 10^{-5}$ g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains no hydrolysable functionalities
Partition Coefficient (n-octanol/water)	log Pow > 6.20	Measured
Adsorption/Desorption	log K_{oc} = 6.5	Calculated using KOCWIN v2.0 (US EPA, 2012).
Dissociation Constant	Not determined	Contains no dissociable functions
Flash Point	181 °C at 101.325 kPa	Measured
Flammability	Not determined	The notified chemical is not expected to be flammable as two diastereomers have a relatively high flash point 181 °C
Autoignition Temperature	256 °C	Measured
Explosive Properties	Non explosive	Not expected to have explosive properties based on the chemical structure
Oxidising Properties	Non oxidising	Not expected to have oxidising properties based on the chemical structure

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia either in pure form or as a component in fragrance formulations.

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

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

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited

TRANSPORTATION AND PACKAGING
The imported notified chemical will be transported by road via truck to the notifier's warehouse or customers' facilities for storage or reformulation. After reformulation, the formulated products containing the notified chemical will be distributed in drums of varying sizes: 180 (typical size), 100, 50, 25, 10 or 5 kg. They will be then transported by road for retail sale.

USE
The notified chemical will be used as a fragrance component in a variety of cosmetic and household products at typical final use concentrations of ≤ 0.2% in leave-on/rinse-off cosmetics, ≤ 0.48% in fine fragrances and ≤ 1% in household cleaning products (use details claimed as Exempt Information).

OPERATION DESCRIPTION
The reformulation procedures for incorporating the notified chemical into end-use products will likely vary depending on the nature of the cosmetic and personal care/household cleaning products formulated. This may involve both automated and manual processes including transferring and blending the notified chemical with other formulations. However, a typical blending operation will be highly automated and occur in a fully enclosed/contained environment, followed by automated filling using sealed delivery systems into containers of various sizes.

The end-use products containing the notified chemical may be used by consumers and professionals such as hairdressers, workers in beauty salons or cleaners.

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	Unknown	Unknown
Mixing	4	2
Drum handling	4	2
Drum cleaning	4	2
Maintenance	4	2
Quality Control	0.5	1
Packaging	4	2
Salon	Unspecified	Unspecified
Cleaners	Unspecified	Unspecified

EXPOSURE DETAILS
Transport and storage
Transport and storage workers may come into contact with the notified chemical in neat form or as a component of the imported preparations, only in the event of accidental rupture of containers. Incidental exposure to the notified chemical may occur via skin or eye during the clean-up of accidental spills.

Formulation of end use products
At the reformulation sites, workers will involve in transferring, weighing and blending of the notified chemical or preparations containing the notified chemical, periodic sampling for quality control analysis and cleaning and maintenance of equipment operations. During these operations, dermal, ocular and potentially inhalation exposure of workers to the notified chemical may occur. Exposure is expected to be minimised through the use of local exhaust ventilation, automated and enclosed systems, including sealed delivery systems and through the use of personal protective equipment (PPE) such as gloves, respirator, eye protection and protective clothing.

Beauty care and cleaning professionals
Dermal and ocular repeated exposure to the notified chemical in end-use products may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. hairdressers, workers in beauty salons) or in the cleaning industry. Exposure is expected to be minimised by the use of PPE and good hygiene practices in place.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of a variety of cosmetic and household products at various concentrations. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

For use of the notified chemical in cosmetic and household products, a combined internal dose of 0.5706 mg/kg bw/day was estimated using data on typical use patterns of the product categories in which the notified chemical may be used (SCCS, 2012; Cadby *et al.*, 2002; Loretz *et. al.*, 2006; ACI, 2010; specific use details of the notified chemical are considered as exempt information). This estimation assumed a worst case scenario and is for a person who is a simultaneous user of a selection of cosmetic and household products that may contain the notified chemical.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,500 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
HRIPT at 1%	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 1,000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity *in vitro* mammalian chromosome aberration test	non genotoxic
Genotoxicity *in vitro* mammalian cell gene mutation test	non genotoxic
Reproduction/Developmental Toxicity Screening	Systemic toxicity: NOAEL = 1,000 mg/kg bw/day Reproductive toxicity: NOEL = 1,000 mg/kg bw/day

Toxicokinetics, metabolism and distribution
No toxicokinetic data on the notified chemical were submitted. Based on the low molecular weight (< 500 Da), water solubility (≤ 9.3×10^{-5} g/L) and partition coefficient (log Pow > 6.20) of the two stereoisomers of the notified chemical, absorption across biological membranes may occur.

Acute toxicity
Based on the data provided, the notified chemical is expected to have low acute oral and dermal toxicity. No information was provided on acute inhalation toxicity.

Irritation and sensitisation
Based on the data in studies conducted in rabbits, the notified chemical is expected to be non-irritating to the skin and slightly irritating to the eyes.

Based on the data provided, the notified chemical is expected to be sensitising in a Local Lymph Node Assay. The EC_3 value for the notified chemical was calculated to be 23.6%. The two stereoisomers of the notified chemical were not sensitising in a repeated insult patch test with challenge when tested at 1%.

Repeated dose toxicity
A repeated dose oral (gavage) toxicity study provided was conducted in rats, in which the two stereoisomers of the notified chemical were administered at 30, 300 and 1,000 mg/kg bw/day for 28 consecutive days. The No

Observed Adverse Effect Level (NOAEL) was established as 1,000 mg/kg bw/day in the study as no treatment related changes were observed at this highest dose tested.

Mutagenicity/Genotoxicity
Provided study data on two stereoisomers of the notified chemical showed negative results in a bacterial reverse mutation assay, an *in vitro* chromosomal aberration test using human lymphocytes and an *in vitro* mammalian cell gene mutation test using the Chinese hamster ovary (CHO) cells.

Developmental toxicity
The NOAEL for systemic toxicity and the NOEL for reproductive toxicity of the two stereoisomers of the notified chemical were established as the highest dose tested (1,000 mg/kg bw/day) in a reproduction/developmental toxicity screening study in rats, with no significant treatment-related effects observed.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Sensitisation, Skin (Category 1)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R43: May cause skin sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available information the critical health effect of the notified chemical is skin sensitisation.

Reformulation
During reformulation workers may be at risk of sensitisation when handling the notified chemical at up to 100% concentration. It is anticipated by the notifier that engineering controls such as enclosed and automated processes and local ventilation will be implemented where possible, and appropriate PPE (coveralls, imperious gloves, eye protection and respiratory protection) will be used to limit workers exposure.

Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use
Cleaners, hair and beauty care professionals will handle the notified chemical in a variety of cosmetic and household products (at various concentrations). 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 (for details of the public health risk assessment, see Section 6.3.2).

6.3.2. Public Health
Members of the public may be repeatedly exposed to the notified chemical during the use of a variety of cosmetic and household products at various concentrations.

Sensitisation
Methods for the quantitative risk assessment of dermal sensitisation have been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). Using fine fragrance as an example product that may contain the notified chemical at a maximum concentration of 0.48%, as a worst case scenario, the Consumer Exposure Level (CEL) for the notified chemical is estimated to be 18.00 µg/cm^2/day (Cadby *et al.,* 2002). When tested in an LLNA study, the notified chemical was a skin sensitiser with an EC_3 value of 23.6%. Consideration of each of the studies and application of appropriate safety factors, allowed the derivation of an Acceptable Exposure Level (AEL) of 18.11 µg/cm^2. In this instance, the factors employed included an

interspecies factor (3), intraspecies factor (10), a matrix factor (3.16), a use/time factor (3.16) and database factor (1), giving an overall safety factor of ~300.

As the AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of fine fragrances (a worst case example of a leave-on cosmetic product) is not considered to be unreasonable. Based on the significantly lower expected exposure level from other leave-on cosmetic products, rinse-off products and household products, by inference, the risk of induction of sensitisation associated with the use of these products is also not considered to be unreasonable. It is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on aggregate exposure has not been conducted.

Repeated-dose toxicity
The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario from use of multiple products of 0.5706 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 1,000 mg/kg bw/day derived from a 28-day repeated dose oral toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 1,752. A MOE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical in a variety of cosmetic and household products at various concentrations assessed is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will not be manufactured in Australia, so there will be no environmental release associated with this activity. The notified chemical will be imported into Australia as a component of fragrance formulations that will be further reformulated into end-use cosmetic and household cleaning products. In the event of a spill, the notified chemical is expected to be contained and collected in an inert absorbent material and disposed of in accordance with local regulations.

A typical blending operation will be highly automated in a fully enclosed/contained environment. Potential sources of release include spills, equipment washing, and container residues. A total of 0.1% of waste may be generated as a result of spills. It is expected that equipment will be cleaned using water which will be reused for subsequent operations. The average amount of residue in empty containers after removal by vacuum pump is estimated to be < 0.1%. Therefore, a total of < 0.2% (20 kg) of waste will be generated each year from reformulation processes.

RELEASE OF CHEMICAL FROM USE
The notified chemical will enter the aquatic compartment during use of the various products into which it will be incorporated. Cosmetic products are expected to be washed off the hair and skin and will enter the aquatic environment diluted in water. Cleaning products will also be diluted in water and will enter the aquatic environment. It is anticipated that the majority of the notified chemical released will enter into sewer systems.

RELEASE OF CHEMICAL FROM DISPOSAL
Empty containers containing the notified chemical at blending facilities will be recycled or disposed of through an approved waste management facility. It is estimated that a maximum of 3% (300 kg) of the notified chemical may remain in the consumer containers that will be sent for disposal.

7.1.2. Environmental Fate
Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system through its use as a component of cosmetics and household cleaning products before potential release to surface waters nationwide. Based on the data provided, the notified chemical is not considered to be readily biodegradable (26% in 28 days). For details of the environmental fate studies, please refer to Appendix C. The calculated adsorption/desorption coefficient (log K_{oc} = 3.08 – 3.11) indicates that the notified chemical may sorb to soil and sediment.

The half-life of the notified chemical in air is calculated to be 8.04 hours based on reactions with hydroxyl radicals (AOPWIN v1.92; US EPA, 2011). Therefore, in the event of release to atmosphere, the notified chemical is not expected to persist in the atmospheric compartment.

The majority of the notified chemical will be released to sewer after use. A small proportion of 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 as collected spills and empty containers. The notified chemical is expected to have low water solubility and predicted to be hydrophobic. Therefore, in the waste water treatment processes in the sewage treatment plant (STP), most of the notified chemical is expected to partition to sludge or to suspended solids where it will be removed for disposal to landfill. In landfill the notified chemical is expected to slowly decompose by abiotic and biotic processes to form water and oxides of carbon. Therefore, the notified chemical is not expected to be bioavailable to aquatic organisms despite its potential for bioaccumulation.

7.1.3. Predicted Environmental Concentration (PEC)

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

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	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	200.0	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:	6.06	µg/L
PEC - Ocean:	0.61	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1,500 kg/m^3). Using these assumptions, irrigation with a concentration of 6.06 µg/L may potentially result in a soil concentration of approximately 40.3 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years may be approximately 201.9 µg/kg and 403.9 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 > 0.1 mg/L	Not harmful to fish up to the limit of its water solubility
Fish Toxicity	LC50 > 100 mg/L	Not harmful to fish
Daphnia Toxicity	EC50 > 0.1 mg/L	Not toxic to aquatic invertebrates up to the limit of its water solubility
Algal Toxicity	EC50 > 0.1 mg/L	Not harmful to algae up to the limit of its solubility
Inhibition of Bacterial Respiration	3h EC50 > 1000 mg/L	Not inhibitory to bacterial respiration

Based on the above ecotoxicological endpoints, the notified chemical is not expected to be harmful to aquatic life up to the limit of its water solubility. Therefore, the notified chemical is not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* (United Nations, 2009) for acute and chronic toxicities.

7.2.1. Predicted No-Effect Concentration

A predicted no effect concentration (PNEC) has not been calculated as the notified chemical is not considered to be harmful to aquatic life up to the limit of its solubility in water.

7.3. Environmental Risk Assessment

A risk quotient RQ (PEC/PNEC) has not been derived since the PNEC has not been calculated. The notified chemical is expected to be neither readily biodegradable, nor bioaccumulative. Therefore, on the basis of the, maximum annual importation 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: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 19 ± 0.5 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Determination of crystallisation point
Test Facility	Firmenich (2004)

Boiling Point > 294 °C at 98.0 kPa

Method	OECD TG 103 Boiling Point.
Remarks	The test substance decomposed from 294 °C at 98.0 kPa prior to boiling using Siwoloboff method.
Test Facility	Firmenich (2004)

Density 921 kg/m^3 at 20 ± 0.5 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Oscillating density meter method.
Test Facility	Firmenich (2004)

Vapour Pressure 7.5×10^{-9} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Vapour pressure balance method. Linear regression analysis used for the calculation of the vapour pressure.
Test Facility	Safepharm (2006a)

Water Solubility ≤ 9.3×10^{-5} g/L at 20 °C

Method	Method A6 of Commission Directive 92/69/EEC
Remarks	Flask Method. Quantification was conducted by GC-MS.
Test Facility	Safepharm (2004a)

Partition Coefficient (n-octanol/water) log Pow > 6.2 at 20 °C

Method	EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method
Test Facility	Safepharm (2004a)

Flash Point 181 ± 2 °C at 101.325 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup equilibrium method.
Test Facility	Firmenich (2004)

Autoignition Temperature 256 ± 5 °C

Method	EC Commission Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Determination by heating the aliquots of the test substance in a flask and observing for any ignition.
Test Facility	Safepharm (2006a)

Explosive Properties

Method	EC Commission Directive 92/69/EEC A.14 Explosive Properties.
Remarks	Based on the chemical structure, the test substance is not expected to have explosive properties.
Test Facility	Safepharm (2006a)

Oxidizing Properties

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	Based on the chemical structure, the test substance is not expected to have oxidising properties.
Test Facility	Safepharm (2006)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Two stereoisomers of the notified chemical

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method (2001).
- Species/Strain: Rat/Sprague-Dawley CD
- Vehicle: None
- Remarks - Method: None protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	2,000	0/3
2	3 F	2,000	0/3

- LD50: > 2,500 mg/kg bw
- Signs of Toxicity: No signs of systemic toxicity were noted.
- Effects in Organs: No abnormalities were observed at necropsy.
- Remarks - Results: Expected bodyweight gain was noted for all animals during the study period.

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

TEST FACILITY: Safepharm (2004b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Two stereoisomers of the notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test (1987).
- Species/Strain: Rat/Sprague-Dawley CD
- Vehicle: None
- Type of dressing: Semi-occlusive.
- Remarks - Method: No protocol deviations

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 - Local: No signs of skin irritation were noted.
- Signs of Toxicity - Systemic: No signs of systemic toxicity were noted.
- Effects in Organs: No abnormalities were observed at necropsy.
- Remarks - Results: Expected bodyweight gain was noted for all animals during the study period.

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

TEST FACILITY: Safepharm (2005)

B.3. Irritation – skin

TEST SUBSTANCE: Two stereoisomers of the notified chemical

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion (1992).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Vehicle	Moistened with water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No protocol deviations

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	0	0	1	< 24 h	0
Oedema	0	0	0	0	-	0

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

Remarks - Results	Very slight erythema was observed in all treated skin sites one hour after patch removal. All treated skin sites appeared normal at the 24-hour observation.
CONCLUSION	The test substance is non-irritating to the skin.
TEST FACILITY	Safepharm (2004c)

B.4. Irritation – eye

TEST SUBSTANCE	Two stereoisomers of the notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion (2002).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Observation Period	72 hours
Remarks - Method	No protocol deviations

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	0.7	0.7	0.7	2	< 72 h	0
Conjunctiva: chemosis	0	0	0.7	1	< 72 h	0
Conjunctiva: discharge	0.3	0	0.3	2	< 48h	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	No corneal or iridial effects were observed. Minimal to moderate conjunctival irritation was observed in all treated eyes one hour after treatment with minimal conjunctival irritation observed in all treated eyes at the 24 and 48-hour observations. All treated eyes appeared normal at the 72-hour observation.
CONCLUSION	The test substance is slightly irritating to the eye.
TEST FACILITY	Safepharm (2004d)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Two stereoisomers of the notified chemical
METHOD	Similar to OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Female Mouse/CBA/J
Vehicle	Acetone/olive oil
Preliminary study	Yes
Positive control	Isoeugenol
Remarks - Method	Minor deviations did not affect the validity of the study. Because an initial assay found no sensitivity among mice treated with a 5.0% concentration of isoeugenol, a known sensitiser, the sensitisation potential of the test substance could not be determined. The initial assay was therefore rejected and the study repeated.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	8 F	26.5	-
1	5 F	26.7	1
5	5 F	41.3	1.56
10	5 F	40.6	1.53
20	5 F	65.3	2.46
40	5 F	140.4	5.30
Positive Control			
0.5	5 F	47.5	1.79
1.0	5 F	57.9	2.18
5.0	5 F	410.4	15.49

EC3	23.6% (20.71% using linear regression as reported by the study authors)
Remarks - Results	None of the mice assigned experienced visible irritation or other adverse toxic effects after dosing. Three mice, each in a different treatment group, lost minimal amounts of weight between randomisation and lymph node harvest. None of the tested mice experienced ≥ 10% increases in ear thickness between day 1 and 3, thus there was no irritation reaction to potentially affect the LLNA stimulation indices.
CONCLUSION	There was evidence of skin sensitisation to the test substance.
TEST FACILITY	BRT (2004)

B.6. Skin sensitisation – human volunteers

TEST SUBSTANCE	Two stereoisomers of the notified chemical
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: nine consecutive applications of the test substance were applied for approximately 24 hours and subsequent evaluations of the patch sites were conducted at 24 or 48 hours after the patch removal. Rest Period: 10-15 days Challenge Procedure: identical patches were applied to sites previously unexposed to the test substance. The patches were removed by subjects after 24 hours and the sites were graded after additional 24- and 48-hour periods.
Study Group	90 F, 23 M; age range 18.5-70.4 years

Vehicle	Diethyl Phthalate (DEP)
Remarks - Method	Occluded. The test substance at 1% was spread on a 2 cm × 2 cm patch.

RESULTS

Remarks - Results	Fourteen (14) subjects between ages of 22 and 73 enrolled in the pilot study and 13 completed the study with one voluntary withdrawal. One hundred and thirteen (113) subjects enrolled in the main study and 106 subjects completed the study with 4 voluntary withdrawals and 2 lost to follow up. One subject was discontinued due to heart attack unrelated to treatment. There was no evidence of sensitisation for the pilot study and main study.
CONCLUSION	The test substance at 1% was non-sensitising under the conditions of the test.
TEST FACILITY	TKL (2004)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Two stereoisomers of the notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (1995).
Species/Strain	Rat/Sprague-Dawley Crl:CD (SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Arachis oil BP
Remarks - Method	No significant protocol deviations. Preliminary fourteen day repeated dose (gavage) range-finder in the rat was performed to establish the maximum tolerated dose level (up to 1,000 mg/kg bw/day) of the test substance following repeated oral administration and to provide information for selection of dose levels for use in the twenty-eight day oral toxicity study.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0/10
low dose	5 per sex	15	0/10
mid dose	5 per sex	150	0/10
high dose	5 per sex	1,000	0/10

Mortality and Time to Death

There were no unscheduled deaths in the study.

Clinical Observations

No toxicological significant clinical signs were observed. There were no treatment-related changes in the behaviour parameters, functional performance parameters, sensory reactivity, bodyweight gain, food consumption or food efficiency and water consumption measured.

Isolated incidences of increased salivation immediately post dose, generalised fur staining and noisy respiration were not considered to be treatment-related. A statistically significant ($p < 0.05$) decrease in activity and mobility in the last 20% of the period for males treated with 1,000 and 150 mg/kg bw/day was noted. This was considered to be incidental and unrelated to treatment by study authors since similar findings were not noted in females at these dose levels.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no treatment related changes in haematological and blood chemistry parameters measured.

A significant decrease ($P < 0.05$) in plasma potassium was noted for females treated with 1,000 and 150 mg/kg bw/day. This was considered to be incidental and unrelated to treatment by study authors as it was not a dose related response.

Effects in Organs

No treatment-related macroscopic abnormalities and histopathological changes were noted.

A significant increase in both absolute and relative kidney ($P < 0.01$) and liver ($p < 0.05$) weights were noted in males treated with 1,000 mg/kg bw/day. The effect on liver weight extended to males treated with 150 mg/kg bw/day. As there were no histological correlates, these differences were considered not to be treatment related by study authors.

One male treated with 1,000 mg/kg bw/day showed pale kidneys; one male treated with 150 mg/kg bw/day showed small testes and epididymides and one control female showed reddened lungs. These observations were considered by the study authors to be incidental and not treatment related.

CONCLUSION

The No Observed Effect Level (NOEL) was established by the study authors as 1,000 mg/kg bw/day in this study, based on the fact that no treatment related changes were observed at the highest dose tested.

TEST FACILITY Safepharm (2006b)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD

	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver S-9 mix
Concentration Range in Main Test	a) With metabolic activation: 0, 50, 150, 500, 1,500 and 5,000 µg/plate b) Without metabolic activation: 0, 50, 150, 500, 1,500 and 5,000 µg/plate
Vehicle	Acetone
Remarks - Method	No significant protocol deviations. *E. coli* strains were not used.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5,000			
Test 1		> 5,000	≥ 1,500	negative
Test 2		> 5,000	≥ 1,500	negative
Present	> 5,000			
Test 1		> 5,000	≥ 1,500	negative
Test 2		> 5,000	≥ 1,500	negative

Remarks - Results

The test substance caused no visible reduction in the growth of the bacterial background lawn at any dose level. An oil precipitate was noted at and above 1,500 µg/plate however this did not prevent the scoring of revertant colonies.

No toxicological significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains, with any dose of the test substance, either with or without S9.

A small statistically significant increase in revertant colony frequency was noted in TA100 strain at 1,500 µg/plate without S9 in test 1. However, the plate counts were within the acceptable range for the strain. There was no evidence of a dose-response relationship or reproducibility. Therefore the response was considered to of no biological or toxicological significance by study authors.

The positive and negative controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

CONCLUSION The test substance was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY Safepharm (2004e)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test (1997).

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	Phenobarbitone/β-naphthoflavone-induced rat liver S-9 mix
Vehicle	Acetone
Remarks - Method	No protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 123.44, 246.88, 493.75*, 987.5*, 1,975*, 3,950*	4	24
Test 2	0*, 123.44, 246.88*, 493.75*, 987.5*, 1,975, 3,950	24	24
Present			
Test 1	0*, 123.44, 246.88, 493.75*, 987.5*, 1,975*, 3,950*	4	24
Test 2	0*, 123.44, 246.88, 493.75*, 987.5*, 1,975*, 3,950*	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	> 3,950			
Test 1		> 3,950	≥ 61.72	negative
Test 2		> 3,950	≥ 30.86	negative
Present	> 3,950			
Test 1		≥ 3,950	≥ 30.86	negative
Test 2		> 3,950	≥ 30.86	negative

Remarks - Results The test substance did not include a statistically significant increase in the frequency of cells or in the number of polyploid cells with chromosome aberration in either the absence or presence of a liver enzyme metabolising system.

The test substance induced some evidence of cytotoxicity in the study; however there was no clear dose-response relationship.

The positive and negative controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial

strains.

CONCLUSION The test substance was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY Safepharm (2006c)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Cell Type/Cell Line Chinese hamster ovary (CHO) cells
Metabolic Activation System Phenobarbitone/β-naphthoflavone-induced rat liver S-9 mix
Vehicle Acetone
Remarks - Method No protocol deviations

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0*, 123.44*, 246.88*, 493.75*, 987.5*, 1975*, 3950*	4	7 d	7 d
Test 2	0*, 123.44*, 246.88*, 493.75*, 987.5*, 1975*, 3950*	24	7 d	14 d
Present				
Test 1	0*, 123.44*, 246.88*, 493.75*, 987.5*, 1975*, 3950*	4	7 d	7 d
Test 2	0*, 123.44*, 246.88*, 493.75*, 987.5*, 1975*, 3950*	4	7 d	14 d

*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	≥ 987.5			
Test 1		> 3,950	≥ 987.5	negative
Test 2		> 3,950	≥ 987.5	negative
Present	> 3,950			
Test 1		> 3,950	≥ 987.5	negative
Test 2		> 3,950	≥ 987.5	negative

Remarks - Results No significant increases in mutant colony frequency in the exposure groups were observed in the tests up to the highest concentration in the presence and absence of metabolic activation.

The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.

CONCLUSION The test substance was not clastogenic to CHO cells at the HPRT locus treated in vitro under the conditions of the test.

TEST FACILITY Harlan (2012a)

B.11. Reproductive/developmental toxicity

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 421 Reproductive/Developmental Toxicity Screening Test (1995).
Species/Strain Rat/Wistar
Route of Administration Oral – gavage
Exposure Information Exposure period - female: 20 days

	Exposure period - male: 43 days
Vehicle	Arachis oil BP
Remarks – Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	10 per sex	0	0/20
Low	10 per sex	30	0/20
Intermediate	10 per sex	300	0/20
High	10 per sex	1,000	0/20

Mortality and Time to Death

There were no unscheduled deaths in the study.

Effects on Parental (P) animals:

Clinical signs were limited to post-dose increased salivation for animals of either sex treated with 1,000 mg/kg bw/day and for two meals treated with 300 mg/kg bw/day. In the absence of any supporting data to suggest irritancy, this isolated finding was considered not to represent an adverse health effect.

No adverse effects on body weight change, food consumption, food efficiency and water consumption were observed.

No treatment-related effects in mating performance, fertility and length of gestation were observed.

Effects on 1st Filial Generation (F1)

No significant differences in litter size, sex ratio, viability parameters ad litter weights were observed for litters from treated animals when compared with control animals. There no clinically notable signs of toxicity observed in offspring.

Remarks - Results

No treatment-related macroscopic abnormalities were noted for adults or offspring. No treatment-related changes were detected for testis and epididymis weights for treated males when compared with controls. No treatment-related histopathological effects were noted.

Post-mortem examinations did not show any treatment-related findings in offspring from treated litters. For the two interim deaths of offspring, no milk was present in the stomach. This was considered by the study authors to be common in offspring found dead soon after parturition and unrelated to treatment. At terminal kills, one male offspring from a 300 mg/kg bw/day litter had a reddened left testis. This was an isolated finding and considered by study authors to be unrelated to treatment. Two litters from the 1,000 mg/kg bw/day showed one small male. These were considered by study authors to be unrelated to substance treatment.

One male treated with 300 mg/kg bw/day showed small epididymides and small and flaccid testes. This animal did not show deficiency of mating with its female partner.

Histopathological examination of the male and female pair which failed to produce a pregnancy showed tubular degeneration of the testes and azoospermia of the epididymides for the male. This was considered by the study authors to be the contributing factor to the failure of mating and pregnancy in this pair. This however was considered by the study authors not to be test substance related.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) for systemic toxicity was established as 1,000 mg/kg bw/day in this study, based on that fact that minor clinical signs observed at this level were not considered to be adverse.

The No Observed Effect Level (NOEL) for reproductive toxicity was established as 1,000 mg/kg bw/day in this study, based on that fact that no treatment-related effects were observed.

TEST FACILITY Harlan (2012b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Two stereoisomers of the notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Carbon Dioxide ($ThCO_2$)
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
6	0	6	67
14	9	14	81
22	14	22	91
29*	21	29*	93

*Corrected for the last gas wash

Remarks - Results	All validity criteria for the test were satisfied. The toxicity control attained more than 46% degradation up to day 28 thereby confirming that the test substance was not toxic to the sewage treatment micro-organisms used in the study. After 28 days the toxicity control had attained 51% degradation. The test material attained 26% degradation after 28 days and, therefore, cannot be considered as readily biodegradable under the conditions of OECD Guideline 301B.
CONCLUSION	The test substance is not ready biodegradable
TEST FACILITY	Safepharm (2004f)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Two stereoisomers of the notified chemical
METHOD	OECD TG 305 I Aqueous Exposure Bioconcentration Fish Test
Species	Carp (*Cyprinus carpio*)
Exposure Period	Exposure: 28 days
Auxiliary Solvent	N,N-dimethylformamide
Concentration Range	Nominal: 0.0002 mg/L Actual: Not reported
Analytical Monitoring	Liquid chromatography-tandem-mass spectrometry
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The test was conducted at nominal concentrations of 0.02 and 0.2 µg test substance /L. No significant deviations to the test protocol were reported. Due to very low solubility of the test substance, the solvent N,N-dimethylformamide, was used to prepare the stock solutions. The test

substance (500 mg) and 25 g of a dispersant (HCO-40) were dissolved in N,N-dimethylformamide to prepare 500 mL stock solution at the test concentration of 1000 mg/L.

RESULTS

Bioconcentration Factor	BCF ≤ 3.9-22 at low concentration (0.02 µg/L) and < 38 at higher concentration (0.2 µg/L).
Remarks - Results	The validity criteria for the test were met. At higher concentration, a steady state of bioaccumulation was attained. At low concentration, a steady state of bioaccumulation was not attained. However, at days 10–19, the bioconcentration factor reached its peak value and progressively decreased through to day 28. Therefore, at low concentration, days 10-19 were assigned to have achieved a steady state of bioaccumulation.

CONCLUSION Under the conditions of this test, the test substance is not considered to be bioaccumulative.

TEST FACILITY Kurume (2014)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi static

Species	*Oncorhynchus mykiss* (Rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	Dimethylformamide
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC)
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The test substance (100 mg/mL) was prepared in dimethylformamide and the volume adjusted to 10 ml to give a 100 mg/10 mL solvent stock solution from which a dilution was made to give a further solvent stock solution of 10 mg/10 mL. An aliquot (500 µL) of this 10 mg/10 mL solvent stock solution was dispersed in 5 L of dechlorinated tap water with the aid of magnetic stirring for approximately 10 minutes to give the required test concentration of 0.10 mg/L.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
0.1	0.07	14	0	0	0	0	0

LC50	> 0.1 mg/L at 96 hours.
NOEC	0.1 mg/L at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. Chemical analysis of the freshly prepared test media at 0, 24, 48 and 72 hours showed measured concentrations to range from 100% to 127% of nominal. Therefore, the results are based on nominal concentrations.

CONCLUSION The test substance is not harmful to fish up to the limit of its water solubility

TEST FACILITY Safepharm (2006d)

C.2.2. Acute toxicity to fish

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi static
Species *Brachydanio rerio* (Zebra fish)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness 10 - 250 mg $CaCO_3$/L
Analytical Monitoring Gas Chromatography (GC)
Remarks – Method Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

The test medium with loading rate of 100 mg/L was prepared by weighing and stirring 300 mg of the test substance into 3 L of test water. No auxiliary solvent or dispersant were used. This mixture was filtered through a 0.45 µm membrane filter.

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*				
Nominal		*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
100	10	0	0	0	0	0

LC50 >100 mg/L at 96 hours.
NOEC 100 mg/L at 96 hours.
Remarks – Results All validity criteria of the test guideline were satisfied. The study results were based on nominal loading rates.

CONCLUSION The test substance is not harmful to fish up to the limit of its water solubility

TEST FACILITY Environmental Testing Laboratory (2007)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent Dimethylformamide
Water Hardness 250 mg $CaCO_3$/L
Analytical Monitoring GC-MS
Remarks - Method Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

The test material (100 mg) was dissolved in dimethylformamide and the volume adjusted to 10 mL to give a 100 mg/10 mL solvent stock solution. An aliquot (1.0 mL) of the 100 mg/10 mL solvent stock solution was dispersed in a final volume of 10 mL of dimethylformamide to give a further solvent stock solution of 10 mg /10 mL. An aliquot (500 µL) of the 10 mg/10 mL solvent stock solution was dispersed in a final volume of 5 litres of reconstituted water with the aid of magnetic stirring for approximately 10 minutes to give a nominal concentration of 0.10 mg/L.

RESULTS

Concentration mg/L	*Number of D. magna*	*Number Immobilised*	
Nominal		*24 h*	*48 h*
0.1	20	0	0

EC50 >0.1 mg/L at 48 hours
NOEC 0.1 mg/L at 48 hours

Remarks - Results All validity criteria of the test guideline were satisfied.

CONCLUSION The test substance is not toxic to aquatic invertebrates up to the limit of its water solubility

TEST FACILITY Safepharm (2006e)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
Species *Scenedesmus subspicatus*
Exposure Period 72 hours
Concentration Range Nominal: 0.1 mg/L
Auxiliary Solvent Dimethylformamide
Water Hardness Not reported
Analytical Monitoring HPLC
Remarks - Method Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

An amount of test material (l00 mg) was dissolved in dimethylformamide and the volume adjusted to 10 mL to give a 100 mg/10 mL solvent stock solution from which a dilution was made to give a further solvent stock solution of 10 mg/10 mL. An aliquot (400 µL) of the 10 mg/10 mL solvent stock solution was dispersed in 4 L of algal suspension to give the required test concentration of 0.10 mg/L.

RESULTS

Biomass		*Growth*	
NOEC	*EC50*	*NOEC*	*EC50*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
0.1	> 0.1	0.1	>0.1

Remarks - Results All validity criteria of the test guideline were satisfied.

CONCLUSION The test substance is not harmful to algae up to the limit of its water solubility.

TEST FACILITY Safepharm (2006f)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE Two stereoisomers of the notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 87/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum Activated sludge

Exposure Period	3 hours
Concentration Range	Nominal: 100 and 1,000 mg/L Actual: Not reported
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The test material (500 mg) was dispersed in approximately 250 mL of water and subjected to ultrasonication for approximately 30 minutes. Synthetic sewage (16 mL), activated sewage sludge (200 mL) and water were added to a final volume of 500 mL to give the required concentration of 1,000 mg/L.

RESULTS

IC50	> 1,000 mg/L
NOEC	1,000 mg/L
Remarks – Results	The study satisfied all the validity criteria of the guideline except the initial and final dissolved oxygen concentrations were below those recommended in the test guidelines (6.5 mg O_2/L and 2.5 mg O_2/L respectively). This was considered to have had 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. In the reference test an EC50 of 10 mg/L was obtained, which is in the recommended validity range of 5 – 30 mg/L.

CONCLUSION	The test substance is not inhibitory to microbial respiration
TEST FACILITY	Safepharm (2006g)

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