File No: LTD/2039

September 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Bicyclo[3.1.1]hept-2-ene-2-propanenitrile, α,α,6,6-tetramethyl-

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment and Energy.

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

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2039	International Flavours and Fragrances (Australia) Pty Ltd	Bicyclo[3.1.1]hept-2-ene-2-propanenitrile, α,α,6,6-tetramethyl-	No	1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

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 2	H401 – Toxic to aquatic life
Chronic Category 2	H411 – Toxic to aquatic life with long lasting effects

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

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, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself. However, these should be selected on the basis of all ingredients in the formulation.

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the 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;

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.

Safety Data Sheet
The SDS of the notified chemical and products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
International Flavours and Fragrances (Australia) Pty Ltd (ABN: 77 004 269 658)
310 Frankston-Dandenong Road
DANDENONG VIC 3175

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: particle size, dissociation constant, and hydrolysis as a function of pH.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
EU (2017)
China (2018)
Japan (2018)
Philippines (2018)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Pinyl nitrile

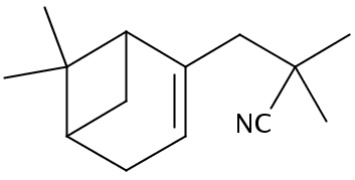
CAS NUMBER
2003244-43-5

CHEMICAL NAME
Bicyclo[3.1.1]hept-2-ene-2-propanenitrile, α,α,6,6-tetramethyl-

OTHER NAME
ES421 pinyl nitrile

MOLECULAR FORMULA
$C_{14}H_{21}N$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
203.32 g/mol

ANALYTICAL DATA
Reference NMR, IR, HPLC, GC-MS, GC, and UV spectra were provided and determination of optical activity.

3. COMPOSITION

DEGREE OF PURITY
≥ 99 %

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS

Chemical Name	Bicyclo[3.1.1]hept-2-ene-2-propanal, α,α,6,6-tetramethyl-		
CAS No.	33885-52-8	*Weight %*	≤ 1
Hazardous Properties	H317 (Causes skin sensitisation)		

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white solid

Property	Value	Data Source/Justification
Melting Point	38 °C	Measured
Boiling Point	271.2 °C at 101.3 kPa	Measured
Density	1,010 kg/m^3 at 20 °C	Measured
Vapour Pressure	3.4 x 10^{-3} kPa at 25 °C	Measured
Water Solubility	8.38 × 10^{-3} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionality, but hydrolysis is not expected in the environmental pH range (4-9)
Partition Coefficient (n-octanol/water)	log Pow = 4.35 at 30 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.87 at 35 °C	Measured
Dissociation Constant	Not determined	No dissociable functionality
Surface Tension	70.6 mN/m at 20 °C	Measured
Flash Point	117 °C	Measured
Flammability	Combustible liquid# *	Based on measured flash point
Pyrophoric Properties	Not pyrophoric	Expert statement based on chemical structure
Autoignition Temperature	275 °C	Measured
Explosive Properties	Not explosive	Expert statement based on chemical structure
Oxidising Properties	Not oxidising	Expert statement based on chemical structure

Based on *Australian Standard AS1940* definitions.
* The notified chemical was supplied as a solid. However, under the conditions of the test, the notified chemical would have presumably become a liquid (starting temperature of the test was 50 °C).

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.

The notified chemical has a flash point of 117 ºC. Based on *Australian Standard AS1940* definitions for combustible liquids, a liquid that has a flash point which is both greater than 93 °C and is less than its boiling point is a Class C2 combustible liquid.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported as a component of fragrance oils at ≤ 10% 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
Melbourne

IDENTITY OF RECIPIENTS
International Flavours and Fragrances (Australia) Pty Ltd

TRANSPORTATION AND PACKAGING
Fragrance mixtures containing the notified chemical at ≤ 10% concentration will be packaged in polypropylene-lined steel drums (usually ~ 208 L in size). Finished products containing the notified chemical at ≤ 1.25% concentration will be packaged in containers suitable for retail sale and transported to retailers, primarily by road.

USE
The notified chemical will be used as a fragrance ingredient in cosmetic and household products at ≤ 1.25% concentration.

OPERATION DESCRIPTION
The procedures for reformulating the fragrance oils containing the notified chemical at ≤ 10% concentration will likely vary depending on the nature of the cosmetic and household products, and may involve both automated and manual transfer steps. In general, it is expected that the reformulation processes will involve blending operations that will normally be automated and occur in an enclosed system, followed by automated filling of the finished products into retailcontainers of various sizes.

End Use
Finished household cleaning products containing the notified chemical at ≤ 1.25 % concentration may be used by consumers and professional cleaners. The cleaning products will be generally applied with a cloth or sponge, mop or brush, or by spray followed by wiping. In some cases the cleaning product will be diluted with water prior to application.

The finished cosmetic products containing the notified chemical at ≤ 1.25 % concentration will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the product, application of products could be by hand, sprayed or through the use of an applicator.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehouse	None	Incidental
Mixing and compounding	4	250
Drum handling	1	250
Drum cleaning	2	200
Equipment cleaning	2	250
Quality control	1	250
Professional cleaners	2	250

Hairdressers and beauticians	2	250
Retailers	None	Incidental

EXPOSURE DETAILS

Transport and storage

Transport, storage and warehouse workers may come into contact with the notified chemical at ≤ 10% concentration (in fragrance oils) or at ≤ 1.25 % concentration (in final end-use products), only in the unlikely event of an accidental rupture of containers.

Reformulation

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical at ≤ 10% concentration may occur during handling of drums, during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of local ventilation and/or enclosed systems, and through the use of personal protective equipment (PPE) such as protective clothing, eye protection and impervious gloves.

End use professionals

Exposure to the notified chemical at ≤ 1.25 % concentration in end-use products may occur in professions where the services provided involve the application of cosmetic products to clients or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure are 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 ≤ 1.25 % concentration) through the use of the cosmetic and household products. The main route of exposure will be dermal, while ocular and inhalation exposure are also possible, particularly if products are applied by spray.

Data on typical use patterns of product categories (SCCS, 2012; Cadby *et al*., 2002; ACI, 2010; Loretz *et al.,* 2006) in which the notified chemical may be used are shown in the following tables. For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. A dermal absorption (DA) rate of 100% was assumed for the notified chemical for calculation purposes. For the inhalation exposure assessment, a 2-zone approach was used (Steiling *et al*., 2014; Rothe *et al*., 2011; Earnest, Jr, 2009) with an adult inhalation rate of 20 m^3/day (enHealth, 2012). It was conservatively assumed that the fraction of the notified chemical inhaled is 50%. A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

Cosmetic products (dermal exposure)

Product Type	**Amount** (mg/day)	**C** (%)	**RF** (unitless)	**Daily Systemic Exposure** (mg/kg bw/day)
Body lotion	7820	1.25	1.000	1.5273
Face cream	1540	1.25	1.000	0.3008
Hand cream	2160	1.25	1.000	0.4219
Fine fragrances	750	1.25	1.000	0.1465
Deodorant (non-spray)	1500	1.25	1.000	0.2930
Shampoo	10460	1.25	0.010	0.0204
Conditioner	3920	1.25	0.010	0.0077
Shower gel	18670	1.25	0.010	0.0365
Hand wash soap	20000	1.25	0.010	0.0391
Hair styling products	4000	1.25	0.100	0.0781
Total				**2.8712**

C = maximum intended concentration of notified chemical; RF = retention factor

Daily systemic exposure = (Amount × C × RF × Dermal Absorption) / Body Weight

Household products (indirect dermal exposure – from wearing clothes)

Product type	Amount (g/use)	C (%)	Product Retained (%)	Transfer (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	1.25	0.95	10	0.0427
Fabric softener	90	1.25	0.95	10	0.0167
Total					**0.0594**

C = maximum intended concentration of notified chemical
Daily systemic exposure = (Amount × C × Product Retained × Transfer × Dermal Absorption) / Body Weight

Household products (direct dermal exposure)

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	1.25	1980	0.01	0.01	0.007	0.0004
Dishwashing liquid	3	1.25	1980	0.009	0.01	0.03	0.0031
All-purpose cleaner	1	1.25	1980	1	0.01	0.007	0.0271
Total							**0.0306**

C = maximum intended concentration of notified chemical
Daily systemic exposure = (Frequency × C × Contact area × Product Use Concentration × Film Thickness on skin × Time Scale Factor × Dermal Absorption)/Body Weight

Hairspray (Inhalation exposure):

Product type	Amount (g/use)	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	1.25	20	1	20	50	1	10	0.0402
Total									**0.0402**

C = maximum intended concentration of notified chemical
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 2 [(amount × C × inhalation rate × exposure duration (zone 2) × fraction inhaled)/(volume (zone 2) × 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 at the maximum intended concentration as specified by the notifier in various product types. This would result in a combined internal dose of 3.0014 mg/kg bw/day.

It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household cleaning products (in addition to hair spray) may occur. However, it is considered that the combination of the conservative hair spray inhalation exposure assessment parameters, and the aggregate exposure from 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 low exposures (e.g. air fresheners and deodorants).

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 > 4.28 mg/L/4 hour; low toxicity
Skin corrosion - *in vitro* (EpiDerm™ model)	non-corrosive
Rabbit, skin irritation	non-irritating
Eye irritation - *in vitro* (BCOP)	non-corrosive and non-irritating
Rabbit, eye irritation	slightly irritating

Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Human, skin sensitisation – RIPT (25%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 337 mg/kg bw/day (males); 1,102 mg/kg bw/day (females)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosomal aberration	non clastogenic

Toxicokinetics
Given the low molecular weight of the notified chemical (203.32 g/mol) absorption across the gastrointestinal and respiratory tracts may occur. Dermal absorption is expected to be limited given the low water solubility (0.00838 g/L) and high lipophilicity (log Pow = 4.35) of the notified chemical, limiting penetration of the hydrophilic epidermis.

Acute toxicity
The notified chemical is of low acute oral, dermal, and inhalation toxicity based on studies conducted in rats.

Irritation and sensitisation
The notified chemical was found to be non-corrosive and non-irritating to the skin based on an *in vitro* study conducted using a reconstructed human epidermis model and a study conducted in rabbits, respectively.

In an *in vitro* bovine corneal opacity and permeability (BCOP) test it was determined that the notified chemical required no classification for serious eye damage or eye irritation. In a study conducted in rabbits, the notified chemical was found to be slightly irritating to eyes with minimal to moderate conjunctival irritation observed for up to 72 hours after treatment with the notified chemical.

The notified chemical, at up to 50% concentration, was not a skin sensitiser in a mouse local lymph node assay. The notified chemical also tested negative in a human repeat insult patch test when tested at 25% concentration.

Repeated dose toxicity
A repeated dose oral (diet) toxicity study was conducted in rats. The test substance was administered at 500, 1,500 and 5,000 ppm (34, 104 and 339 mg/kg bw/day) in males and 1,500, 5,000, and 15,000 ppm (108, 358 and 1,107 mg/kg bw/day) in females for 28 days, with a 14-day recovery period for high dose (336 and 1098 mg/kg for males and females, respectively) and control animals. The average intake in males and females for the high dose and high dose recovery groups combined was 337 and 1,102 mg/kg bw/day, respectively.

The overall food consumption at 5,000 ppm in males and at 5,000 and 15,000 ppm in females was 7%, 14% and 11% lower (but not statistically significant) as compared to controls, respectively. These changes in food consumption were not considered to be adverse as there were no significant changes in body weight gain.

High-dose males showed statistically significantly higher mean liver weight and increased mean kidney weight. These effects were reversible after recovery. All high dose males and females presented with hepatocellular hypertrophy, which regressed after the recovery period. In females, heptatocellular hypertrophy was associated with increased triglycerides, cholesterol, high density lipoprotein, low density lipoprotein and gamma glutamyl transpeptidase levels.

Half of the high dose males displayed minimal to mild kidney basophilia and all high dose males presented with granular tubular casts and an increased presence of hyaline droplets. Following recovery, there was a reduced severity of kidney basophilia and no hyaline droplets were observed. However, granular casts were still present in nearly all high dose males.

The increased liver and kidney weight and the presence of hepatocellular hypertrophy, mild to minimal kidney basophilia and granular tubular casts were considered to be related to the test substance. The noted hepatic changes were seen to be adaptive. All renal changes were considered as not toxicologically relevant for humans. The decreases in combined uterus and cervix weight were considered to be incidental.

The No Observed Adverse Effect Level (NOAEL) for the notified chemical was established by the study authors as 337 mg/kg bw/day in males and 1,102 mg/kg bw/day in females (highest dose tested).

Mutagenicity/Genotoxicity
The notified chemical tested negative in a bacterial reverse mutation assay and in an *in vitro* mammalian cell chromosome aberration test in Chinese hamster lung (V79) cells.

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.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the toxicological information provided, the notified chemical is of low hazard presenting only as a slight eye irritant. The notified chemical has the potential to cross biological membranes; however, the toxicity of the notified chemical following repeated exposure is considered to be low.

Reformulation
The notifier anticipates that worker exposure will be limited through the use of engineering controls such as enclosed systems, automated processes and mechanical ventilation. The use of appropriate PPE (coveralls, imperious gloves and eye protection) will also be used to limit worker exposure.

End-Use
Workers involved in professions which involve cleaning or the application of cosmetic products containing the notified chemical to clients (*e.g.* beauty salon workers) may be exposed to the notified chemical at ≤ 1.25% concentration. Dermal, and to a lesser extent, ocular exposure may occur. PPE may be employed by workers to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the risk to such workers is expected to be of a similar or lesser extent than that for consumers using the various products containing the notified chemical.

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

6.3.2. Public Health
Members of the public will experience widespread and frequent exposure to the notified chemical at ≤ 1.25% concentration through daily use of cosmetic and household cleaning products. The main route of exposure is expected to be dermal, while ocular and inhalation exposure are also possible, particularly if products are applied by spray.

Based on the toxicological information provided, the notified chemical is of low hazard. Therefore, the risk to the public associated with use of the notified chemical at ≤ 1.25% concentration 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 at ≤ 10% concentration as a component of fragrance preparations for local reformulation into a variety of cosmetics and household products. Release during reformulation in Australia is expected to arise from spills (0.1%), formulation equipment cleaning (no release estimate as cleaning water is recycled) and residues in import containers (0.1%). Accidental spills during transport or reformulation are expected to be collected with inert material and disposed of to landfill. Import containers will either be recycled or disposed of through an approved waste management facility. Therefore, up to 0.2% (≡ 2 kg per annum) of the import volume is estimated to be released to landfill as a result of reformulation in Australia.

RELEASE OF CHEMICAL FROM USE

The notified chemical is expected to be released to sewers in domestic situations across Australia as a result of its use in cosmetic and household products, which are either washed off the hair and skin of consumers, or disposed of following cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL

It is estimated that a maximum of 3% of the consumer products containing the notified chemical will remain in end-use containers. These containers will be disposed of through domestic garbage disposal and will enter landfill or be recycled. The washings from the recycling process are expected to be sent to sewer.

7.1.2. Environmental Fate

Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system before potential release to surface waters on a nationwide basis. The notified chemical will enter the sewer system as a result of the use of the chemical as a fragrance ingredient in cosmetic and household products. The notified chemical is not readily biodegradable based on the provided test reports. For the details of the environmental fate studies refer to Appendix C. Based on the log Pow (4.35), there is an indication of the potential of the notified chemical to bioaccumulate, but the bioconcentration study demonstrated that this potential was low (bioconcentration factor of 210 L/kg).

The half-life of the notified chemical in air is calculated to be 11.7 hours based on reactions with hydroxyl radicals (AOPWIN v1.92; US EPA, 2011). The notified chemical is volatile (vapour pressure = 3.4 Pa) and will volatilise to the atmosphere, but it is not expected to persist in the atmospheric compartment.

Most of the notified chemical will be released to the sewer after use and directed to sewage treatment plants (STPs) nationwide. A small amount of the notified chemical may be sent to landfill as collected spills or container residues. In STPs, the majority of the notified chemical is expected to be removed from the water column via adsorption to sludge sediment given the hydrophobic structure and the measured log KOC of 3.87, and eventually be sent to landfill. In landfill or water, the notified chemical is expected to undergo biotic or abiotic degradation processes, forming water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated assuming a worst case scenario of 100% release of the notified chemical into sewer systems nationwide and no removal from 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)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.56	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 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.562 µg/L may potentially result in a soil concentration of approximately 0.0037 mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil (PEC_{soil}) in 5 and 10 years may be approximately 0.018 mg/kg (=18 µg/kg) and 0.036 mg/kg (= 36 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 = 2.48 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 2.5 mg/L	Toxic to invertebrates
Algal Toxicity	72 h E_rC50 = 4.1 mg/L	Toxic to algae
Earthworm	14 day LC50 = 373 mg/kg dry weight	Slightly toxic to earthworms
Inhibition of Bacterial Respiration	3 h IC50 > 1,000 mg/L	Not inhibitory to bacterial respiration

Based on the above acute ecotoxicological endpoints, the notified chemical is expected to be toxic to aquatic life. 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 2: Toxic to aquatic life". Based on its acute toxicity and lack of ready biodegradability, under the GHS the notified chemical is formally classified as "Chronic Category 2: Toxic to aquatic life with long lasting effects".

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)	2.48	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	24.8	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	0.56	24.8	0.022
Q - Ocean	0.056	24.8	0.0022

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 earthworm study report indicates that the notified chemical is expected to be slightly toxic to terrestrial organisms and a $PNEC_{soil}$ of 373 µg/kg is estimated using the endpoint for earthworms and an assessment factor of 1000. From this value, the worst case risk quotient for the terrestrial environment (PEC_{soil} ÷ $PNEC_{soil}$), is estimated as 0.1. The notified chemical is not readily biodegradable. The notified chemical has low potential to bioaccumulate as it is not expected to be significantly bioavailable in the aquatic environment due to its low water solubility. On the basis of the PEC/PNEC ratios calculated using the maximum annual importation volume and the assessed use pattern in cosmetic 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 38 °C

Method	OECD TG 102 Melting Point/Melting Range (1995) EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature (2008)
Remarks	Determined using differential scanning calorimetry. When the test substance was heated an initial endotherm was noted at approximately 21°C, prior to the melting point endotherm. This endotherm was representative of "enthalpy relaxation" (softening) of the test substance. The onset temperature of this endotherm was deemed by the authors of this study as the glass transition temperature.
Test Facility	Envigo (2016a)

Boiling Point 271.2 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point (1995) EC Council Regulation No 440/2008 A.2 Boiling Temperature (2008)
Remarks	Determined using differential scanning calorimetry.
Test Facility	Charles River (2017a)

Density 1,010 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids (2012) EC Council Regulation No 440/2008 A.3 Relative Density (2008)
Remarks	Determined using a gas comparison stereopycnometer.
Test Facility	Charles River (2017a)

Vapour Pressure 3.4 x 10^{-3} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure (2006) EC Council Regulation No 440/2008 A.4 Vapour Pressure (2008)
Remarks	Determined using a vapour pressure balance.
Test Facility	Envigo (2017a)

Water Solubility 8.38 × 10^{-3} g/L at 20 °C

Method	OECD TG 105 Water Solubility (1995) EC Council Regulation No 440/2008 A.6 Water Solubility (2008)
Remarks	Flask Method. The preliminary water solubility test indicated that the column elution method was the most suitable (solubility less than 1 × 10^{-2} g/L). However due to the physical nature of the test substance, it was not possible to use this method. The test substance was waxlike at ambient temperature and coated onto the glass beads in the column, causing the beads to adhere together to form a plug within the column. The standard and sample solutions were analysed by gas chromatography.
Test Facility	Envigo (2016a)

Partition Coefficient (n-octanol/water) log Pow = 4.35 at 30 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water) (2004). EC Council Regulation No 440/2008 A.8 Partition Coefficient (2008).
Remarks	HPLC Method. The determination was performed at an approximately neutral pH.
Test Facility	Envigo (2016a)

Adsorption/Desorption log K_{oc} = 3.87 at 35 °C

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) (2001) EC Council Regulation No 440/2008 C.19 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)

(2008)
Remarks The HPLC method using soil-adsorption-reference data was applied for the determination of the adsorption coefficient of the test substance.
Test Facility Charles River (2017a)

Surface Tension 70.6 mN/m at 20 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions (1995)
EC Council Regulation No 440/2008 A.5 Surface Tension (2008)
Remarks Concentration: ~ 90% saturated solution of the test substance in distilled water. Ring method was used to determine surface tension. As the surface tension of the test substance is > 60mN/m, the test substance was considered not to be surface active.
Test Facility Charles River (2017a)

Flash Point 117 °C

Method EC Council Regulation No 440/2008 A.9 Flash Point (2008)
Remarks Closed cup method.
Test Facility Charles River (2017a)

Pyrophoric Properties Not pyrophoric in contact with air

Method EC Council Regulation No 440/2008 A.13 Pyrophoric Properties of Solids and Liquids (2008)
Remarks Based on chemical structure.
Test Facility Charles River (2017a)

Autoignition Temperature 275 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases) (2008)
Test Facility Charles River (2017a)

Explosive Properties No explosive properties

Method EC Council Regulation No 440/2008 A.14 Explosive Properties (2008)
Remarks Based on chemical structure.
Test Facility Charles River (2017a)

Oxidizing Properties No oxidising properties

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids) (2008)
Remarks Based on chemical structure.
Test Facility Charles River (2017a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method (2001) EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Wistar (Rcc:Han)
Vehicle	Arachis Oil BP
Remarks - Method	No significant protocol deviations. In the absence of toxicity data, 300 mg/kg was chosen as the starting dose.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	No mortalities or clinical signs of toxicity were observed in the group treated at 300 mg/kg. Two animals treated at 2000 mg/kg were sacrificed for humane reasons at day 1 and day 2 after dosing. This was for humane reasons, as these animals displayed clinical signs of toxicity that exceeded the set regulatory severity limit on the days they were sacrificed. Clinical signs of toxicity displayed by both animals included ptosis, increased salivation, pilo-erection, hunched posture, diarrhoea, lethargy (one animal), laboured respiration and decreased respiratory rate. On day 1 after dosing, one animal treated at 2000 mg/kg displayed ptosis and tiptoe gait for up to one day, and hunched posture for up to two days. Another animal treated at this dose displayed hunched posture on day 1 after dosing, for up to one day.
Effects in Organs	No abnormalities were noted in animals that were sacrificed at the end of the study period. Both animals that were sacrificed during the study period displayed pale liver, haemorrhaged gastric mucosa and blood-filled bladder.
Remarks - Results	All surviving animals in all groups showed expected gains in body weight over the observation period. Based on the results of this study, the LD50 was estimated to be > 2000 mg/kg bw
CONCLUSION	The notified is of low acute toxicity via the oral route.
TEST FACILITY	Envigo (2016b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test (1987) EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal) – Limit Test
Species/Strain	Rat/Wistar (Rcc:Han)

Vehicle	Arachis Oil BP
Type of dressing	Semi-occlusive
Remarks - Method	No deviations from the study plan were noted.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	None observed during the study
Signs of Toxicity - Systemic	None observed during the study
Effects in Organs	None observed
Remarks - Results	Animals showed expected gains in body weight during the study, except for two females, which displayed weight loss during the first week but made expected weight gains during the second week.

CONCLUSION The notified chemical is of low acute toxicity via the dermal route.

TEST FACILITY Envigo (2016c)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 403 Acute Inhalation Toxicity (2009)
EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation)

Species/Strain	Rat Wistar (Rcc:Han)
Vehicle	Ethanol
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	2.94 µm
Remarks - Method	No significant protocol deviations were noted. The test substance was prepared as a 50:50 w/w solution with ethanol to improve its aerolisation properties. Prior to each use and during exposure, the formulation containing the test substance was warmed in a warming bath set at 80 °C to ensure that the formulation didn't solidify.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5F/5M	36.1	4.28	0/10

LC50	> 4.28 mg/L/4 hours
Signs of Toxicity	During exposure to the test substance, all animals presented with wet fur and decreased respiratory rate. In addition, 2 out of 10 animals displayed laboured respiration during the exposure period, which ceased when the animals were removed from the exposure chamber. One animal also displayed red/brown staining around the snout when removed from the exposure chamber. All animals continued to display wet fur and decreased respiratory rate up to one day after exposure to the test substance. Pilo-erection and noisy respiration was also noted in all animals up to one day after exposure. All animals presented with hunched posture for up to two days post exposure in addition to sneezing, which was observed only on

	the first day after exposure to the test substance. All signs of toxicity had regressed by day 2 post-treatment.
Effects in Organs	None observed
Remarks - Results	The authors of this study note that hunched posture pilo-erection and wet fur are commonly seen in animals for short period after removal from the exposure chamber following 4 hour inhalation studies. As such, these observations were considered to be due to the restraint procedure and not related to the test substance. The observations of red-brown staining around the snout, decreased respiratory rate, laboured respiration and sneezing are considered to be related to the test substance. All males and most female (4/5) showed body weight losses or showed no body weight gain on the first day after exposure to the test substance. During the remainder of the recovery period all animals presented expected body weight gains, with the exception of two female animals which exhibited body weight loss from days 1 to 3 after exposure.
CONCLUSION	The notified chemical is of low acute toxicity via inhalation.
TEST FACILITY	Envigo (2016d)

B.4. Corrosion– skin (*in vitro* EPIDERM™ Skin Corrosion Test)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 431 *In vitro* Skin Corrosion – Reconstructed Human Epidermis Test Method (2015) EC Council Regulation No 440/2008 B.40 BIS. *In vitro* Skin Corrosion - Human Skin Model Test
Vehicle	None
Remarks - Method	No significant deviations to the study plan were noted. Test system: EpiDerm Skin Model. The test substance (50 µL) was applied to the tissues in duplicate. Following exposure periods of 3 minutes (room temperature; test 1) and 1 hour (37 °C; test 2), the tissues were rinsed, treated with MTT and incubated (37 °C, 3 hours) to test cell viability. After extraction, optical densities were determined at 562 nm. A preliminary test had been conducted, which found that the test substance was able to directly reduce MTT. As such, an additional corrosion test using freeze-killed tissues (which do not possess metabolic activity but absorb and bind the test item like viable tissues) was performed in parallel with the main test. The results showed that the test item only produced negligible interference through direct reduction of MTT. Therefore, it was unnecessary to use the results of this additional test to correct the results obtained in the main test. Furthermore, an additional preliminary test found that the solution containing the test item did not have the potential to cause colour interference under the conditions of the main study. Positive and negative controls were run in parallel with the test substance: - Negative control (NC): Sterile distilled water - Positive control (PC): Potassium hydroxide (8M)

RESULTS

Test material	*Test 1 (3 minute exposure period)*		*Test 2 (1 hour exposure period)*	
	Mean OD_{562} of duplicate tissues	*Relative mean viability (%)*	*Mean OD_{562} of duplicate tissues*	*Relative mean viability (%)*
Negative control	1.467	100	1.781	100
Test substance	1.728	117.8	2.153	120.9
Positive control	0.061	4.1	0.067	3.8

OD = optical density

Remarks - Results: The positive and negative controls performed as expected and variation between tissue replicates was within acceptable range, confirming the validity of the test system.

CONCLUSION: The viability of the notified chemical was ≥ 50% after 3 minutes exposure and ≥ 15% after 1 hour exposure, indicating that the notified chemical is non-corrosive to the skin under the conditions of the test.

TEST FACILITY: Envigo (2016e)

B.5. Irritation – skin

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion (2015)
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)

Species/Strain: Rabbit/New Zealand White
Number of Animals: 2
Vehicle: None.
Observation Period: 72 hours
Type of Dressing: Occlusive
Remarks - Method: The study authors note that the test substance appeared as a white solid. For the purpose of the study, the test substance was ground to a powder. At the test site, 0.5g of the ground "test item, moistened sufficiently with 0.5mL of distilled water" was applied on the test site of each animal.

Remarks - Results: No evidence of skin irritation or oedema was noted in any of the animals at any of the observational time points in this study. The primary irritation index was zero. Both animals showed expected gains in body weight during the study.

CONCLUSION: The notified chemical is non-irritating to the skin.

TEST FACILITY: Envigo (2016f)

B.6. Irritation – eye (*in vitro* Bovine Corneal Opacity and Permeability Test)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage (2013)
EC Council Regulation No 440/2008 B.47 Bovine Corneal Opacity And Permeability Test Method For Identifying Ocular Corrosives And Severe Irritants

Vehicle: None
Remarks - Method: No significant protocol deviations.

The test item was heated to 45 °C prior to use.
Corneas were exposed to the test substance for 10 minutes at 32 °C. Saline

(0.9% w/v) was used as a negative control and ethanol (> 99%) was used as a positive control.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Negative control	1.3	0.023	1.7
*Test substance**	0.0	0.006	0.1
*Positive control**	26.0	0.612	35.2

IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks - Results

The positive and negative controls performed as expected, confirming the validity of the test system.

Corneas treated with the test substance or negative control appeared clear after treatment.

The test substance produced an IVIS of 0.1. Under the OECD TG 437 no GHS hazard classification is required for IVIS ≤ 3.

CONCLUSION

The notified chemical did not require hazard classification for eye irritation or serious eye damage under the conditions of the test.

TEST FACILITY

Envigo (2016g)

B.7. Irritation – eye

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion (2012)
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation)

Species/Strain: Rabbit/New Zealand White
Number of Animals: 2
Observation Period: 72 hours
Remarks - Method: No significant protocol deviations. The test substance was ground to a powder and approximately 90mg was applied to each animal.

RESULTS

Lesion	*Mean Score** *Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: redness	0.67	0.33	2	< 72 hours	0
Conjunctiva: chemosis	0.33	0.33	1	< 48 hours	0
Conjunctiva: discharge	0	0	2	< 24 hours	0
Corneal opacity	0	0	0	-	0
Iridial inflammation	0	0	0	-	0

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

Remarks - Results

At one hour after treatment with the test substance, both animals displayed diffuse, deep-red coloured conjunctiva. This had regressed to a slight red colour at the 24 and 48 hour time points. Slight swelling of the conjunctiva was noted in both animals up to 72 hours after treatment. At the one hour timepoint, the animals presented either moderate or slight conjunctival discharge. All signs of irritation were resolved at the 72 hour observation.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY Envigo (2016h)

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

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2010)
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain	Mouse/CBA (Ca Ola Hsd)
Vehicle	Acetone:olive oil (4:1)
Preliminary study	Yes
Positive control	25% v/v dilution of α-Hexylcinnamaldehyde (85% purity) in acetone:olive oil mixture (4:1).
Remarks - Method	No significant deviations from the study protocol or test guideline were noted. A preliminary screening test using the test substance at 50% concentration was conducted to determine dose concentrations for the main study. Based on these results, 50% was chosen as the high dose for the main study as it was not expected to induce any systemic toxicity, a 25% or more increase in ear thickness or moderate to severe erythema.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (mean DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5F	746.81	1.00
10	5F	732.46	0.98
25	5F	1399.63	1.87
50	5F	817.64	1.09
Positive Control			
25	5F	6214.93	8.32

Remarks - Results No mortalities and no signs of systemic toxicity were noted in the test or control animals during the study. No signs of irritation were observed in any treatment group.

The positive control performed as expected confirming the validity of the study. Body weight changes of the treated animals were comparable to that seen in the vehicle control animals.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical at up to 50% concentration.

TEST FACILITY Envigo (2016i)

B.9. Skin sensitisation – human volunteers

TEST SUBSTANCE Notified chemical (25% w/w)

METHOD Repeated insult patch test with challenge

Study Design Induction Procedure: Patches containing 0.15 mL test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed by the applicants after 24 hours and graded after an additional 24 h (or 48 h for patches applied on Friday).

Rest Period: 10 - 21 days
Challenge Procedure: Patches were applied to previously untreated test sites. Patches were removed by a laboratory technician after 24 h. Sites

	were graded immediately following, and 48 h and 72h post-patch removal. Subjects not present at the 48 h or 72 h time points were asked to return for a grading at 96 h post-patch removal.
Study Group	83 F, 32 M; age range 18 - 70 years
Vehicle	Alcohol (19% w/w) and diethyl phthalate (56% w/w) was combined with the notified chemical to produce a formulation of the notified chemical at a 25% w/w concentration.
Remarks - Method	Occluded patches were used. The vehicle control was a formulation consisting of distilled water (6% w/w), alcohol (23.5% w/w) and diethyl phthalate (70.5% w/w). The test substance or vehicle control was spread on a 3.63 cm × 3.63 cm patch. The test substance or vehicle control was allowed to evaporate on the patch for at least 30 mins, but no longer than 90 mins, prior to patch application.

RESULTS

Remarks - Results	103/115 subjects completed the study. The remaining subjects discontinued their participation for reasons unrelated to the application of the test material. No visible skin reaction was observed on any of the subjects during the induction or challenge phases.

CONCLUSION	The test substance was non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2016)

B.10. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (2008)
Species/Strain	Rat/Sprague Dawley
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	None
Remarks - Method	No significant protocol deviations Dose levels were selected based on results of a 14-day dose-range finding study performed previously (Syngene Study No. U-17021) using dose levels of 1,000, 5,000 and 15,000 ppm in males and females. Hepatocellular hypertrophy was observed at ≥ 5,000 ppm in males and at 15,000 ppm in females, which is considered adaptive and non-adverse. Kidneys in male rats at 15,000 ppm showed increased hyaline droplets in the proximal tubules, tubular basophilia, single cell necrosis in the tubular epithelium and presence of granular cast in the tubules. Similar changes were observed at 5,000 and 1,000 ppm dose levels, with decreased severity and incidence. These changes in male rats were considered as species and sex specific. Based on these results the highest concentration of 5,000 ppm in males and 15,000 ppm in females was selected.

RESULTS

Group	*Number and Sex of Animals*	*Dose ppm(mg/kg bw/day)*	*Mortality*
control	6M/6F	0	0/12
control recovery	6M/6F	0	0/12
low dose	6M/6F	500/1,500 (34/108)	0/12

mid dose	6M/6F	1,500/5,000 (104/358)	0/12
high dose*	6M/6F	5,000/15,000 (339/1107)	0/12
high dose recovery*	6M/6F	5,000/15,000 (336/1098)	0/12

* The average intake in males and females for the high dose and high dose recovery groups combined was 337 and 1,102 mg/kg bw/day, respectively.

Mortality and Time to Death
No unscheduled deaths occurred.

Clinical Observations
No clinical signs or toxicologically significant changes were noted in the clinical appearance and functional observations for all animals in this study.

No noticeable or significant differences in average body weight and average body weight change for main and recovery group males and females were observed. The overall food consumption at 5,000 ppm in males and at 5,000 and 15,000 ppm in females was 7, 14 and 11% lower (but not statistically significant) as compared to controls, respectively. These changes in food consumption were test substance-related. However, they were not considered to be adverse as they were not accompanied by significant changes in body weight parameters.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No changes in haematological and urine parameters were noted in treated males and females compared to controls. Treated males did not present any clinical chemistry changes.

Females treated at 15,000 ppm displayed statistically significantly higher triglyceride, total cholesterol, high density lipoprotein and low density lipoprotein concentrations and gamma glutamyl transpeptidase (GGT) activity (increases of 103% 35%, 26%, 64% and 29% respectively) compared with the control group. All changes were reversed at the end of the recovery period. The increase in GGT was considered to be test substance related but not adverse due to no significant increases in alanine aminotransferase and alkaline phosphatase, or adverse microscopic changes in the liver" (see 'effects in organs' below for more details). The increases in lipid species were considered to be an adaptive effect.

Recovery group females treated at 15,000 ppm showed a statistically significant 38% increase in neutrophil count compared to recovery controls. Recovery group males treated at 5,000 ppm presented statistically significantly lower red blood cell count and glucose concentration (decreases of 8% and 6% respectively), compared with the control group and statistically significantly higher blood urea nitrogen and urea concentrations and alanine aminotransferase activity (increases of 16%, 16% and 33% respectively, compared with the control group).

Effects in Organs
Males treated at 5,000 ppm showed a statistically significantly higher absolute mean liver weight and a noticeable increase in kidney weight compared to controls (increases of 21% and 18%, respectively). These effects were reversible in recovery males treated at 5,000 ppm.

When corrected for body weight, females treated at 5,000 ppm and 15,000 ppm showed statistically significant dose-dependent increases in liver weight (23% and 41%, respectively) and dose-dependent decreases in combined uterus and cervix weight (14% and 25%, respectively), compared to controls.

The changes in liver weights for all males and females treated at 5,000 ppm and 15,000 ppm respectively, were accompanied by the presence of hepatocellular hypertrophy, which regressed after the recovery period. Changes in kidney weight were accompanied with minimal to mild kidney basophilia in half of the males treated at 5,000 ppm. All males treated at this dose also presented with granular tubular casts and an increased presence of hyaline droplets. Following recovery, there was a reduced severity of kidney basophilia with all males treated at 5,000 ppm presenting with mild basophilia. No males presented with hyaline droplets at the end of recovery. Nearly all males in this group however, continued to display granular casts following recovery.

The increases in liver weight (absolute and relative) and the presence of hepatocellular hypertrophy in male and female animals were regarded as related to the test item but were considered to be adaptive and non-adverse. All renal changes were considered as species and sex specific and hence not toxicologically relevant for humans. The observed decreases in combined uterus and cervix weight in females treated at 5,000 ppm and 15,000 ppm were considered to be incidental due to biological variance and hence not toxicologically relevant.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 5,000 ppm (equivalent to 337 mg/kg bw/day) for males and 15,000 ppm for females (equivalent to 1,102 mg/kg bw/day) in this study.

TEST FACILITY Syngene (2017)

B.11. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1997)
Plate incorporation procedure
Species/Strain *Salmonella typhimurium*: TA1535, TA1537, TA98, TA100
Escherichia coli: WP2uvrA
Metabolic Activation System S9 fraction from Aroclor 1254 induced rat liver.
Concentration Range in Main Test
Test 1
a) With metabolic activation: 1.5 – 1500 µg/plate
b) Without metabolic activation: 1.5 – 1500 µg/plate

Test 2
a) With metabolic activation: 15 – 1500 µg/plate
b) Without metabolic activation: 15 – 1500 µg/plate

Vehicle DMSO
Remarks - Method No significant deviations from the study plan. A preliminary experiment was conducted to determine the dose range for the main test.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 1500	-	≥ 5000	Negative
Test 2	-	≥ 5000	≥ 5000	Negative
Present				
Test 1	≥ 500	-	≥ 5000	Negative
Test 2	-	≥ 1500	≥ 5000	Negative

Remarks - Results No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level, in the presence or absence of metabolic activation. Vehicle and positive controls performed as expected, confirming the validity of the test system.

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

TEST FACILITY BioReliance (2016a)

B.12. Genotoxicity – *in vitro* mammalian chromosome aberration

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (2014)
Species/Strain Hamster/Chinese
Cell Type/Cell Line Lung/V79
Metabolic Activation System S9 fraction from Aroclor 1254 induced rat liver.
Vehicle DMSO
Remarks - Method A preliminary experiment was conducted to determine the dose range for

the main test. No metaphase analysis was conducted on the cells used in this experiment. Historical control data for the vehicle and positive controls was not available for the cell line used in this study. To determine if the validity of this study was impacted by this, a small non-GLP trial was conducted comparing the endpoint of the cell line in this study with Chinese Hamster Ovary cells. The study indicated that the endpoints between the two cell lines had comparable values. As such, it was concluded that this lack of historical data had no impact on the validity o the study.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	5, 10*, 20*, 30, 35*, 40, 45, 50, 55, 60	4h	20h
Test 2	0.5, 1, 2.5, 5*, 10, 20*, 30, 35*, 40, 45, 50	20h	20h
Present			
Test 1	20*, 40*, 60, 80, 100*, 120, 140, 160, 180, 200	4h	20h
Test 2	-	-	-

*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	≥ 20	≥ 40	> 60	Negative
Test 2	≥ 20	≥ 45	> 50	Negative
Present				
Test 1	≥ 60	≥ 140	≥ 80	Negative
Test 2	-	-	-	-

Remarks - Results

In the presence and absence of metabolic activation, there were no biologically relevant increases in structural chromosomal aberrations after treatment with the test substance.

In the presence and absence of metabolic activation, there were no biologically relevant increases in polypoid cells after treatment with the test substance.

The solvent and positive controls performed as expected, confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic to Chinese hamster lung (V79) cells treated *in vitro* under the conditions of the test.

TEST FACILITY

BioReliance (2016b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 310 Ready Biodegradability; CO2 in Sealed Vessels (CO_2 Headspace Test)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	Acetone
Analytical Monitoring	CO2 production in the vessels was determined by measuring the increase in the concentration of Inorganic Carbon (IC) in the headspace.
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. The test substance was heated to 45 °C prior to weighing. A nominal amount of test substance (1036 mg) was dissolved in 10 mL of acetone to give a 1036 mg/10 mL solvent stock solution. An aliquot (25 µL) on filter paper was added to inoculated mineral medium to give a final concentration of 24.2 mg/L, equivalent to 20 mg carbon/L.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	67
14	0	14	70
21	0	21	73
28	0	28	84

Remarks - Results	The mean total inorganic carbon (TIC) in the inoculum control vessels on Day 28 was 1.05 mg/L, equivalent to 5% of the organic carbon added initially as test item to the test vessels, proving the validity of the test. The reference compound reached the threshold of 67% by day 7, indicating the suitability of the inoculums. The toxicity control attained 39% degradation by day 14 of the study thereby confirming that the notified chemical was not toxic to the sewage treatment micro-organisms used in the study. The notified chemical attained 0% degradation after 28 days and therefore cannot be considered as readily biodegradable under the conditions of OECD Guideline 310.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Envigo (2016j)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301C Ready Biodegradability; Modified MITI Test (I) (1992)

Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biological Oxygen Demand (BOD)
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0.67	7	76
14	0.67	14	99
21	0.00	21	100
28	-1.00	28	99

Remarks - Results	The reference compound reached the 10 day window threshold and biodegradability pass level, demonstrating the suitability of the inoculums. The maximum difference between replicate values of percentage biodegradation was 2% and the oxygen uptake in the control blank was 32 mg/L. Therefore, the test was valid. The notified chemical attained < 0% degradation after 28 days and, therefore, cannot be considered as readily biodegradable under the conditions of OECD Guideline 301C MITI Test (I).
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	CERI (2017)

C.1.3. Inherent biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302C Inherent Biodegradability; Modified MITI Test (II) (2009)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical Oxygen Demand (BOD)
Remarks – Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	-10.1	7	76.7
14	-11.0	14	80.4
21	-5.1	21	81.7
28	-3.5	28	82.8

Remarks – Results	The reference compound reached the threshold of 76.7% by day 7 and 80.4% by day 14 indicating the suitability of the inoculums. The recovery of the test substance in the inoculum blank was 50%. Therefore, the test was valid.

	The notified chemical attained < 0% degradation after 28 days and therefore, cannot be considered as readily biodegradable under the conditions of OECD Guideline 302C MITI Test (II)
CONCLUSION	The notified chemical is not inherently biodegradable.
TEST FACILITY	CTI (2017a)

C.1.4. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	"Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body" stipulated in the "Testing Methods for New Chemical Substances": Flow-through Fish Test (2011).
Species	*Cyprinus carpio* (Common carp)
Exposure Period	Exposure: 28 days
Auxiliary Solvent	DMSO and Hydrogenated castor oil (HC0-40)
Concentration Range	Nominal: High exposure level (Level 1) 40 µg/L Low exposure level (Level 2) 4 µg/L
Analytical Monitoring	GC-MS
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. Based on the results of the range finding test, the test was conducted at nominal concentrations of 40 µg and 4 µg notified chemical/L. No significant deviations to the test protocol were reported. Level 1 - The required nominal concentrations of the test substance were prepared from a stock solution (200 mg/L, 20 g/L co-solvent HCO-40 in DMSO). Level 2 - The required nominal concentrations of the test substance were prepared from a stock solution (200 mg/L, 20 g/L co-solvent HCO-40 in DMSO). Control - 20 g/L HCO-40 in DMSO.
RESULTS	
Bioconcentration Factor (Steady state)	Level 1 = 210 L/kg Level 2 = 210 L/kg
Remarks - Results	The validity criteria for the test were met. The water temperature was 25 ± 2°C. The concentration of dissolved oxygen did not fall below 60% of the saturated concentration (8.1 mg/L at 25°C). The concentration of the test substance in the test tank was maintained within ± 20% of the mean of the measured values during the uptake phase. The mortality or other adverse effects/disease in both control and test group was less than 10% at the end of the test. The average test substance concentration in test fish made from samples taken at 14, 20, 23 and 28 days were within ± 20% of each other for all peaks. Therefore, it was confirmed that a steady-state was reached in the uptake phase. Concentrations of the test substance were maintained at ≥ 91 % of nominal concentrations. Variation between replicates was within ± 20% of the average measured concentrations. Concentrations of the test substance in test water before the uptake phase were 40.4 µg/L (Level 1) and 3.87 µg/L (Level 2).

CONCLUSION	Under the conditions of this test, the notified chemical is not considered to be bioaccumulative.
TEST FACILITY	CERI (2018)

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 (1992)
Species	Rare minnow (*Gobiocypris rarus*)
Exposure Period	120 hours
Auxiliary Solvent	None
Water Hardness	160 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography
Remarks – Method	One day before the exposure, 1.0002, 1.0005 and 1.0003 g of test substance were respectively weighed and added to 10 L of test water. The test solutions were ultrasonicated for 40 min and stirred for 24 hours. After standing for 2 hours, the middle layer of the above three sets of solution with nominal concentration of 100 mg/L were drawn, combined, mixed thoroughly and filtered to obtain the stock solution (water accommodated fraction). The definitive test was conducted at nominal test substance concentrations of 1.40, 1.82, 2.37, 3.08 and 4.00 mg/L. Trimmed Spearman-Karber Method (TSK) VERSION 1.5 was used to calculate the LC50 and its 95% confidence limits. No significant protocol deviations were reported.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Time-Weighted Mean Measured*		*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	10	0	0	0	0
1.4	1.19	10	0	0	0	0
1.82	1.54	10	0	0	0	0
2.37	2.13	10	0	1	1	2
3.08	2.76	10	0	0	2	7
4.00	3.65	10	0	9	10	10

LC50	2.48 mg/L (95% CI 2.23-2.76 mg/L) mg/L at 96 hours
NOEC	Not determined
Remarks – Results	All validity criteria for the test were satisfied. During the test, the concentration of dissolved oxygen was 67.9-98.1%. The concentration of test solutions could not be maintained in the 80-120% range of the nominal concentration during the test. Therefore, the test result was calculated as based on time-weighted mean measured concentrations. After 48 hours of exposure, the measured concentrations of test solutions were 64%-69% of the initial measured concentrations. Therefore, additional test solution renewal was performed at 72 hours of exposure and the concentration of test substance was also measured before and after the test solution renewal. The 96 h LC50 for fish was determined to be 2.48 mg/L, as based on time-weighted mean measured concentrations. In the definitive test, the temperature of test solution was 22.00-24.32 °C. This temperature variation deviated from the study plan as it was not within a range of 2 °C. This deviation did not affect the test result.

CONCLUSION Under the study conditions, the notified chemical is considered to be toxic to fish.

TEST FACILITY CTI (2017b)

C.2.2. Fish Embryo Acute toxicity (FET) Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 236 Fish embryo, Acute Toxicity (FET) Test – Semi-static (2013)
Species Fathead minnow *(Pimephales promelas)*
Exposure Period 120 hours
Auxiliary Solvent None
Water Hardness 140 - 144 mg $CaCO_3$/L
Analytical Monitoring Gas Chromatography/Mass Spectrometry (GC/MS)
Remarks - Method A primary stock solution at 10 mg/mL was prepared in HPLC-grade dimethylformamide (DMF); the solution was inverted to mix and appeared clear and colourless. Additional secondary stock solutions at 0.63, 1.3, 2.5 and 5.0 mg/L were prepared from the primary stock solution in DMF. The solutions were inverted to mix and appeared clear and colourless. Test solutions were prepared at nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg/L.

New test solutions were prepared daily and were added to each well at renewal approximately 24-hours interval after the old test solutions were removed. A small amount of old solutions was left in each well to ensure that embryos remain covered with old test solutions. The internal and negative control solutions were dilution water only.

Test solutions of each treatment, negative control and solvent control group were added to 20 wells of each well plate assigned to group for a total of 20 replicates per group. Dilution water was added to the remaining 4 wells in each plate to serve as an internal control.

RESULTS

Time-Weighted Mean Measured Concentration (mg/L)	*Number of Observations[1] in 24-Hour Period / Number Embryos Originally Exposed*									
	72 hours					96 hours				
	CE	SF	TD	HB	Hatch	CE	SF	TD	HB	Hatch
Negative control	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	5/20
Solvent control	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	12/20
Internal control	0/28	0/28	0/28	0/28	2/28	0/28	0/28	0/28	0/28	10/28
0.028	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	11/20
0.043	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	13/20
0.071	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	8/20
0.12	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	5/20
0.24	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	3/20

[1]CE = coagulation of the embryo; SF = absence of somite formation; TD = absence of tail detachment; HB = absence of heart beat; Hatch = embryo has hatched.

LC50 > 0.24 mg/L at 96 hours
NOEC (or LOEC) ≥ 0.24 mg/L
Remarks - Results All validity criteria for the test were met. The overall fertilization rate of all eggs collected was 90%. The water temperature was maintained at 25 ± 1ºC in test chambers throughout the test. Overall survival of embryos in the negative control and solvent control were 95% and 100%, respectively. Hatching rate in the negative control and solvent control were 95% and 100%, respectively. The dissolved oxygen concentration in the negative control and highest test concentration were ≥86% throughout the test.

CONCLUSION	Under the study conditions, the notified chemical is not considered to be toxic to fish embryo.
TEST FACILITY	EAG (2016)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Semi Static (2004) EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia – Semi Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	GC/FID
Remarks - Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported. Test substance (at a nominal concentration of 100 mg/L) was stirred in test water for 48 hours. Any undissolved test substance was removed by filtration (0.2 µm filter) to make a 100% w/v saturated solution. Serial dilution from this saturated solution produced test concentrations of 56, 32, 18 and 10% w/v saturated solution.

RESULTS

Geometric Mean Measured Concentration mg/L	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
Control	20	0	0
0.44	20	0	0
0.77	20	0	0
1.20	20	0	0
2.10	20	1	1
4.20	20	20	20

EC50	2.5 mg/L at 48 hours
NOEC	2.1 mg/L
Remarks - Results	The validity criteria for the test were met. The oxygen concentration at the end of the test was ≥ 3 mg/L in the control and test vessels. The EC50 values at 24 and 48 hours and the slope of the response curve and its standard error were calculated by Probit analysis using Linear Maximum-Likelihood regression. This is not regarded as standard methodology when there is only one partial response for the concentrations tested. However, the result is regarded as a reasonable estimate. Chemical analysis of the freshly prepared test samples at 0 and 24 hours showed measured test concentrations of 0.52 - 4.8 mg/L and nominal concentrations of 0.53 - 5.4 mg. A decline of 0.37 - 3.5 mg/L in measured test concentrations was observed in the old or expired media at 24 and 48 hours. Given this decline, it was considered appropriate to calculate the results based on the geometric mean measured test concentrations.

CONCLUSION	The notified chemical is toxic to aquatic invertebrates
TEST FACILITY	Envigo (2016k)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test (2006) EC Council Regulation No 761/2009 C.3 Algal Inhibition Test.
Species	*Pseudokirchneriella subcapitata* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 3.2, 10, 32, 100% v/v saturated solution Actual: 0.04, 0.15, 0.37, 1.4, 4.0 mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography
Remarks - Method	After a range finding test, a definitive test was conducted in accordance with the guidelines above and in compliance with GLP standards and principles. No significant deviations to the test protocol were reported. The test substance (nominal concentration 100 mg/L) was stirred for 48 hours in test water. Any undissolved test substance was removed by filtration (0.2 µm filter) to give a 100% w/v saturated solution. This saturated solution was serially diluted to make solutions of 32, 10, 3.2 and 1.0% w/v concentration. An aliquot (900 mL) of each solution was separately inoculated with 8.0 mL of algal suspension to give the required test concentrations of 1.0, 3.2, 10, 32 and 100% w/v saturated solution.

RESULTS

Biomass		*Growth*	
EyC50 *mg/L at 72 h*	*NOEC* *mg/L*	*ErC50* *mg/L at 72 h*	*NOEC* *mg/L*
1.9	1.4	4.1	1.4

Remarks - Results	The validity criteria for the test were met. The cell concentration of the control cultures increased by a factor of 137 over the test period. The mean of the coefficients of variation of the section by section daily growth rates in the control cultures during the course of the test was 11%. The coefficient of variation of the average specific growth rate in replicate control was 1%. Analysis of the sample taken from the solution prepared at 0 hours showed measured test concentrations of 0.066 - 6.8 mg/L. Declines in measured concentrations were 0.056 -5.6 mg/L at 24 hours, 0.045 - 4.5 mg/L at 48 hours and less than the limit of quantification (LOQ), determined to be 0.0034 - 0.098 mg/L at 72 hours. Given this decline in measured concentrations, it was considered appropriate to calculate the results based on the geometric mean measured test concentrations.
CONCLUSION	The notified chemical is toxic to algae.
TEST FACILITY	Envigo (2016l)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test (Carbon and

	Ammonium Oxidation) (2010)
Inoculum	Aerated activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 320, 560 and 1000 mg/L Actual: Not determined
Remarks – Method	3,5-dichlorophenol was used as the reference control. The respiration rate was determined by measurement of biological oxygen demand during the test after following exposure to the test substance. No significant deviations in protocol were reported.
RESULTS	
EC50	> 1000 mg/L at 3 hours
NOEC	1000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The 3h EC50 was therefore determined to be >1000 mg/L, based on nominal concentrations.
CONCLUSION	The notified chemical is not inhibitory to microbial activity.
TEST FACILITY	Envigo (2017b)

C.2.6. Acute toxicity to earthworms

TEST SUBSTANCE	Notified chemical (98%).
METHOD	OECD TG 207 Earthworm, Acute Toxicity Tests (1984)
SPECIES	*Eisenia foetida* (earthworm)
Remarks - Method	The test involved acute exposure of test species in artificial soil with 10% sphagnum peat, 20% kaolin clay and 70% quartz sand. Based on the results of the preliminary range-finding test, nominal concentrations for the definitive test were 100, 145, 210, 30 and 450 mg/kg (dry weight). The reference substance used to determine the sensitivity of the test was chloracetamide. A control group was run concurrently with the test substance. There were 4 replicates per test treatment and control group, and 10 earthworms in each vessel. Mortality was recorded after 7 and 14 days of exposure. The earthworms were kept under continuous light and the test system was maintained at a temperature of 20 °C ± 2 °C, a soil moisture content of 32 - 35%, and a soil pH range of 6.29 – 6.43. Trimmed Spearman-Karber Version 1.5 was used to calculated LC50 and 95 % confidence limits.
RESULTS	
14 day LC50	373 mg/kg dry weight
Remarks - Results	The mortality of the blank control and solvent control was 0% at the end of the test, confirming the validity of the test. The wet weights of the earthworms were 381 mg - 597 mg at the start of the test, which was within the recommended weight range. At the end of the test, the wet weights of the earthworms were 270 mg - 500 mg. The 14-day LC50 (95% confidence limits) of the notified chemical was 373 (356 – 391) mg/kg dry weight.
CONCLUSION	The notified chemical is slightly toxic to earthworms.
TEST FACILITY	CTI (2017c)

BIBLIOGRAPHY

ACI (2010) Consumer Product Ingredient Safety, Exposure and Risk Screening Methods for Consumer Product Ingredients, 2nd Edition, American Institute, Washington DC.

BioReliance (2016a) Bacterial Reverse Mutation Assay (Study No. AE15LG.503.BTL, October, 2016) Maryland, U.S.A., BioReliance Corporation (Unpublished report submitted by the notifier).

BioReliance (2016b) *In Vitro* Mammalian Chromosomal Abberation Assay in Chinese Hamster Lung (CHL) or V79 Cells (Study No. AE15LG.371.BTL, October, 2016) Maryland, U.S.A., BioReliance Corporation (Unpublished report submitted by the notifier).

Cadby, P.A., Troy, W.R., Vey, M.G.; Consumer Exposure to Fragrance Ingredients: Providing Estimates for Safety Evaluation, Regulatory Toxicology and Pharmacology 36 (2002) 246-252.

CERI (2017) Biodegradation Study of ES421 Pinyl Nitrile (Study No. 16426, October, 2017). Kurume, Japan, Chemicals Evaluation and Research Institute. (Unpublished report submitted by the notifier).

CERI (2018) Bioconcentration study of ES421 Pinyl Nitrile in Common Carp (Study No. 46204, January, 2018). Kurume, Japan, Chemicals Evaluation and Research Institute. (Unpublished report submitted by the notifier).

Charles River (2017a) Determination of the Physico-Chemical Properties of ES421 Pinyl Nitrile (Study No. 517232, July, 2017). 's-Hertogenbosch, The Netherlands, Charles River Laboratories Den Bosch B.V. (Unpublished report submitted by the notifier).

CRL (2016) Repeated Insult Patch Test (RIPT) – Shelanski Method (Study No. CRL2016-0626, October, 2016). New Jersey, USA, Clinical Research Laboratories LLC (Unpublished report submitted by the notifier).

CTI (2017a) Inherent Biodegradability Test of Pinyl Nitrile (302C Modified MITI Test (II)) (Study No. G16302001A, July, 2017). Suzhou, China, Centre Testing International-Safety Evaluation Technical Service Co. (Unpublished report submitted by the notifier).

CTI (2017b) Pinyl Nitrile Fish Acute Toxicity Test (Rare Minnow) (Study No. G16203020A, March, 2017). Suzhou, China, Centre Testing International-Safety Evaluation Technical Services Co. (Unpublished report submitted by the notifier).

CTI (2017c) Pinyl Nitrile Earthworm Acute Toxicity Test (Study No. G16207011A, March, 2017). Suzhou, China, Centre Testing International -Safety Evaluation Technical Services Co. (Unpublished report submitted by the notifier).

EAG (2016) Pinyl Nitrile: A Static-Renewal Fish Embryo Acute Toxicity (FET) Test with the Fathead Minnow (*Pimephales promelas*) (Study No. 549A-102, November, 2016). Maryland, USA, EAG Laboratories (Unpublished report submitted by the notifier).

Earnest, C.W., Jr. (2009) A Two-Zone Model to Predict Inhalation Exposure to Toxic Chemicals in Cleaning Products, MSCEng thesis, The University of Texas at Austin

enHealth (2012) Australian Exposure Factor Guide, companion document to: Environmental Health Risk Assessment: Guidelines for assessing human health risks from environmental hazards, EnHealth, Commonwealth of Australia.

Envigo (2016a) ES421 Pinyl Nitrile: Determination of General Physico-Chemical Properties (Study No. YF21WX, June, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016b) ES421 Pinyl Nitrile: Acute Oral Toxicity in the Rat – Acute Toxic Class Method (Study No. PM77PB, June, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016c) ES421 Pinyl Nitrile: Acute Dermal Toxicity (Limit Test) in the Rat (Study No. NM52TF, June, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016d) ES421 Pinyl Nitrile: Acute Inhalation Toxicity (Nose Only) Study in the Rat (Study No. RR42HY, June, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016e) ES421 Pinyl Nitrile: *In Vitro* EPIDERM™ Skin Corrosion Test (Study No. KW91WW, May, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016f) ES421 Pinyl Nitrile: Acute Dermal Irritation in the Rabbit (Study No. YL63QK, September, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016g) ES421 Pinyl Nitrile: The Bovine Corneal Opacity and Permeability (BCOP) Assay (Study No. SS74YC, June, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016h) ES421 Pinyl Nitrile: Acute Eye Irritation in the Rabbit (Study No. RK90WC, September, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016i) ES421 Pinyl Nitrile: Local Lymph Node Assay in the Mouse – Individual Method (Study No. RY47VY, June, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016j) ES421 Pinyl Nitrile: Daphnia sp., 48-Hour Acute Immobilization Test (Study No. KT58VV, October, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016k) ES421Pinyl Nitrile: Assessment of Ready Biodegradability; CO_2 in Sealed Vessels (CO_2 Headspace Test) (Study No. DJ44TH, August, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016l) ES421 Pinyl Nitrile: Algal Growth Inhibition Test (Study No. LP44DX, August, 2016). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2017a) 1st Re-Issued Report ES421 Pinyl Nitrile: Determination of Vapour Pressure (Study No. XR08NG, January, 2017). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2017b) ES421 Pinyl Nitrile: Toxicity to Activated Sludge in a Respiration Inhibition Test (Study No. ND22HL, April, 2017). Derbyshire, UK, Envigo Research Limited (Unpublished report submitted by the notifier).

Loretz, L., Api, A.M., Barraj, L., Burdick, J. Davis, D.A., Dressler, W., Gilberti, E., Jarrett, G., Mann, S., Pan, Y.H.L., Re, T., Renskers, K., Scrafford, C., Vater, S. (2006) Exposure data for personal care products : Hairspray, spray perfume, liquid foundation, shampoo, body wash, and solid antiperspirant, Food and Chemcial Toxicology 44 (2006) 2008-2018.

Rothe, H., Fautz, R., Gerber, E., Neumann, L., Rettinger, K., Schuh, W., Gronewold, C (2006) Special aspects of cosmetic spray evaluations: Principles on inhalation risk assessment, Toxicology Letters 205 (2011) 97-104.

SCCS (2012) Notes of Guidance for testing of Cosmetic Ingredients and Their Safety Evaluation (8th revision). European Commission - Scientific Committee on Consumer Safety.

Steiling, W., Bascompta, M., Carthew, P., Catalano, G., Corea, N., D'Haese, A., Jackson, P., Kromidas, L., Meurice, P., Rothe, H., Singal, M. Principle considerations for the risk assessment of sprayed consumer products, Toxicology Letters 227 (2014) 41-49.

Syngene (2017) ES421 Pinyl Nitrile: 28-Day Oral Dietary Toxicity Study with 14-Day Recovery Period in Sprague Dawley Rats (Study No. U-17043, October, 2017). Bangalore, India, Syngene International Limited (Unpublished report submitted by the notifier).

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >