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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Propanoic acid, 2,2-dimethyl-, 1-methyl-2-(1-methylethoxy)-2-oxoethyl ester

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/1896	Firmenich Limited	Propanoic acid, 2,2-dimethyl-, 1-methyl-2-(1-methylethoxy)-2-oxoethyl ester	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*
Flammable liquids	H227 – Combustible liquid

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

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

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

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

  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.

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

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 as changed from 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: other names, analytical data, degree of purity, residual monomers, impurities, use details, and import volume.

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

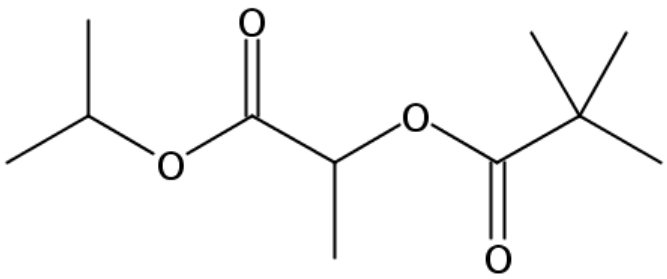
MARKETING NAME(S)
1-Isopropoxy-1-oxo-2-propanyl pivalate

CAS NUMBER
1821051-37-9

CHEMICAL NAME
Propanoic acid, 2,2-dimethyl-, 1-methyl-2-(1-methylethoxy)-2-oxoethyl ester

MOLECULAR FORMULA
$C_{11}H_{20}O_4$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
216.27 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -80 °C	Measured
Boiling Point	207.5 °C at 101.3 kPa	Measured
Density	949.1 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.05 kPa at 25 °C	Measured
Stability Testing	Stable at 300 °C	Measured
Water Solubility	0.492 g/L at pH 4.7 at 20 °C	Measured
Hydrolysis as a Function of pH	Stable at pH 4-9 Unstable at pH 2, 12	Measured (in-house method)*
Partition Coefficient (n-octanol/water)	log Pow = 3.32 at 22.8 °C	Measured
Surface Tension	45.64 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.0 at 23.6 °C for soil log K_{oc} = 2.1 at 23.6 °C for sewage sludge	Measured
Dissociation Constant	Not determined	Contains no dissociable functionalities
Flash Point	84 °C at 101.3 kPa	Measured
Autoignition Temperature	415 °C	Measured
Explosive Properties	Not determined	Not expected to be explosive based on chemical structure.
Oxidising Properties	Not determined	Not expected to be oxidative based on chemical structure.

* Study report not provided

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 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*
Flammable Liquids (Category 4)	H227 – Combustible liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia in its pure form or as a component in a fragrance formula (at a concentration ≤ 1%).

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

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

PORT OF ENTRY
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in either its pure form or as a component of fragrance preparations containing the notified chemical (at ≤ 1% concentration). The notified chemical will be imported and distributed in tightly closed lacquered drums of 180, 100, 50, 25, 10 or 5 kg in size. They will be transported by road to the Firmenich Ltd warehouse for storage and then distributed to reformulation sites. It is also possible that the notified chemical will be transported directly to the customer's facilities from the port of entry. End-use products will be packaged in containers suitable for retail sale.

USE
The notified chemical is intended to be used as a fragrance ingredient for a variety of cosmetic and household products at < 1% concentration.

OPERATION DESCRIPTION
The procedures for incorporating the imported preparations (in pure form or at < 1% concentration) into end-use products will likely vary depending on the nature of the cosmetic and personal care/household cleaning products formulated, and may involve both automated and manual transfer steps. It is expected that the reformulation processes will involve blending operations that will be highly automated and occur in a fully enclosed/contained environment, followed by automated filling (using sealed delivery systems) of the reformulated end-use products into containers of various sizes.

The end-use products containing the notified chemical (at < 1% concentration) may be used by consumers and professionals such as hairdressers, workers in beauty salons or 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 workers	Unknown	Unknown
Mixer	4	2
Drum handling	4	2
Drum cleaning	4	2
Maintenance	4	2
Quality Control	0.5	1
Packaging	4	2
Salon Workers	Unspecified	Unspecified
Cleaners	Unspecified	Unspecified

EXPOSURE DETAILS
Transportation and Storage
Transport and storage workers may come into contact with the notified chemical, in its pure form or as a component of the imported preparations (at a concentration of < 1%), only in the event of accidental rupture of containers.

Mixing/Blending/Filling/Packaging
During reformulation, dermal, ocular and potentially inhalation exposure of workers to the notified chemical (in pure form) may occur during weighing and transfer stages, equipment preparation, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of

mechanical exhaust ventilation and/or automated/enclosed systems and through the use of personal protective equipment (PPE) such as gloves, respirator, eye protection and protective clothing.

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 and personal care products to clients (e.g. hairdressers, workers in beauty salons) or in the cleaning industry. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical (at a concentration ≤ 1%) through the use of the household cleaning products, perfumes and both rinse-off and leave-on cosmetic and personal care products. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible particularly if products are applied by spray.

Data on typical use patterns of cosmetic product categories in which the notified chemical may be used are shown in the following table (SCSS, 2012; Cadby *et al*., 2012). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. An adult bodyweight of 64 kg was used for calculation purposes. Based on absence of dermal absorption data on the notified chemical, a dermal absorption of 100% was assumed for the notified chemical.

- Cosmetic products (Dermal exposure)

Product type	**Amount** (mg/day)	**C** (%)	**RF** (unitless)	**Daily systemic exposure** (mg/kg bw/day)
Body lotion	7820	1	1	1.2219
Face cream	1540	1	1	0.2406
Hand cream	2160	1	1	0.3375
Fragrances	750	1	1	0.1172
Deodorant (non-spray)	1500	1	1	0.2344
Shampoo	10460	1	0.01	0.0163
Hair conditioner	3920	1	0.01	0.0061
Shower gel	18670	1	0.01	0.0292
Hand wash soap	20000	1	0.01	0.0313
Hair styling products	4000	1	0.1	0.0625
Total				**2.2970**

C = concentration (%); RF = Retention Factor
Daily Systemic Exposure = (Amount × C × RF x dermal absorption)/body weight

- Hair spray (inhalation exposure)

Product type	**Amount** (g/day)	**C** (%)	**Inhalation Rate** (m^3/day)	**Exposure Duration (Zone 1)** (min)	**Exposure Duration (Zone 2)** (min)	**Fraction Inhaled** (%)	**Volume (Zone 1)** (m^3)	**Volume (Zone 2)** (m^3)	**Daily systemic exposure** (mg/kg bw/day)
Hairspray	9.89	1	20	1	20	50	1	10	**0.0322**

Total Daily systemic exposure = Daily systemic exposure in Zone 1 [(amount × C × inhalation rate × exposure duration (zone 1) × fraction inhaled)/(volume (zone 1) × body weight)] + Daily systemic exposure in Zone 1 [(amount × C × inhalation rate × exposure duration (zone 2) × fraction inhaled)/(volume (zone 2) × body weight)]
Note - conversion factors of 0.1 [to account for C/Bioavailability as a % and unit conversion (g to mg) ((1/100 × 1/100) × 1000)] and 1440 [to account for mins to day conversion, i.e. 1440 mins/day]

- Household products (Indirect dermal exposure – from wearing clothes)

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	1	0.95	10	0.0341
Fabric softener	90	1	0.95	10	0.0134
Total					**0.0475**

Daily Systemic Exposure = (Amount × C × PR × PT)/body weight

- Household products (Direct dermal exposure – from wearing clothes)

Product type	Frequency (use/day)	C (%)	Contact area (cm^2)	Product use C (g/cm^3)	Film thickness (cm)	Time scale factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	1	1980	0.01	0.01	0.007	0.0003
Dishwashing liquid	3	1	1980	0.009	0.01	0.03	0.0025
All-purpose cleaner	1	1	1980	1	0.01	0.007	0.0217
Total							**0.0245**

Daily Systemic Exposure = (Frequency × C × Contact area × Product Use Concentration × Film Thickness on skin × Time Scale factor × dermal absorption)/body weight

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical. This would result in a combined internal dose of 2.4011 mg/kg bw/day. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products (in addition to hair spray) may occur. However it is considered that he combination of conservative hair spray inhalation exposure assessment parameters, (in particular assuming an airspace volume of 2 m^3), and the aggregate exposure form the use of the dermally applied products, (which assumes a conservative 100% absorption rate), is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray cosmetic and household products with lower exposure factors (e.g. air fresheners).

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 6.74 mg/L/4 hour; low toxicity
Skin irritation (in vitro: Reconstructed Human *Epidermis* Test)	non-irritating
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Guinea pig, skin sensitisation – adjuvant test.	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 mammalian chromosome aberration test	non genotoxic

Toxicokinetics, metabolism and distribution.
No information on the toxicokinetics of the notified chemical was provided. For dermal absorption, molecular weights below 100 Da. are favourable for absorption and molecular weights above 500 Da. do not favour absorption (ECHA, 2014). Dermal uptake is likely to be moderate to high if the water solubility is between 100-10,000 mg/L and log P values between 1 and 4 also favour dermal absorption (ECHA, 2014). Based on the water solubility (0.492 g/L at 20 °C), partition coefficient (log Pow = 3.32) and the low molecular weight (216.27 Da) of the notified chemical, passive diffusion across the gastrointestinal (GI) tract and dermal absorption are possible. The notified chemical may also be absorbed across the respiratory tract if inhaled.

Acute toxicity.
The notified chemical was found to have low acute toxicity in rats via the oral, dermal and inhalation routes.

Irritation.
No evidence of irritation was observed in an *in vitro* skin irritation study (EpiSkin). In an acute dermal irritation study in rabbits the notified chemical was slightly irritating with well-defined-slight erythema and very slight oedema observed. The notified chemical was also slightly irritating to the eye when tested in rabbits.

Sensitisation
The notified chemical showed no evidence of reactions indicative of skin sensitisation when challenged at 100% in a guinea pig maximisation test.

Repeated dose toxicity.
In a 28 day repeat dose study by oral gavage, rats were administered the notified chemical at doses of 30, 300 or 1000 mg/kg bw/day. The study authors concluded that the transient clinical, haematological, plasma biochemistry, urinalysis and organ/bodyweight effects observed in both sexes exposed to the highest dose of test substance were indicative of an adaptive response by the liver and kidneys. Minimal to slight microvesicular vacuolation in periportal liver cells was also recorded in both sexes in the high-dose group. No associated hepatocellular damage or necrosis was observed and the study authors concluded that the vacuolation was indicative of increased storage of fat associated with altered lipid metabolism.
Recovery from clinical and toxicological effects was indicated at the end of the two week recovery period.

The effects noted for animals in the high dose group were not considered by the study authors to be toxicologically significant. Therefore, the No Observed Adverse Effect Level (NOAEL) was established by the study authors as 1,000 mg/kg, based on their determination of an absence of adverse effects.

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in a bacterial reverse mutation study and non-clastogenic in an *in vitro* mammalian chromosome aberration test.

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Reformulation
Exposure of workers to the notified chemical (in its pure form) may occur during blending operations. The notified chemical has the potential to cause slight skin and eye irritation. In addition, harmful effects following inhalation and/or repeated exposure to the notified chemical are possible. Therefore, caution should be exercised when handling the notified chemical during reformulation processes.

However, provided that control measures are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use
Cleaners and beauty care professionals will handle the notified chemical at ≤ 1% concentration, similar to public use. Therefore the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experienced by members of the public who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health

Irritation

The notified chemical has the potential to cause slight skin and eye irritation. However, skin and eye irritation effects are not expected from use of the notified chemical at the proposed concentrations in cosmetic and household products.

Repeated dose toxicity

Members of the public may experience repeated exposure to the notified chemical through the use of the cosmetic and household products (containing the notified chemical at ≤ 1% concentration).

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 2.4011 mg/kg bw/day (see Section 6.1.2) and the NOAEL of 1000 mg/kg bw/day, as determined by the study authors in a 28-day repeated dose toxicity study on the notified chemical. Using the abovementioned NOAEL, a MoE of 416 was estimated. A MoE value ≥ 100 is considered acceptable to account for intra- and inter-species differences, and to account for long-term exposure; therefore, the MoE is considered to be acceptable. However, if the treatment related effects such as the liver effects and changes in the biochemical parameters in both sexes in the 1,000 mg/kg bw/day dose group are considered adverse and the next lowest dose of 300 mg/kg bw/day is used as the NOAEL the MOE estimate would still be > 100 (125).

Therefore, based on the information available, the risk to the public associated with the 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 be imported neat, or as a component of fragrance formulations, for reformulation into finished cosmetic formulations and household products. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills and leaks. It is estimated by the notifier that a maximum of 0.001% (or up to 10 g) of the notified chemical may be released from accidental spills and leaks. In the event of spills, the product containing the notified chemical is expected to be collected with adsorbents, and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve blending operations that will be highly automated, and expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. The process will be followed by automated filling of the formulated products into containers of various sizes suitable for retail use. Wastes containing the notified chemical generated during reformulation include equipment wash water, empty import containers and spilt materials. These are expected to be collected and disposed of to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The notified chemical is expected to be released to the aquatic compartment through sewers during its use in various cosmetic formulations and household products.

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated by the notifier that a maximum of 0.003%, or up to 30 g of the notified chemical, may remain in end-use containers once the consumer products are used up. Wastes and residue of the notified chemical in empty containers are likely to either share the fate of the container and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system through cosmetic formulations and household products, before potential release to surface waters nationwide. Based on the results of a ready biodegradability study, the notified chemical is considered readily biodegradable (71% in 28 days). For details of the environmental fate study, please refer to Appendix C. Based on its water

solubility and adsorption coefficient (log K_{OC} = 2.0-2.1), release to surface waters may occur as only partial partitioning to sludge and sediment is expected under environmental pH. The notified chemical is not expected to bioaccumulate due to its low partition coefficient (log P_{OW} = 3.32), surfactant properties and ready biodegradability. Therefore, in surface waters the notified chemical is expected to disperse and degrade through biotic and abiotic processes to form water and oxides of carbon.

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, when sewage sludge is used for soil remediation, or when disposed of to landfill as collected spills and empty container residue. The notified chemical residues in landfill, soil and sludge are expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated to assume a worst case scenario, with 100% release of the notified chemical into sewer systems nationwide and no removal within sewage treatment plants (STPs).

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:	0.606	µg/L
PEC - Ocean:	0.061	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 0.61 µg/L may potentially result in a soil concentration of approximately 4.04 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years may be approximately 20.19 µg/kg and 40.39 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 52 mg/L	Harmful to fish
Daphnia Toxicity	48 h EC50 = 108 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	72 h E_rC50 > 67.9 mg/L 72 h E_bC50 = 30.2 mg/L	Harmful to algae
Inhibition of Bacterial Respiration	3 h IC50 > 1000 mg/L	Not inhibitory to bacterial respiration

Based on the above ecotoxicological endpoints for the notified chemical, it is expected to be harmful to fish and algae, but not harmful to aquatic invertebrates. 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, ready biodegradability and low bioaccumulation potential of the notified chemical, it is not formally classified under the GHS for chronic toxicity.

7.2.1. Predicted No-Effect Concentration

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

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish, 96 h)	52	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	520	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated based on the predicted PEC and PNEC.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.606	520	**0.001**
Q - Ocean	0.061	520	**< 0.001**

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment 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 is considered readily biodegradable, and is expected to have a low potential for bioaccumulation. On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern in cosmetic formulations and household 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 < -80 °C

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	Differential scanning calorimetry
Test Facility	Consilab (2014a)

Boiling Point 207.5 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	Differential scanning calorimetry
Test Facility	Consilab (2014a)

Density 949.1 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	Pycnometer
Test Facility	Noack (2014a)

Vapour Pressure 0.036 kPa at 20 °C
0.05 kPa at 25 °C
0.234 kPa at 50 °C

Method	OECD TG 104 Vapour Pressure. EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks	Dynamic method: Vapour-liquid equilibrium.
Test Facility	Consilab (2014b)

Stability Testing Stable at 300 °C

Method	OECD TG 113 Screening Test for Thermal Stability and Stability in Air.
Remarks	Differential scanning calorimetry.
Test Facility	Consilab (2014b)

Water Solubility 0.492 g/L at pH 4.7 at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method
Test Facility	Noack (2014c)

Partition Coefficient (n-octanol/water) log Pow = 3.32 at 22.8 °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	Noack (2015a)

Surface Tension 45.64 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks	Concentration: 90% saturated aqueous solution
Test Facility	Noack (2014b)

Adsorption/Desorption log K_{oc} = 2.0 at 23.6 °C for soil
log K_{oc} = 2.1 at 23.6 °C for sewage sludge

Method	OECD TG 121 Adsorption - Desorption (log K_{OC}).
Remarks	HPLC Method.
Test Facility	Noack (2015b)

Flash Point 84 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Pensky Martens
Test Facility	Consilab (2014e)

Autoignition Temperature 415 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Cylindrical resistance furnace with Erlenmeyer flask ignition chamber.
Test Facility	Consilab (2014f)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/ Crl:CD (SD)
Vehicle	Corn oil
Remarks - Method	No protocol deviations. GLP compliant.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	6 F	300	0/6
2	6 F	2,000	0/6

LD50	> 2,000 mg/kg bw
Signs of Toxicity	One female in group 2 exhibited salivation up to 2 hr post dosing. No other clinical signs noted.
Effects in Organs	No abnormalities observed.
Remarks - Results	All animals exhibited satisfactory body weight gain.

CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Huntingdon (2014)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/ Wistar
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	No protocol deviations. GLP compliant.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	Skin irritation effects were observed in 3/5 females. One subject exhibited very slight erythema on day 1 (not observed on day 2). One subject exhibited very slight erythema on days 1 and 2 (not observed on day 3) in addition to small superficial scattered scabs and glossy skin on days 6, 7 and 8 and crust formation on days 9 and 10. Full recovery from these effects in this subject was observed on day 11. One subject exhibited very slight erythema (days 4 and 5) with small superficial scattered scabs and glossy skin (days 4, 5, 6, 7 and 8), full recovery was observed on Day 9. No signs of dermal irritation were observed in the remaining two females or in any of the male test subjects.
Signs of Toxicity - Systemic	None.
Effects in Organs	No abnormalities observed.

Remarks - Results	All animals exhibited satisfactory body weight gain.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Harlan (2014a)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity.
Species/Strain	Rat/ RccHan™:WIST
Vehicle	None
Method of Exposure	Nose-only exposure.
Exposure Period	4 hours
Physical Form	Solid aerosol (particulate).
Particle Size	2.91 µm
Remarks - Method	The mean MMAD* of the notified chemical was 2.91 µm with an inhalable fraction (< 4 µm) of 66.6%. No significant protocol deviations. GLP compliant. **Mass Median Aerodynamic Diameter*

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
1	5 M, 5 F	26.8	6.74 ± 1.10	0/10

LC50	> 6.74 mg/L/4 hours
Signs of Toxicity	All animals exhibited wet fur during the exposure period with hunched posture, pilo-erection and an increased respiratory rate on removal from and at one hour post exposure. All animals exhibited hunched posture and an increased respiratory rate up to 24 hours after exposure. Increased respiratory rate was observed at 2, 3 (all animals), 4 (9/10 animals) and 5 (6/10 animals) days after exposure. All animals appeared to have recovered by day 6 post-exposure. No other adverse effects observed.
Effects in Organs	Dark patches on the lungs of 2/5 males and 2/5 females were observed. No other macroscopic abnormalities were detected.
Remarks - Results	All males and 2/5 females exhibited a loss in body weight 1 day post-exposure (with one of the females exhibiting weight loss between days 3 and 7 post-exposure). However, the losses were not significant. No weight gain was observed in 1/5 females between days 1 and 3. All animals gained body weight during the recovery period.
CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	Harlan (2015a)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method (EpiSkin)
Vehicle	None
Remarks - Method	Triplicate tissues were treated with 10 µL of the test substance. Following

an exposure period of 15 minutes at room temperature, the tissues were rinsed and then incubated in maintenance medium at 37 °C for 42 hours. The tissues were then treated with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) and incubated at 37 °C for 3 hours. Following extraction, the optical densities were determined (562 nm).

The study authors noted that a preliminary test had been conducted, which indicated that the test substance does not directly reduce MTT.

Positive and negative controls were run in parallel with the test substance:
- Negative control: Dulbecco's Phosphate Buffered Saline;
- Positive control: 5% Sodium Dodecyl Sulphate.

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.828	100	0.3
Test substance	0.819	98.9	8.8
Positive control	0.046	5.5	2.3

OD = optical density; SD = standard deviation

Remarks - Results: The positive and negative controls gave satisfactory results, confirming the validities of the test systems.

CONCLUSION: The notified chemical was non-irritating to the skin under the conditions of the test.

TEST FACILITY: Harlan (2014b)

B.5. Irritation – skin

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	3
Vehicle	None
Observation Period	7 days
Type of Dressing	Semi-occlusive.
Remarks - Method	No deviations from the protocol. GLP compliant

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	1.7	0	0.3	2	< 7 d	0
Oedema	1	0	0.3	1	< 7 d	0

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

Remarks - Results: One animal exhibited well-defined erythema and very slight oedema up to 48 h post-exposure with a lessening of effects (very slight erythema and oedema) observed at 72 h post-exposure. Very slight erythema and oedema was observed in a second animal 24 h post-exposure with apparent recovery at 48 h post-exposure.

All animals showed expected body weight gains.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Harlan (2014c)

B.6. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals 3 (1 F, 2 M)
Observation Period 7 days
Remarks - Method GLP compliant.

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	1.7	0.7	0.7	2	< 7 days	0
Conjunctiva: chemosis	1	0.3	0.3	1	< 7 days	0
Conjunctiva: discharge	0.3	0	0	1	< 48 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	1	< 24 hours	0

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

Remarks - Results All animals exhibited moderate conjunctival irritation 1 h post-exposure. One animal exhibited moderate conjunctival up to 48 h post-exposure with a lessening of effects (minimal irritation) observed at 72 hr post-exposure and apparent recovery at the day 7 observation. The remaining two animals exhibited minimal conjunctival irritation up to 48 h post-exposure with apparent recovery at 72 h observation.

Moderate iridial inflammation was recorded in 1/3 animals 1 h post-exposure, with apparent recovery at the 24 h observation where no iridial inflammation was observed in 3/3 animals. No corneal effects were observed in any of the animals.

All animals showed expected body weight gains.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Harlan (2015b)

B.7. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test.
Species/Strain Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY Maximum Non-irritating Concentration:
intradermal: 20%
topical: 100%
MAIN STUDY
Number of Animals Test Group: 10 F Control Group: 5 F
Vehicle Intradermal: Olive oil
Topical: Liquid paraffin
Positive control Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.

INDUCTION PHASE	Induction Concentration: intradermal: 20% topical: 100%
Signs of Irritation	None
CHALLENGE PHASE	
1st challenge	topical: 100%
Remarks - Method	The concentration of the test substance for intradermal induction was determined as 20% based on necrosis observed with test substance concentrations of 50% and 100%. Freund's Complete Adjuvant (50% in olive oil) used at intradermal induction phase. Sodium lauryl sulfate (10%) used to create local irritation at topical induction phase. There was a 10 day rest phase between the induction and challenge phases. The challenge phase was performed under occlusive dressing for 24 hr. Liquid paraffin served as negative control. No significant protocol deviations. GLP compliant.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions at challenge*	
		24 h	*48 h*
Test Group	100	0/5	0/5
Control Group	100	0/10	0/10

Remarks - Results	None
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Phycher (2015)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/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	GLP compliant. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 F, 5 M	0	0/10
low dose	5 F, 5 M	30	1/10
mid dose	5 F, 5 M	300	0/10
high dose	5 F, 5 M	1000	0/10
control recovery	5 F, 5 M	0	1/10
high dose recovery	5 F, 5 M	1000	0/10

Mortality and Time to Death

Two female animals died, one from the control recovery group (day 26) and one from the low-dose group who failed to recover from anaesthesia (during routine blood sampling on day 29). However, these deaths were considered to be to be incidental and unrelated to exposure to the test substance as there were no preceding clinical signs or significant macroscopic or microscopic findings.

Clinical Observations

Transient clinical effects including reddening of the ears (males and females in the high dose group), reddening of the limbs (one male, high-dose group), swaying gait (one male, high-dose group), and salivation (one male in mid-dose group, both sexes in high-dose group) with associated chin rubbing in some animals, were observed within the first two weeks of the exposure period. Low motor activity scores were observed in mid-and high-dose females. However, while a dose-relationship was observed and the scores were lower than those obtained from control animals, the study authors did not consider the differences to the controls to be of an adverse magnitude and being within the historical control data range the effect was not considered to be attributable to the test substance by them.

All animals gained body weight over the course of the study. However, females in the high-dose group showed slightly low body weight gains in the first week, but exhibited an overall increase similar to animals in the control group. Recovery from other clinical effects was indicated at the end of the two week recovery period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Females in the high-dose group recorded low haematocrit, haemoglobin concentration and erythrocyte count and high mean cell haemoglobin and mean cell haemoglobin concentration. Males in the high-dose group recorded a higher mean cell haemoglobin concentration, large unstained cell counts and a higher incidence of ketones in the urine. A decreased platelet count and an increased activated partial thromboplastin time was seen in female animals in the high dose group after the 14 day recovery period but not at the end of the dosing period and were therefore considered by the study authors to be of no biological or toxicological significance.

Males in the high dose group exhibited low glucose and chloride concentrations and females in the high dose group low glucose, high triglyceride, sodium and phosphorous concentrations. Calcium concentration was low in all dose groups although a dose-relationship was not observed. Low urine pH was recorded for animals in the mid- and high-dose groups.

Recovery from toxicological effects was indicated at the end of the two week recovery period. Plasma calcium concentration was slightly low in high-dose recovery males, but as the values were within the normal background range, the study authors did not consider the result to be of toxicological significance.

Effects in Organs

Both sexes in the high-dose group and males in the mid-dose group recorded high absolute and bodyweight-relative liver and kidney weights. Males in the high-dose group also recorded high absolute and bodyweight-relative heart weights. No macroscopic test substance related lesions were observed. Both sexes in the high-dose group exhibited minimal to slight reversible microvesicular vacuolation in periportal liver cells.

Remarks – Results

The two deaths were considered to be to be incidental and unrelated to exposure to the test substance as there were no preceding clinical signs or significant macroscopic or microscopic findings.

Females in the high-dose group exhibited changes in red blood cell parameters. However, as these were not

supported with adverse pathology the effect was not considered to be adverse at the magnitude of change observed.

The study authors concluded that the transient clinical, haematological, plasma biochemistry, urinalysis and organ/bodyweight effects observed in both sexes exposed to the highest dose of test substance were indicative of an adaptive response by the liver and kidneys. Minimal to slight microvesicular vacuolation in periportal liver cells was also recorded in both sexes in the high-dose group. No associated hepatocellular damage or necrosis was observed and the study authors concluded that the vacuolation was indicative of increased storage of fat associated with altered lipid metabolism (glucose, chloride, triglyceride, phosphorous (both sexes in high-dose group), creatinine and bile acid (females in high-dose group) concentrations were different to that of control animals). Similar biochemistry effects were not recorded in males in the mid-dose group.

Recovery from clinical and toxicological effects was indicated at the end of the two week recovery period. Plasma calcium concentration was slightly low in high-dose recovery males, but as the values were within the normal background range, the study authors did not consider the result to be of toxicological significance.

The effects noted for animals in the high dose group were not considered by the study authors to be toxicologically significant. Therefore, the No Observed Adverse Effect Level (NOAEL) was established by the study authors as 1,000 mg/kg, based on their determination of an absence of adverse effects.

CONCLUSION
The NOAEL was established as 1000 mg/kg bw/day in this study, based on the determination by the study authors of an absence of adverse effects at all doses.

TEST FACILITY Huntingdon (2015)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2*uvrA*
Metabolic Activation System	Rat S9 fraction from phenobarbitone/β-Naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: TA98, TA1535, TA1537, *WP2uvrA*: 1.5 - 1500 µg/plate TA100: 5 - 5000 µg/plate b) Without metabolic activation: TA100: 1.5 - 1500 µg/plate TA98, TA1535, TA1537, *WP2uvrA*: 0.5 - 500 µg/plate
Vehicle	Dimethyl sulphoxide
Remarks - Method	GLP compliant. No deviations from protocol. Positive controls: absence of metabolic activation: N-ethyl-N'-nitro-N-nitrosoguanidine (TA100, TA1535, WP2*uvrA*), 9-Aminoacridine (TA1537) and 4-Nitroquinoline-1-oxide (TA98); presence of metabolic activation: 2-Aminoanthracene (TA100, TA1535, TA1537, WP2*uvrA*) and Benzo(a)pyrene (TA98). A preliminary range-finding test (1.5 – 5000 µg/plate) performed on all strains. TA1537 exhibited a small, but statistically significant, increase in frequency of revertant colonies observed at 150 µg/plate (absence of metabolic activation). Increase was considered to be of no biological relevance based on absence of a dose-response relationship or reproducibility and an associated decrease in bacterial background lawn.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	≥ 150	> 1500	negative
Present				
Test 1	> 5000	≥ 500	> 5000	negative

Remarks - Results

Reduction in bacterial background lawn observed from 150 µg/plate (TA1537) and 500 µg /plate (TA100, TA1535, TA98, WP2*uvrA*) in the absence of S9-mix. Weakened bacterial lawns were observed from 500 µg/plate (TA1535) and 1500 ug/plate (TA100, TA98, WP2*uvrA*, TA1537) in the presence of S9-mix. No precipitate was observed at any of the doses tested in either the presence or absence of S9-mix.

No toxicologically significant increase in the frequency of revertant colonies was recorded in the presence or absence of metabolic activation for any of the bacterial strains at the doses tested.

Negative and positive controls performed as expected.

CONCLUSION

The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY

Harlan (2014d)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

Metabolic Activation System: Rat S9 fraction from phenobarbitone/β-Naphthoflavone induced rat liver

Vehicle: Dimethyl sulphoxide

Remarks - Method: GLP compliant.
No deviations from protocol.

A preliminary cell-growth inhibition test was performed (8.45 – 2163 µg/mL) to determine the maximum dose level in the main test

Positive controls: absence of metabolic activation – mitomycin C; presence of metabolic activation - cyclophosphamide

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	67.5*, 135*, 270*, 405*, 540, 1080	4 h	24 h
Test 2	33.75, 67.5, 135*, 202.5*, 270*, 405*, 540	24 h	24 h
Present			
Test 1	135, 270*, 405*, 540*, 810*, 1080	4 h	24 h
Test 2	135, 270, 405*, 540*, 810*, 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	≥ 540.75 µg/mL	≥ 405 µg/mL	≥ 1080 µg/mL	negative
Test 2	≥ 1081.5 µg/mL	> 405 µg/mL	≥ 405 µg/mL	negative
Present				
Test 1	≥ 1081.5 µg/mL	> 810 µg/mL	≥ 1080 µg/mL	negative
Test 2		≥ 1080 µg/mL	≥ 810 µg/mL	negative

Remarks - Results

No statistically significant increase in the frequency of chromosomal aberrations in the presence or absence of metabolic activation was recorded.

Negative and positive controls performed as expected.

CONCLUSION

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

TEST FACILITY

Harlan (2015c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Oxygen Demand (ThOD)
Remarks - Method	Ammonium chloride was omitted from the test medium to prevent oxygen consumption due to nitrification. All other validity criteria were met and satisfied.

RESULTS

Test substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
7	5	7	80
14	48	14	89
21	55		
28	71		

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound surpassed the threshold level of 60% by 7 days (80%) and reached 89% degradation by 14 days. Therefore, the test indicates the suitability of the inoculums. The test substance attained 71% degradation by 28 days, and a degradation plateau was not achieved. As the test substance is surface active, the 10-day window is not applicable. Therefore, the test substance is considered to be readily biodegradable according to the OECD (301 D) guideline.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	Akzo Nobel (2015)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static.
Species	*Danio rerio* (zebra fish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	123 mg $CaCO_3$/L
Analytical Monitoring	GC
Remarks – Method	The definitive test was conducted at nominal concentrations of 21.4, 31.5, 46.3, 68.0, and 100 mg/L of the test substance. No significant deviation in protocol was reported.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	10	0	0	0	0	0
21.4	24.6	10	0	0	0	0	0
31.5	32.2	10	0	0	0	0	0
46.3	41.0	10	0	20	20	20	20
68.0	ND	10	100	100	100	100	100
100	ND	10	100	100	100	100	100

ND: Not determined

LC50	52.0 mg/L (95% CI 47.1-57.3 mg/L) at 96 hours.
NOEC	31.5 mg/L at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. The test solutions were renewed every 48 hours during the 96 h test period. The actual concentrations of the test substance were measured at 0, 48, and 96 hours during the 96 h test period. The 96 h LC50 and NOEC for fish were determined to be 52.0 mg/L and 31.5 mg/L, based on measured concentrations.

CONCLUSION The notified chemical is considered to be harmful to fish.

TEST FACILITY Guangdong Detection Center of Microbiology (2015)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Semi-static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	160-180 mg $CaCO_3$/L
Analytical Monitoring	GC-MS
Remarks - Method	The definitive test was conducted at nominal concentrations of 12.5, 25, 50, 100, and 200 mg/L of the test substance. A total of 20 daphnids (5 daphnids/replicate across 4 replicates) were used. No significant deviation in protocol was reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
12.5	9.51	20	0	0
25	23.5	20	0	0
50	46.1	20	0	10
100	86.0	20	30	45
200	161	20	50	80

EC50	108 mg/L (95% CI 85.2-145 mg/L) at 48 hours
NOEC	Not determined
Remarks - Results	All validity criteria for the test were satisfied. The test solutions were renewed every 24 hours during the 48 h test period. The actual concentrations of the test substance were measured at 0 and 48 hours during the 48 h test period. The 48 h EC50 for *Daphnia* was determined to be 108 mg/L, based on measured concentrations.

CONCLUSION	The notified chemical is not considered to be harmful to aquatic invertebrates.
TEST FACILITY	Noack (2014d)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 3.125-100 mg/L Actual: 2.14-67.9 mg/L (geometric mean)
Auxiliary Solvent	None
Water Hardness	0.24 mmol Ca + Mg/L
Analytical Monitoring	GC-MS
Remarks - Method	The definitive test was conducted at nominal concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 mg/L of the test substance. No significant deviation in protocol was reported.

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
30.2	16.6	> 67.9	16.6

Remarks - Results	All validity criteria for the test were satisfied. The actual concentrations of the test substance were at 0 and 72 hours within the 72 h test period. The test solutions were not renewed during the 72 h test period. The 72 h E_rC50, E_bC50, and NOEC were determined to be > 67.9 mg/L, 30.2 mg/L, and 16.6 mg/L, respectively, based on the geometric mean measured concentrations.
CONCLUSION	The notified chemical is considered to be harmful to algae.
TEST FACILITY	Noack (2014e)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge.
Exposure Period	3 hours
Concentration Range	Nominal: 46-1000 mg/L Actual: Not determined
Remarks – Method	No significant deviation in protocol was reported. Copper (II) sulphate pentahydrate was used as the reference control. The respiration rate was determined by measurement of Biochemical Oxygen Demand during the test after 3 hours of exposure.
RESULTS	
IC50	> 1000 mg/L at 3 hours
NOEC	100 mg/L at 3 hours
Remarks – Results	All validity criteria for the test were satisfied. The 3 h IC50 was determined to be > 1000 mg/L, based on nominal concentrations.
CONCLUSION	The notified chemical is not inhibitory to microbial activity.
TEST FACILITY	Noack (2014f)

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