File No: LTD/1899

May 2016

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2-Butanone, 4-(dodecylthio)-4-[2,6,6-trimethyl-1(*or* 2)-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.

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
LTD/1899	Firmenich Limited	2-Butanone, 4-(dodecylthio)-4-[2,6,6-trimethyl-1(*or* 2)-cyclohexen-1-yl]-	Yes	< 1 tonne per annum	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*
Skin Sensitisation (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 phrase:

R43: May cause sensitisation by skin contact

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

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

Human health risk assessment
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 at ≤ 1% in cosmetic and household products, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern, 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:
  - 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
  - Local exhaust ventilation

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

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

  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 one tonne per annum notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed 1% in cosmetic and household products;

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
BALGOWLAH NSW 2093

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: marketing name, other names, analytical data, degree of purity, impurities, additives/adjuvants and identity of manufacturer.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

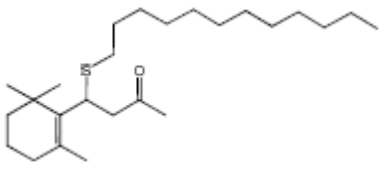
2. IDENTITY OF CHEMICAL

CAS NUMBER
1803467-44-8

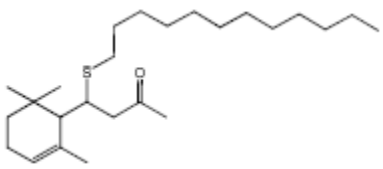
CHEMICAL NAME
2-Butanone, 4-(dodecylthio)-4-[2,6,6-trimethyl-1(*or* 2)-cyclohexen-1-yl]-

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

STRUCTURAL FORMULA
The notified chemical consists of two isomers:



Isomer 1



Isomer 2

Isomer 1: 2-butanone, 4-(dodecylthio)-4-[2,6,6-trimethyl-1-cyclohexen-1-yl]-
Isomer 2: 2-butanone, 4-(dodecylthio)-4-[2,6,6-trimethyl-2-cyclohexen-1-yl]-

MOLECULAR WEIGHT
394.7 Da

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

3. COMPOSITION

DEGREE OF PURITY
The notified chemical is never isolated from the product mixture. The product mixture contains the notified chemical at 50-70% concentration.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Pale yellow liquid*

Property	Value	Data Source/Justification
Freezing Point	< -20 °C at 97.3 kPa*	Measured
Boiling Point	273 °C at 97.4 kPa*	Measured
Density	930 kg/m^3 at 20 °C*	Measured
Vapour Pressure	7.39×10^{-7} kPa at 60 °C (Isomer 1) 6.12×10^{-7} kPa at 60 °C (Isomer 2)	Measured
Water Solubility	$< 3.3 \times 10^{-2}$ g/L at 20 °C*	Measured
Hydrolysis as a Function of pH	Hydrolytically stable*	Measured
Partition Coefficient (n-octanol/water)	log Pow > 6.5 at 20 °C*	Measured
Adsorption/Desorption	log K_{oc} < 1.25 at 20 °C*	Measured
Dissociation Constant	Not determined	The notified chemical does not contain any functional groups that are expected to dissociate in water.
Flash Point	128 °C at 101.3 kPa*	Measured
Flammability	Not determined	Will not be isolated from the marketed product which is expected to be not flammable based on the measured flash point
Autoignition Temperature	246 °C*	Measured
Explosive Properties	Not determined	Predicted to be negative based on the structure of the notified chemical
Oxidising Properties	Not determined	Predicted to be negative based on the structure of the notified chemical

* For the product mixture containing the notified chemical at 50-70% concentration

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured within Australia. The notified chemical will be imported into Australia as a component of fragrance formulations at ≤ 3% concentration for reformulation into cosmetic and household products.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 1	< 1	< 1	< 1	< 1

PORT OF ENTRY
Sydney (wharf or airport)

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited

TRANSPORTATION AND PACKAGING
The notified chemical will be imported and distributed as a component of fragrance formulations in closed lacquered drums of 5-180 kg size. After reformulation, finished cosmetic and household products will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient in cosmetic and household products at ≤ 1% concentration.

OPERATION DESCRIPTION
The notified chemical will be imported into Australia as a component of fragrance formulations at ≤ 3% concentration for reformulation into cosmetic and household products.

Reformulation
When reformulated, the notified chemical will be blended into end-use consumer products at customer sites. Procedures will vary depending on the nature of the cosmetic or household product being formulated. Both manual and automated steps will likely be involved. For example, a chemist will sample and test the notified chemical for QA purposes manually; a compounder will weigh an appropriate amount of the notified chemical into a container then add the amount directly into a mixing tank, with periodic sampling for quality control purposes also carried out during the manufacturing process. Automated processes may include mixing and filling of end-use containers with products.

End-use
Finished products containing the notified chemical at ≤ 1% concentration will be used by the public and may also be used by professionals such as hairdressers and workers in beauty salons and cleaners. Depending on the nature of the product, these could be applied in a number of ways, such as by hand, using an applicator or sprayed.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	4	12
Mixing	4	2
Drum handling	4	2
Drum cleaning	4	2
Maintenance	4	2
Quality control	0.5	1
Packaging	4	2
End users (workers)	8	365

EXPOSURE DETAILS
Transport and storage
Transport and storage and retail workers may come into contact with the notified chemical either in fragrance formulations at ≤ 3% concentration or at various concentrations (≤ 1%) in cosmetic and household products only in the event of an unlikely accidental rupture of containers.

Reformulation
During reformulation into cosmetic and household products, dermal, ocular and inhalation exposure of workers to the notified chemical at ≤ 3% concentration may occur. Exposure is expected to be minimised through the use of exhaust ventilation and/or automated/enclosed systems as well as through the use of personal protective equipment (PPE) such as coveralls, eye protection, impervious gloves and respiratory protection (as appropriate).

End use
Exposure to the notified chemical in end-use products at ≤ 1% concentration may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. hair dressers, workers in beauty salons), or in the cleaning industry. The principal route of exposure will be dermal and inhalation, while accidental oral and ocular exposure is also possible. Such professionals may use some PPE to minimise repeated exposure and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the notified chemical at ≤ 1% concentration through the use of cosmetic and household cleaning products. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible particularly if products are applied by spray.

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the product mixture containing the notified chemical at 50-70% concentration are summarised in the following table. The other major components of the product mixture have similar structure to the notified chemical and are expected to have the same hazard profile. Therefore the results from the studies conducted on the product mixture are assumed to reflect that of the notified chemical for risk assessment purposes. For full details of the studies, refer to Appendix B.

*Endpoint**	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 5.11 mg/L/4 hour; low toxicity
Rabbit, skin irritation	non-irritating
Eye irritation (in vitro)	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation (EC_3 = 23%)
Human, skin sensitisation – RIPT (5%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration	non genotoxic

*Test substance: product mixture containing the notified chemical at 50-70% concentration

Toxicokinetics.
No toxicokinetic data on the notified chemical were submitted. Based on the low molecular weight of the notified chemical, absorption across biological membranes may occur. However, dermal absorption may be limited by the relatively high partition coefficient (log Pow > 6.5).

Acute toxicity.
The notified chemical is expected to have a low acute oral, dermal and inhalation toxicity based on studies conducted in rats on the product mixture containing the notified chemical at 50-70% concentration.

Irritation.
A skin irritation study using reconstructed human Epidermis model found the test substance incompatible with the test system. The product mixture containing the notified chemical at 50-70% concentration was found to be non-irritating to the skin in a study conducted in rabbits.

The product mixture containing the notified chemical at 50-70% concentration was found to be non-irritating to eyes in a bovine corneal opacity and permeability test, and slightly irritating in a study conducted in rabbits.

Sensitisation.
The skin sensitising potential of the product mixture containing the notified chemical at 50-70% concentration was investigated in a local lymph node assay (LLNA) and in a human repeat insult patch test (HRIPT).

In a local lymph node assay (LLNA), the product mixture was found to be a sensitiser (stimulation indices of 3.40, 6.69 and 13.56 at 25%, 50% and 100%, respectively) when tested at up to 100% concentration. The EC_3 value was calculated to be 23%.

In a human repeat insult patch test (HRIPT), a cosmetic formulation containing 5% of the product mixture (2.5-3.75% notified chemical) did not elicit a positive response.

Repeated dose toxicity.
A No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day , the highest dose tested, for the product mixture containing the notified chemical at 50-70% concentration in a 28-day repeat dose oral toxicity study, based on noted treatment-related effects were either adaptive or human irrelevant.

The changes in body weight gains and laboratory parameters and the finding of centrilobular hypertrophy for the high dose group were considered by the study authors to be adaptive due to the reversible nature of these findings. The finding of hyaline droplets in male kidneys was considered by the study authors to have no relevance to human health.

Mutagenicity/Genotoxicity.
The product mixture containing the notified chemical at 50-70% concentration was negative in a bacterial reverse mutation assay and in an *in vitro* chromosomal aberration study in human lymphocytes.

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*
Skin sensitisation (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 sensitisation by skin contact

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 systemic toxicity, presenting as a mild eye irritant and a skin sensitiser.

Reformulation
During reformulation workers may be at risk of sensitisation when handling the notified chemical at ≤ 3% concentration (eye irritation effects are not expected at this low concentration). This risk should be reduced through the expected use of engineering controls such as enclosed, automated processes and personal protective equipment (PPE), including impervious gloves and coveralls.

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 at ≤ 1% concentration. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, risk to these workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical on a regular basis (for details of the public health risk assessment, see Section 6.3.2.).

6.3.2. Public Health

The general public will be repeatedly exposed to the notified chemical during the use in a variety of cosmetic and household products containing the notified chemical at ≤ 1% concentration.

Local effects

The notified chemical is slightly irritating to eyes. However, eye irritation effects are not expected from use of the notified chemical at the proposed concentrations (≤ 1%) in cosmetic and household products.

The notified chemical is considered a sensitiser based on the results of a LLNA study.

Proposed 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 fragrances (containing 1% notified chemical) as worst case scenario example of products that may contain the notified chemical, the Consumer Exposure Level (CEL) is estimated to be 37.5 μg/cm^2 (Cadby *et al.*, 2002).

When tested at 5% concentration in a human repeat insult patch study [0.3 mL applied to 25 mm Hill Top Chamber patch (2.54 cm^2 assumed)], the product mixture containing the notified chemical at 50-70% concentration (2.5-3.75% notified chemical) was not a skin sensitiser. Although this study has been used for the purposes of quantitative risk assessment of the notified chemical, the availability of additional information on the sensitisation potential of the product mixture (i.e., the LLNA study) was taken into account when determining the safety assessment factors that should be applied. For risk assessment purposes, it is assumed that there is no contribution to sensitisation by the other components in the product mixture. Thus consideration of each of the studies and application of appropriate safety factors, allowed the derivation of an Acceptable Exposure Level (AEL) of 84.8 μg/cm^2. In this instance, the factors employed included an interspecies factor (1), intraspecies factor (10), a matrix factor (1), a use and time factor (3.16) and a database factor (1), giving an overall safety factor of ~30.

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) at ≤ 1% concentration is not considered to be unreasonable. Based on the generally lower expected exposure level from other leave-on and rinse-off cosmetic products, and household products, containing the notified chemical at ≤ 1% concentration, 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 the aggregate exposure has not been conducted.

Systemic effects

Based on the low concentration in end use products and low systemic toxicity of the notified chemical (NOAEL 1,000 mg/kg bw/day), systemic effects from repeated exposure are not expected.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 1% concentration in cosmetic and household 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 not be manufactured in Australia, so there will be no environmental release associated with this activity. The notified chemical will be imported into Australia at 100% concentration, in the form of fragrance preparations for further reformulation into end-use cosmetic and household products or as a component of end-use 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 that 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% (2 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.

It is estimated that a maximum of 3% (30 kg) of the consumer products may remain in the consumer containers that will be sent for disposal.

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. Empty product containers are expected to be disposed of to landfill.

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, consumer products and fine fragrances, before potential release to surface waters nationwide. The product mixture containing the notified chemical at 50-70% concentration is not considered readily biodegradable (58% in 28 days), but shows inherent biodegradability (70% in 60 days). For details of the environmental fate studies, please refer to Appendix C. The reported adsorption/desorption coefficient (log K_{oc} < 1.25) indicates that the notified chemical will not strongly adsorb to soil and sediment in the sludge fraction. In either landfill or water, the notified chemical will ultimately decompose to water, oxides of carbon and sulphur. The notified chemical is expected to have potential for bioaccumulation in the aquatic organisms given its low molecular weight and high log Pow.

The half-life of the notified chemical in air is calculated to be 0.672 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 to landfill as collected spills and empty containers. The notified chemical has 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 it is expected to slowly decompose by abiotic and biotic processes to form water and oxides of carbon and sulphur. Therefore, the notified chemical is not expected to be bioavailable to the 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, it is conservatively assumed that 100% of the notified chemical will be released to sewer on a nationwide basis over 365 days per year. It is also assumed that under a worst-case scenario there is no removal of the notified chemical during STP processes.

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

PEC - Ocean:	0.06	µg/L

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

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the product mixture containing the notified chemical at 50-70% concentration are summarised in the table below. The other major components of the product mixture have similar structure to the notified chemical and are expected to have the same hazard profile. Therefore the results from the studies conducted on the product mixture are assumed to reflect that of the notified chemical for risk assessment purposes. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96h LC50 = 30.6 mg/L	Harmful to fish
Daphnia Toxicity	48h EC50 = 10.9 mg/L	Harmful to *Daphnia*
Algal Toxicity	72h E_rC50 = 56.5 mg/L	Harmful to algae
Inhibition of Bacterial Respiration	3h EC50 > 1000 mg/L	Not inhibitory to bacterial respiration

Based on the ecotoxicological endpoints for the product mixture, the notified chemical is expected to be harmful to fish, daphnids and algae on an acute basis. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is formally classified as "Acute Category 3; Harmful to aquatic life". Based on the acute toxicity and inherent biodegradability of the notified chemical, it is formally classified as "Harmful to aquatic life with long lasting effects" under the GHS.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive endpoint for *Daphnia*. A safety factor of 100 was used given acute endpoints for three tropic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	10.90	mg/L
Assessment Factor	100	
PNEC:	109.00	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC, the following Risk Quotient has been calculated:

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	0.61	109	0.006
Q - Ocean:	0.06	109	0.001

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment (Q < 1) indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters based on its maximum annual importation quantity. The notified chemical may be persistent in the environment due to its hydrolytic stability, and a lack of ready biodegradability. However, the notified chemical is not expected to be bioavailable to the aquatic organisms due to its low water solubility. On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Note: The following physical and chemical properties were determined using the product containing the notified chemical at 50-70% concentration as the test substance.

Melting Point/Freezing Point < -20 ± 0.5 °C at 97.3 kPa

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	The test substance did not solidify when cooled to -20 °C
Test Facility	Firmenich (2013)

Boiling Point 273 ± 2 °C at 97.4 kPa

Method	OECD TG 103 Boiling Point.
Remarks	Siwoloboff method
Test Facility	Firmenich (2013)

Density 930 kg/m^3 at 20 ± 0.5 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Determined using an oscillating density meter
Test Facility	Firmenich (2013)

Vapour Pressure 7.39×10^{-7} kPa at 25 °C (Isomer 1)
6.12×10^{-7} kPa at 60 °C (Isomer 2)

Method	OECD TG 104 Vapour Pressure.
Remarks	Determined using gas saturation method
Test Facility	Harlan (2014a)

Water Solubility < 33×10^{-3} g/L at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method. Water solubility of test material is < 33mg/L at 20± 0.5°C according to limit of detection and limit of quantification of the system.
Test Facility	Firmenich (2013)

Hydrolysis as a Function of pH

Method	Internal method
Remarks	Hydrolytically stable substance, but unstable at pH 12 and pH 2.
Test Facility	Firmenich

Partition Coefficient (n-octanol/water) log Pow > 6.5 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method
Test Facility	Firmenich (2013)

Adsorption/Desorption log K_{oc} < 1.25

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).
Remarks	The adsorption coefficient (Koc) has been determined to be in the range of < 17.8 to 1.85 x 10^4, log10 Koc < 1.25 to 4.27, using the HPLC method
Test Facility	Harlan (2014b)

Flash Point 128 ± 2 °C at 98.0 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup
Test Facility	Firmenich (2013)

Autoignition Temperature 246 ± 5 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility	Harlan (2014c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure.
Species/Strain	Rat/Wistar
Vehicle	None for 1000 mg/kg dose and Arachis oil BP for 300 mg/kg dose
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1F	300	0/1
2	1F	2000	0/1
3	4F	2000	0/4

LD50	> 2000 mg/kg bw
Signs of Toxicity	Hunched posture was noted in 3/5 animals at a dose of 2000 mg/kg during the day of dosing, which persisted in one animal one day after dosing. No signs of systemic toxicity were noted in the remaining animals.
Effects in Organs	There were no abnormalities at necropsy.
Remarks - Results	All animals showed expected body weight gains.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	Harlan (2013a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Wistar
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 per sex	2000	0/10

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	There were no signs of dermal irritation.
Signs of Toxicity - Systemic	There were no signs of systemic toxicity.
Effects in Organs	There were no abnormalities at necropsy.
Remarks - Results	All animals showed expected body weight gains.
CONCLUSION	The test substance is of low toxicity via the dermal route.
TEST FACILITY	Harlan (2014d)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/RccHan:WIST
Vehicle	Air
Method of Exposure	Nose-only exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	3.42 μm (Mean Mass Median Aerodynamic Diameter)
Remarks - Method	No significant protocol deviations. A sighting study was carried out in two animals at 2.03 mg/L concentration.

RESULTS

Group	*Number and Sex of Animals*	*Concentration <units>*		*Mortality*
		Nominal	*Actual*	
1	3 per sex	19.4	5.11	0/6

LC50	> 5.11 mg/L/4 hours
Signs of Toxicity	Clinical signs including hunched posture, pilo-erection, wet fur and increase respiratory rate were not considered by the study authors to be indicative of toxicity but to be associated with the restraint procedure.
Effects in Organs	No macroscopic abnormalities were noted at necropsy.
Remarks - Results	All animals showed body weight losses or no gains on Day 1 post-exposure, with the effects occurring in two female animals during Days 3-7 post-exposure. All animals showed reasonable body weight gains during the remaining period.
CONCLUSION	The test substance is of low toxicity via inhalation.
TEST FACILITY	Harlan (2013b)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method
Vehicle	None
Remarks - Method	EPISKIN™ Reconstructed Human Epidermis Model No significant protocol deviations. In addition to the normal test procedure, the test substance was also applied to three water-killed tissues.

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.947	100.0*	2.2
Test substance	0.351	37.1	8.9
Positive control	0.082	8.7	0.96

OD = optical density; SD = standard deviation
*The mean viability of the negative control tissues was set as 100%.

Remarks - Results	The test substance was found to cause direct reduction of MTT with a degree exceeding 30% upper acceptable limit.

CONCLUSION	The test substance was incompatible with the test system.
TEST FACILITY	Harlan (2015)

B.5. Irritation – skin

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

Remarks - Results	No evidence of skin irritation was noted. All animals showed expected body weight gains.
CONCLUSION	The test substance is non-irritating to the skin.
TEST FACILITY	Harlan (2014e)

B.6. Irritation – eye (in vitro)

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants
Vehicle	None
Remarks - Method	No significant protocol deviations. The optical density was determined at 492 nm. Sodium chloride solution (0.9% w/v) was used as a negative control and ethanol was used as a positive control.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	1.0	0.036	1.5
*Test substance**	0.0	0.016	0.2
*Positive control**	26.7	1.058	42.5

IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results	The corneas treated with the test substance and negative control were clear post treatment and post incubation, whereas the corneas treated with the positive control were cloudy during those periods. The positive and negative controls gave satisfactory results, confirming the validities of the test systems.
CONCLUSION	The test substance was not corrosive or a severe eye irritant under the conditions of the test.
TEST FACILITY	Harlan (2014f)

B.7. Irritation – eye

TEST SUBSTANCE: Product mixture containing the notified chemical at 50-70% concentration

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3
- Observation Period: 72 hours
- Remarks - Method: No significant 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	1.0	0.7	0.7	2	< 72 h	0
Conjunctiva: chemosis	0.3	0.7	0.7	2	< 72 h	0
Conjunctiva: discharge	0.3	0.7	0.7	2	< 72 h	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	1	< 24 h	0

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

Remarks - Results: Two animals showed expected body weight gains but no body weight gain was noted 1 animal. It was stated in the report that these variations were within the laboratory historical data and was confirmed to be of no toxicological relevance.

Iridial inflammation was observed in one animal at the 1 hour observation. Moderate conjunctival irritation was observed in all animals that was fully resolved at the 72 hour observation period. No corneal effects were noted.

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

TEST FACILITY: Harlan (2014g)

B.8. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Product mixture containing the notified chemical at 50-70% concentration

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
- Species/Strain: Mouse/
- Vehicle: Acetone/olive oil (4:1)
- Preliminary study: Yes
- Positive control: Not conducted in parallel with the test substance, but conducted previously in the test laboratory using α-hexylcinnamaldehyde as a 25% dilution in AOO.
- Remarks - Method: No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5	2,074.19 (± 887.21)	-
25	5	7,061.81 (± 6,263.82)	3.40
50	5	13,877.86 (± 7,826.82)	6.69
100	5	28,122.56 (± 10,913.61)	13.56

- EC3: 23%
- Remarks - Results: No signs of systemic toxicity were noted in the test or control animals.

CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.
TEST FACILITY	Harlan (2014h)

B.9. Skin sensitisation – human volunteers

TEST SUBSTANCE	Cosmetic formulation containing 5% of the product mixture containing the notified chemical at 50-70% concentration
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: patches containing 0.3 mL test substance were applied. Patches were removed after 24 hours and sites were graded 24 hours after removal of the patches (or 48 hours for patches removed on weekend). The identical site was then repatched until 9 applications were completed. Rest Period: approximately 14 days Challenge Procedure: patches containing 0.3 mL test substance was applied to the treated site and a naïve site for 24 hours. Sites were graded at removal and at 48hours, 72 hours and 96 hours post application.
Study Group	78 F, 30 M; age range 19-75 years
Vehicle	None
Remarks - Method	Occluded. The test substance was spread on a 25 mm × 25 mm patch. The purity of the notified chemical was 50-70%.
RESULTS	
Remarks - Results	108/119 subjects completed the study. 11 subjects withdrew due to personal reasons. No withdrawals were related to the application of the test substance. No adverse responses were noted.
CONCLUSION	The test substance was non-sensitising under the conditions of the test.
TEST FACILITY	HRL (2015)

B.10. Repeat dose toxicity

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rats/Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations.

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	30	0/10
mid dose	5 per sex	300	0/10
high dose	5 per sex	1000	0/10
control recovery	5 per sex	0	0/10
high dose recovery	5 per sex	1000	0/10

Mortality and Time to Death
There were no unscheduled deaths.

Clinical Observations
No clinical signs of systemic toxicity were noted. There were no treatment-related effects on sensory reactivity, grip strength and motor activity.

Overall body weight gains in all dose groups were similar to the control group. Reduced body weight gain was noted in male animals of the high dose group in the first 2 weeks, with subsequent weight gain (and food consumption) higher than the controls. This was considered by the study authors to be a non-specific toxic response. Body weight gains of other treated animals were considered by the study authors to be unaffected.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Increased platelet numbers and prothrombin were noted in male animals of the high dose group, which was reversed to be similar to the controls after the recovery period.

Electrolyte changes (low chloride and high calcium and phosphorous concentrations) were noted in male animals of the high dose group, which was reversed to be similar to the controls after the recovery period.

An increase in acidity was noted among male animals of the mid dose and high dose groups and decreased potassium and creatinine concentrations were noted in female animals of the high dose group, which had resolved after the recovery period.

Effects in Organs
Absolute organ weights were not affected by treatment. Changes in relative organ weights including the kidney, liver, epididymides, testes, heart and ovaries did not show a dose response and were considered by the study authors to represent biological variations and were of no toxicological significance.

Centrilobular hypertrophy was noted in both male and female animals of the high dose group, which was absent after the recovery period. This finding was considered by the study authors to be an adaptive change in the presence of a xenobiotic and corresponded to the increased liver weight noted at necropsy. This finding was not considered by the study authors to be adverse as it was not supported by the blood chemistry.

Hyaline droplets (intra-cytoplasmic protein droplets) were noted in male kidneys of the mid dose and high dose groups, which were absent after the recovery period. It was stated by the study authors that the protein is synthesised by the parenchymal cells of the liver of the adult male rat, secreted into the blood and freely filtered through the kidney granulomas. Its synthesis is influenced by testosterone and cortisone and the protein is not found in immature male rats, female rats or humans.

Remarks – Results
The changes in body weight gains and laboratory parameters and the finding of centrilobular hypertrophy for the high dose group were considered by the study authors to be adaptive due to the reversible nature of these findings. The finding of hyaline droplets in male kidneys was considered by the study authors to have no relevance to human health.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1000 mg/kg bw/day, the highest dose tested, in this study, based on noted treatment-related effects were either adaptive or human irrelevant.

TEST FACILITY	HLS (2014a)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure/Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA

Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 5-5000 µg/plate b) Without metabolic activation:5-5000 µg/plate
Vehicle	Acetone
Remarks - Method	The purity of the notified chemical was 50-70%. A preliminary study was carried out at 0.15-5000 µg/mL. The dose selection for the main experiments was based on toxicity observed in the preliminary study. Positive controls: With metabolic activation: 2-aminoanthracene (TA1535, TA1537, TA100, WP2uvrA); venzo(a)pyrene (TA98) Without metabolic activation: N-Ethyl-N-nitroso-N'-nitroguanidine (TA1535, TA100, WP2uvrA); 9-aminoacridine (TA1537); 4-nitroquinoline-1-oxide (TA98)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 500	> 500	> 500	negative
Test 2		> 150	> 500	negative
Present				
Test 1	> 500	> 500	> 500	negative
Test 2		> 150	> 500	negative

Remarks - Results	In both tests, no increases in the frequency of revertant colonies were obtained in the presence or absence of metabolic activation. The results from the positive controls confirmed the validity of the test.

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

TEST FACILITY Harlan (2013c)

B.12. Genotoxicity – in vitro

TEST SUBSTANCE Product mixture containing the notified chemical at 50-70% concentration

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat liver
Vehicle	Acetone
Remarks - Method	A preliminary study was carried out at 19.53-4465 µg/mL. The dose selection for the main experiments was based on toxicity observed in the preliminary study. Vehicle and positive controls (mitomycin C and cyclophosphamide) were run concurrently with test substance.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	40, 80*, 160*, 320, 480*, 640	4 h	24 h
Test 2	22.5*, 45*, 90*, 100, 180, 360, 640	24 h	24 h

Present			
Test 1	40, 80, 160, 320*, 640*, 800*	4 h	24 h
Test 2	45, 90, 180, 360*, 640*, 800*, 1080	4 h	24 h

*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	> 39.06	> 640	> 40	negative
Test 2	> 78.13	> 45	> 45	negative
Present				
Test 1	> 625	> 800	> 40	negative
Test 2		> 800	> 45	negative

Remarks - Results

No statistically significant increases in chromosome aberrations were observed in the presence or absence of activation system.

The results of the positive controls confirmed the validity of the test system.

CONCLUSION

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

TEST FACILITY

Harlan (2014i)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge
Exposure Period	28 days.
Auxiliary Solvent	None.
Analytical Monitoring	Biochemical oxygen demand (BOD)
Remarks - Method	The test was conducted in accordance with the test guideline above with no significant deviation from the protocol reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	6.40	4	75.1
7	17.00	7	86.7
14	24.10	14	93.4
21	44.30	21	95.4
28	57.60	28	95.8

Remarks - Results	All validity criteria for the test were satisfied. After 28 days, the percent degradation for the notified chemical was 57.6%. The percent degradation calculated in the reference item up to day 28 was 95.8%. In the toxicity control, more than 25% degradation was observed up to day 14. The percent biodegradation of the test substance did not reach 60% in 10-day window of the test under the conditions.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	Guangdong (2014)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static
Species	Zebra fish (*Danio rerio*)
Exposure Period	96 h
Auxiliary Solvent	None reported
Water Hardness	None reported
Analytical Monitoring	High performance liquid chromatography (HPLC)
Remarks – Method	According to the results of range-finding test, solubility test and stability test, the definitive test was conducted using water accommodated fraction (WAF) method. 34mg of test substance was dissolved with test water to 5000 ml to give the 6.80 mg/L loading rate suspension. 53mg, 82mg, 128mg and 200mg of test substance were prepared in the same way as the above procedure, the 10.6mg/L, 16.4mg/L, 25.6mg/L and 40.0mg/L loading rates WAFs were obtained respectively (3.24mgfL, 5.03mg/L, 1.85mg/L and 12.2mg/L calculated based on 30.6% purity of the 1st constituent present in test substance) and the WAFs were used in the test directly.

RESULTS

Concentration mg/L Nominal (WAF)	*Number of Fish*	*Mortality (%)*				
		3 h	*24 h*	*48 h*	*72 h*	*96 h*
Blank	10	0	0	0	0	0
6.80	10	0	0	0	0	0
10.6	10	0	0	0	0	0
16.4	10	0	0	0	0	0
25.6	10	0	0	0	0	0
40.0	10	60	100	100	100	100

LL50 30.6 mg/L at 96 hours.

Remarks – Results All validity criteria for the test were satisfied.

CONCLUSION The test substance is harmful to fish.

TEST FACILITY Guangdong (2015)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Product mixture containing the notified chemical at 50-70% concentration

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static.

Species *Daphnia magna*
Exposure Period 48 hours.
Auxiliary Solvent None.
Water Hardness 220 mg $CaCO_3$/L.
Analytical Monitoring Gas chromatography with mass spectrum (GC-MS) method
Remarks - Method Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. Samples were prepared with WAF method. The test substance (500, 227.5, 103.5, 47.0 or 21.4 mg) was added to 4 L dilution medium in an aspirator (5 L) and after being mixed, the medium was made up to volume. Additionally 19.4 mg of test substance was added to 9 L dilution medium in an aspirator (10 L) and after being mixed well, the medium was made up to volume. WAFs with nominal loading rates of 100, 45.5, 20.7, 9.39, 4.27 and 1.94 mg/L were used as the test media.

RESULTS

Concentration mg/L Nominal	*Number of D. magna*	*Cumulative Immobilised (%)*	
		24 h	*48 h*
Control	20	0	0
1.94	20	0	0
4.27	20	0	20
9.39	20	5	45
20.7	20	55	85
45.5	20	65	80
100	20	100	100

EL50 10.9 mg/L at 48 hours (95% confidence limits)
NOEL 1.94 mg/L at 48 hours (95% confidence limits)
Remarks - Results All validity criteria for the test were satisfied. The 48 h EL50 and NOEL for daphnids were determined to be 10.9 mg/L and 1.94 mg/L, respectively, based on measured concentrations.

CONCLUSION	The test substance is harmful to aquatic invertebrates.
TEST FACILITY	HLS (2015a)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata* (green alga).
Exposure Period	72 hours.
Concentration Range	Nominal: 100, 40.0, 16.0, 6.40 and 2.56 mg/L
Auxiliary Solvent	None.
Water Hardness	Not reported.
Analytical Monitoring	GC-MS.
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. Samples were prepared with WAF method. The test substance (500, 200, 80.0 or 32.0 mg) was added to 4 L dilution medium in an aspirator (5 L) and after being mixed, the media were made up to volume. Additionally 25.6 mg of test substance was added to 9 L dilution medium in an aspirator (10 L) and after being mixed well, the medium were made up to volume. WAFs with nominal loading rates of 100, 40.0, 16.0, 6.40 and 2.56 mg/L were used as the test media.

RESULTS

Biomass		*Growth*	
E_bL50	*NOEL*	*E_rL50*	*NOEL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
24.3 (95% CL 19.5-30.9)	6.40	56.5 (95% CL 52.1–61.4)	6.40

Remarks - Results	All validity criteria for the test were satisfied. Based on nominal loading rates, algal growth was significantly inhibited to give a 72-hour EL50 of 56.5 mg/L based on growth rate and 24.3 mg/L based on yield.
CONCLUSION	The test substance is harmful to algae.
TEST FACILITY	HLS (2015b)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Product mixture containing the notified chemical at 50-70% concentration
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 100 and 1000 mg/L
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. Chemical 3,5-dichlorophenol was used as the reference control. The respiration rate was determined by measurement of BOD during the test after 3 hours of exposure.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. Concentration-related inhibition of respiration rates were observed at concentrations between 100-1000 mg/L, with 94-96% inhibition at 1000 mg/L. The 3 h EC50 was

	determined to be > 1000 mg/L, based on measured concentrations.
CONCLUSION	The test substance is not inhibitory to microbial activity.
TEST FACILITY	HLS (2014b)

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