File No: LTD/2036 and LTD/2037

August 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

1-Cyclohexene-1-propanoic acid, ethyl ester (LTD/2036)
2-Cyclohexene-1-propanoic acid, ethyl ester (LTD/2037)

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2036 LTD/2037	International Flavours and Fragrances (Australia) Pty Ltd	1-Cyclohexene-1-propanoic acid, ethyl ester (LTD/2036) 2-Cyclohexene-1-propanoic acid, ethyl ester (LTD/2037)	Yes	≤ 1 tonne per annum (each notified chemical)	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

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

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

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

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio the notified chemicals are not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

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

  The above should be used for products/mixtures containing the notified chemicals, if applicable, based on the concentration of the notified chemicals present.

Health Surveillance

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

CONTROL MEASURES

Occupational Health and Safety

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

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure to the notified chemicals during reformulation processes:
  - Avoid skin contact

- 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:
  - Impervious gloves
  - Coveralls

  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 chemicals 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 chemicals in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Storage

- The handling and storage of the notified chemicals 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 chemicals 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 for each notified chemical;
- the combined concentration of the notified chemicals exceeds or is intended to exceed ≤ 1.1% concentration in air freshener products, rinse-off cosmetic products and other household products, ≤ 0.73% in fine fragrances, ≤ 0.5% concentration in body lotion, hand and face creams and ≤ 0.3% concentration in deodorants;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemicals has changed from a fragrance ingredient, or is likely to change significantly;
- the amount of chemicals being introduced has increased, or is likely to increase, significantly;
- the chemicals have begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemicals 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 isomer mixture containing the notified chemicals and products containing the isomer mixture 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
LTD/2036: Limited-small volume: Chemical other than polymer (1 tonne or less per year)
LTD/2037: Limited-small volume (reduced fee notification): Chemical other than polymer (1 tonne or less per year) - Chemical notified at the same time as a similar chemical.

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 for dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
China – Simplified Notification (2015)

2. IDENTITY OF CHEMICAL

MARKETING NAMES
FRET 11-0078 (mixture of the notified chemicals; > 97% combined concentration)

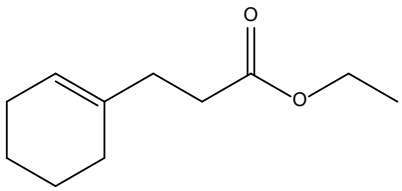
CAS NUMBER
LTD/2036: 65173-43-5
LTD/2037: 109976-49-0

CHEMICAL NAME
LTD/2036: 1-Cyclohexene-1-propanoic acid, ethyl ester
LTD/2037: 2-Cyclohexene-1-propanoic acid, ethyl ester

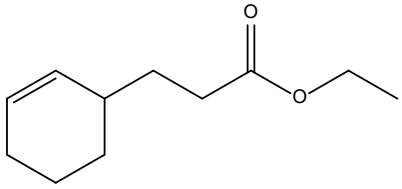
OTHER NAMES
LTD/2036: Ethyl 3-(cyclohex-1-en-1-yl) propanoate
LTD/2037: Ethyl 3-(cyclohex-2-en-1-yl) propanoate

MOLECULAR FORMULA
$C_{11}H_{18}O_2$ (LTD/2036 and LTD/2037)

STRUCTURAL FORMULA
LTD/2036:



LTD/2037:



MOLECULAR WEIGHT
182.26 g/mol (LTD/2036 and LTD/2037)

ANALYTICAL DATA
Reference NMR, FT-IR, HPLC, GC, GC-MS, UV spectra were provided and optical activity was determined. (Analysed as FRET 11-0078: mixture of the notified chemicals)

3. COMPOSITION

DEGREE OF PURITY
The notified chemicals are manufactured as an inseparable isomer mixture with a combined purity of > 96%. The ratio of the two isomers has been determined on two separate batches by GC-MS (IFF, 2013) and GC (IFF, 2017) as follows:

GC-MS
67.9% (LTD/2036)
29.5% (LTD/2037)

GC
81.0% (LTD/2036)
15.0% (LTD/2037)

NON HAZARDOUS IMPURITIES (> 1% BY WEIGHT)

Chemical Name	Propanoic acid, 3-cyclohexylidene-, ethyl ester		
CAS No.	18559-89-2	*Weight %*	2.8 (GC); 0.4 (GC-MS)

ADDITIVES/ADJUVANTS

Chemical Name	2-Propenoic acid, 3-phenyl-, 2-propen-1-yl ester		
CAS No.	1866-31-5	*Weight %*	0.1
Hazardous Properties	H302 – Harmful if swallowed H315 – Causes skin irritation H319 – Causes serious eye irritation		

4. PHYSICAL AND CHEMICAL PROPERTIES
The following measured physicochemical properties were obtained on the isomer mixture of the notified chemicals.

APPEARANCE AT 20 °C AND 101.3 kPa: colourless liquid

Property	Value	Data Source/Justification
Freezing Point	< -20 °C	Measured
Boiling Point	Boiling with decomposition from ~237 °C at 102.9 kPa	Measured
Density	966 kg/m^3 at 20 °C	Measured
Vapour Pressure	7 × 10^{-3} kPa at 25 °C	Measured
Water Solubility	8.17 x 10^{-2} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	t½ at 25 °C: 64.4 days at pH 4; 54.8 days at pH 7; 14.2 days at pH 9	Measured

Partition Coefficient (n-octanol/water)	log Pow = 4.08 – 4.19 at 35 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.96 at 30 °C	Measured
Dissociation Constant	Not determined	No dissociable functionality
Surface Tension	66.5 mN/m at 20 °C	Measured
Flash Point	104 ± 2 °C at 101.3kPa	Measured
Pyrophoric Properties	Non-pyrophoric	Measured
Flammability	Combustible liquid[#] Non-flammable in contact with water	Based on measured flash point Measured
Autoignition Temperature	252 ± 5 °C	Measured
Explosive Properties	Not explosive	Expert prediction based on chemical structure
Oxidising Properties	Not oxidising	Expert prediction based on chemical structure

[#]Based on *Australian Standard AS1940 definitions*

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

Reactivity
The notified chemicals are 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 isomer mixture containing the notified chemicals 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 isomer mixture containing the notified chemicals has a flash point of 104 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 chemicals will be introduced into Australia as components of fragrance oils at a combined concentration of ≤ 11%.

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

LTD/2036

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

LTD/2037

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

PORT OF ENTRY
Melbourne

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

TRANSPORTATION AND PACKAGING
The notified chemicals (at a combined concentration of ≤ 11%) will be imported as components of fragrance oils in 205 L polypropylene-lined steel drums. The imported fragrance oils containing the notified chemicals will be transported to reformulation sites within Australia by road. The end-use products containing the notified chemicals (at a combined concentration of ≤ 1.1%) will be packaged in containers suitable for retail sale.

USE
The notified chemicals will be used as fragrance ingredients for use in cosmetic and household products. The proposed use concentration for both chemicals (combined) is ≤ 1.1% concentration in (spray) air freshener products, rinse-off cosmetic products and other household products, ≤ 0.73% in fine fragrances, ≤ 0.5% concentration in body lotion, hand and face creams and ≤ 0.3% concentration in deodorants.

OPERATION DESCRIPTION
The notified chemicals will not be manufactured in Australia. The notified chemicals (at a combined concentration of ≤ 11%) will be introduced into Australia as components of fragrance oils for reformulation into cosmetic and household products.

Reformulation
The procedures for incorporating the notified chemicals (at a combined concentration of ≤ 11%) into end-use products will likely vary depending on the nature of the formulated products and may involve both automated and manual transfer steps. However, in general, it is expected that for the reformulation process, the notified chemicals will be weighed and added to the mixing tank where it will be blended with additional additives to form the finished cosmetic and household products. This will be followed by automated filling of the reformulated products into containers of various sizes. The blending operations are expected to be highly automated and use closed systems and/or adequate ventilation. During the reformation process, samples of the notified chemicals and the finished end-use products will be taken for quality control testing.

Cosmetic products
The finished cosmetic products containing the notified chemicals (at a combined concentration of ≤ 1.1%) will be used by consumers and professionals (such as beauticians and hair dressers). Depending on the nature of the products, application could be by hand, sprayed or through the use of an applicator.

Household products
Household products containing the notified chemicals (at a combined concentration of ≤ 1.1%) may be used by consumers and professional workers (i.e., cleaners). The products may be used in either closed systems with episodes of controlled exposures, for example automatic washing machines, or open processes and manually by rolling, brushing, spraying and dipping.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehouse workers	Incidental	Incidental
Plant operators - mixing/blending	4	250
Plant operators - drum handling	1	250
Plant operators - drum cleaning/washing	2	200
Plant operators - equipment maintenance	2	250
Quality control workers	1	250
End users (professionals)	1	250

EXPOSURE DETAILS
Transport and storage
Transport, storage and warehouse workers may come into contact with the notified chemicals (at a combined concentration of ≤ 11% in fragrance oils or ≤ 1.1 % concentration in final formulated 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 chemicals (at a combined concentration of ≤ 11%) 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
Workers involved in professions which involve professional cleaning or the application of cosmetic products containing the notified chemical to clients (e.g. beauty salon workers) may be exposed to the notified chemicals at a combined concentration of ≤ 1.1%. 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 chemicals.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemicals (at a combined concentration of ≤ 1.1%) through the use of a wide range of cosmetic and household products. The principal route of exposure will be dermal, while ocular and inhalation exposure (e.g. through the use of spray products) are also possible.

Data on typical use patterns of product categories (SCCS, 2012; Cadby *et al*., 2002; ACI, 2010; Loretz *et al.,* 2006) in which the notified chemicals 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 chemicals 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 chemicals 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	0.500	1.000	0.6109
Face cream	1540	0.500	1.000	0.1203
Hand cream	2160	0.500	1.000	0.1688
Fine fragrances	750	0.730	1.000	0.0855
Deodorant (non-spray)	1500	0.300	1.000	0.0703
Shampoo	10460	1.100	0.010	0.0180
Conditioner	3920	1.100	0.010	0.0067
Shower gel	18670	1.100	0.010	0.0321
Hand wash soap	20000	1.100	0.010	0.0344
Hair styling products	4000	1.100	0.100	0.0688
Total				**1.2158**

C = maximum intended combined concentration of the notified chemicals; 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	1.1	0.95	10	0.0376
Fabric softener	90	1.1	0.95	10	0.0147
Total					**0.0523**

C = maximum intended combined concentration of the notified chemicals
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	1.1	1980	0.01	0.01	0.007	0.0003

Dishwashing liquid	3	1.1	1980	0.009	0.01	0.03	0.0028
All-purpose cleaner	1	1.1	1980	1	0.01	0.007	0.0238
Total							**0.0269**

C = maximum intended combined concentration of the notified chemicals
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**	**C**	**Inhalation rate**	**Exposure duration zone 1**	**Exposure duration zone 2**	**Fraction inhaled**	**Volume zone 1**	**Volume zone 2**	**Daily systemic exposure**
	(g/use)	(%)	(m^3/day)	(min)	(min)	(%)	(m^3)	(m^3)	(mg/kg bw/day)
Hairspray	9.89	1.1	20	1	20	50	1	10	0.0354
Total									**0.0354**

C = maximum intended combined concentration of the notified chemicals
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 chemicals at the maximum intended combined concentration as specified by the notifier in various product types. This would result in a combined internal dose of 1.3304 mg/kg bw/day.

It is acknowledged that inhalation exposure to the notified chemicals 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 chemicals 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 isomer mixture containing the notified chemicals 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 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 4.78 mg/L/4 hour; low toxicity
Skin corrosion – *in vitro* (EpiDerm™ model)	non-corrosive
Skin irritation – *in vitro* (EpiDerm™ model)	non-irritating
Eye irritation – *in vitro* Bovine Corneal Opacity Test (BCOP)	non-irritating
Skin sensitisation – *in chemico* Direct Peptide Reactivity Assay (DPRA)	positive (moderate to high reactivity)
Skin sensitisation – *in vitro* ARE-Nrf2 luciferase test	negative
*S*kin sensitisation – *in vitro* human Cell Line Activation Test (h-CLAT)	positive
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation (EC3 = 34%)
Human, skin sensitisation – RIPT (1.5%)	no evidence of sensitisation
Rat, combined repeated dose oral toxicity with reproduction/developmental screening (first study)	Reproductive effects in females treated at 10,000 ppm (low number of pregnant dams that gave birth and litters that survived until study termination).
Rat, combined repeated dose oral toxicity with reproduction/developmental screening test (second study; repeat of the first study)	The study was terminated due to abnormally low number of pregnant dams that gave birth and had litters that survived until study termination.
Rat, combined oral repeated dose oral toxicity with reproduction/developmental screening test (third	NOAEL parental toxicity: 2,500 ppm (equivalent to 148 mg/kg bw/day in males and 175 mg/kg bw/day in

study)	females) NOAEL reproductive toxicity: 10,000 ppm (equivalent to 553 mg/kg bw/day in males and 687 mg/kg bw/day in females) NOAEL developmental (offspring) toxicity: 2,500 ppm
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration test	non clastogenic

Toxicokinetics
Given the low molecular weight of the notified chemicals (182.26 g/mol) absorption across the gastrointestinal and respiratory tracts may occur. However, dermal absorption is expected to be limited given the low water solubility (0.0817 g/L) and high lipophilicity (log Pow = 4.08 – 4.19) of the notified chemicals, limiting penetration through the hydrophilic epidermis.

Acute toxicity
The isomer mixture containing the notified chemicals is of low acute oral, dermal, and inhalation toxicity based on studies conducted in rats.

Irritation and sensitisation
The isomer mixture containing the notified chemicals was found to be non-corrosive and non-irritating to the skin based on *in vitro* studies conducted using the reconstructed human epidermis model.

In an *in vitro* bovine corneal opacity and permeability (BCOP) eye irritation test the isomer mixture containing the notified chemicals was determined to be non-irritating.

One *in chemico* and two *in vitro* cell based assays were conducted to evaluate the skin sensitisation potential of the isomer mixture containing the notified chemicals. The tests are part of Integrated Approach to Testing and Assessment (IATA) which address specific events of the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation (OECD, 2016). The tests are thus considered relevant for assessment of the skin sensitisation potential of a test substance, along with other supporting information.

The first key event in the AOP, commonly referred to as the molecular initiating event, for sensitisation is the covalent binding to nucleophilic centres in skin proteins. The *in chemico* Direct Peptide Reactivity Assay (DPRA) aims to address this key event by measuring the interaction of a test substance with cysteine and lysine-containing small synthetic peptides (representing the nucleophilic centres in skin protein).

The second key event in the AOP for sensitisation is the activation of keratinocytes which leads to upregulation of stress related proteins (cytokines) via transcriptional upregulation of the genes. The ARE-Nrf2 Luciferase Assay aims to address this key event by measuring the change in expression of luciferase gene under the transcriptional control of a constitutive promoter fused with an Antioxidant Response Element (ARE). The ARE is from a gene that is known to be upregulated by contact sensitisers.

The third key event in the AOP for sensitisation is the activation of dendritic cells resulting in change in cell surface expression of markers such as CD54 and CD86. The *in vitro* h-CLAT assay measures the change in expression of cell surface markers CD54 and CD86 upon activation of human monocyte leukaemia cell line (THP-1) with proper stimuli. The assay addresses the third key event in the AOP for skin sensitisation.

The isomer mixture containing the notified chemicals showed positive responses in two of the three tests (DPRA assay and h-CLAT test), suggesting the notified chemicals could be skin sensitisers. According to the OECD test guidelines (TG 442c, 442d and 442e), the suite of tests based on the AOP may not detect pre-haptens (chemicals that can become sensitisers following auto-oxidation) and pro-haptens (chemicals requiring enzymatic activation to become sensitisers). As such, (i) the notified chemical may be a stronger sensitiser than predicted in the DPRA assay and h-CLAT test (ii) the negative result in the ARE-Nrf2 Luciferase assay could potentially be a false negative.

The isomer mixture containing the notified chemicals was found to be a weak skin sensitiser in a mouse local lymph node assay (EC3 = 34%). However, the notified chemicals tested negative in a human repeat insult patch test when tested at 1.5% concentration.

Based on the results of the LLNA study, the notified chemicals are considered Category 1B skin sensitisers.

Repeated dose toxicity
Three combined repeated dose toxicity studies with reproduction/developmental screening tests were conducted in rats. For information on the first two studies, refer to the table in this section and 'B.13. Repeat dose toxicity (first study)' and 'B.14. Repeat dose toxicity (second study; repeat of first study)'.

In the third repeated dose toxicity study, the test substance was administered at 2,500, 5,000 and 10,000 ppm (148, 289 and 584 mg/kg bw/day) in males for 6 weeks (2 weeks prior to mating, during mating and until approx. 80% of the females had given birth). Females were dosed with the test substance at 2,500, 5,000 and 10,000 ppm (175, 351 and 679 mg/kg bw/day) for approx. 8 weeks (2 weeks prior to mating, during mating and gestation and at least up to post-partum day 14). Recovery group males and females were given the test substance at 10,000 ppm (522 and 694 mg/kg bw/day, respectively) for 6 weeks, followed by a recovery period of 2 weeks. The recovery animals were not mated during the study. The average high dose intake in males and females (main study and recovery groups combined) was 553 and 687 mg/kg bw/day, respectively.

One female animal treated at 2,500 ppm was found dead on day 24 of gestation. No clinical signs of toxicity were noted from any of the main group and recovery animals.

High dose males had decreased mean body weight gain (statistically significant at Weeks 2 and 7) due to decreased food consumption. High dose females had a statistically significantly lower body weight than controls during day 14 of lactation. The mean body weight gain for these females was also 69% lower than controls over the whole lactation period; this was not accompanied by differences in mean food consumption.

Males treated at 10,000 ppm showed notable increases in white blood cell, neutrophil, lymphocyte, monocyte and basophil counts, compared to controls. Females in the 10,000 ppm group showed notable increases in neutrophil and reticulocyte counts. High dose males had notable reductions in lactate dehydrogenase and creatinine kinase activity compared to controls; this trend continued after recovery. Lactate dehydrogenase and creatinine kinase activity was noticeably lower in all female treatment groups (statistically significant decrease only at 10,000 ppm); these trends were partially reversed after recovery. Large decreases in thyroid stimulating hormone were reported in all treated males (statistically significant only at 10,000 ppm). High and low dose female showed notable increases in thyroid stimulating hormone concentrations compared to controls.

No consistent organ abnormalities were detected during necropsy. The mean absolute weight of the Cowper's gland in low and high dose males was statistically significantly decreased; the relative weight of this gland was statistically significantly and notably decreased in low and high dose males, respectively. After recovery, high dose males continued to have notably lower Cowper's gland weight. High dose females had apparent decreases in absolute and relative thymus weight in comparison with controls; but reversed after recovery.

The No Observed Adverse Effect Level (NOAEL) for the test substance was established by the study authors as 148 mg/kg bw/day in males and 175 mg/kg bw/day in females (lowest dose tested).

Toxicity for reproduction
Fertility index, parturition index, percentage of pregnant animals, litter size, pup ano-genital distance, mating index and pup mortality were not affected. No consistent signs of toxicity were noted in the pups belonging to the control or treatment groups. The live birth index however, was statistically significantly lower in the 10,000 ppm group compared to the control group.

The combined mean body weight of pups of both sexes on lactation day 14 was statistically significantly lower in the 5,000 and 10,000 ppm groups compared to the control group. The mean body weight gain over the entire lactation period for pups in these groups was statistically significantly lower than the control group. These body weight and body weight gain changes were seen as test substance-related and adverse.

The NOAEL for reproductive toxicity was established as 10,000 ppm and the NOAEL for developmental (offspring) toxicity was established as 2,500 ppm.

Mutagenicity/Genotoxicity
The isomer mixture containing the notified chemicals tested negative in a bacterial reverse mutation assay and in an *in vitro* mammalian cell chromosome aberration test with human lymphocytes.

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

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available information, the notified chemicals are weak skin sensitisers.

Reformulation
During reformulation, workers may be at risk of skin sensitisation when handling the notified chemicals (at a combined concentration of ≤ 11%) as introduced. The notifier anticipates that worker exposure will be limited through the use of engineering controls such as enclosed systems, automated processes and local exhaust 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 chemicals to clients (*e.g.* beauty salon workers) may be exposed to the notified chemicals (at a combined concentration of ≤ 1.1%). The principal route of exposure will be dermal, while ocular and inhalation exposure are also possible. 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 chemicals.

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

6.3.2. Public Health
Members of the public will experience widespread and frequent exposure to the notified chemicals (at a combined concentration of ≤ 1.1%) 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.

Sensitisation
Based on the results of an LLNA study, the notified chemicals are weak skin sensitisers (EC3 = 34%).

Proposed methods for the quantitative risk assessment of dermal sensitisation have been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). As is shown in the table below, the Consumer Exposure Level (CEL) from use of the notified chemicals in leave-on and rinse-off cosmetic products may be estimated (SCCS, 2012 and Cadby *et al*, 2002). Consideration of available information and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 27.37 µg/cm^2. In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16), a use and time factor (3.16) and a database factor (1), giving an overall safety factor of 300.

Product type	**Proposed usage concentration (%)**	**CEL (µg/cm^2)**	**AEL (µg/cm^2)**
Fine fragrances	0.73	27.37	27.37
Other leave-on cosmetics (assumed: face cream)	0.5	13.63	27.37
Rinse-off cosmetics (assumed: hand wash soap)	1.1	2.56	27.37

As the AEL ≥ CEL, the risk to the public of the induction of sensitisation that is associated with the use of the notified chemicals at a combined concentration of ≤ 0.73% concentration in fine fragrances, at ≤ 0.3% concentration in deodorants and ≤ 0.5% in other leave-on cosmetics (using face cream as a worst case example), and at ≤ 1.1% concentration in rinse-off cosmetic products and household cleaning products (using hand wash soap as a worst case example) is not considered to be unreasonable. It is acknowledged that consumers may be exposed to multiple products containing the notified chemicals, and a quantitative assessment based on the aggregate exposure has not been conducted.

Repeated dose toxicity
The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemicals using the worst case exposure scenario from use of multiple products by an individual with total exposure of 1.3304 mg/kg bw/day (see Section 6.1.2). Using a NOAEL for the notified chemicals of 148 mg/kg bw/day in males and 175 mg/kg bw/day in females derived from combined oral repeated dose oral toxicity with reproduction/developmental screening test, the margin of exposure (MoE) was estimated to be 111 for males and 132 for females. A MoE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences.

Overall, based on the information available, the risk to the public associated with use of the notified chemicals at a combined concentration of ≤ 1.1% in (spray) air freshener products, rinse-off cosmetic products and other household products, ≤ 0.73% in fine fragrances, ≤ 0.5% concentration in body lotion, hand and face creams and ≤ 0.3% in deodorants 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 chemicals will be imported as a component of fragrance oil formulations for local reformulation into finished cosmetic and household products. Environmental release during importation, transport and distribution may occur as a result of accidental spills. In the event of a spill, the notified chemicals are expected to be contained and collected with an inert absorbent material and disposed of to landfill.

The fragrance formulations containing the notified chemicals will be blended with other ingredients in the manufacture of cosmetic and household products within a fully enclosed environment. The process is expected to be followed by automated filling of the formulated products into containers of various sizes suitable for retail sale and end-use. Wastes containing the notified chemicals generated during reformulation include equipment wash water, empty import containers and spilt materials. These will be collected, recycled or released to on-site wastewater treatment facilities or sewers in accordance with local government regulations. Empty containers will be either recycled or disposed of to landfill.

RELEASE OF CHEMICAL FROM USE
The notified chemicals are expected to be released to the aquatic compartment through sewers during its use in various cosmetic and household products.

RELEASE OF CHEMICAL FROM DISPOSAL
Approximately 1% of the import volume of the notified chemicals is expected to remain as residues in end-use containers (or up to 10 kg/year for each notified chemical). Wastes and residue of the notified chemicals 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 their use in cosmetic and household products in Australia, the majority of the notified chemicals are expected to enter the sewer system, before potential release to surface waters nationwide. Based on the result of a biodegradability study, the notified chemicals are considered readily biodegradable (79% in 28 days). For details of the environmental fate studies, please refer to Appendix C. The submitted study by the notifier has also indicated that the notified chemicals are not hydrolytically stable.

The half-life of the notified chemicals in air is calculated to be 1.19 hours based on reactions with hydroxyl radicals (AOPWIN v1.92, US EPA 2011). Therefore, the notified chemicals are not expected to persist in the atmospheric compartment.

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 chemicals into sewer systems nationwide and no removal within sewage treatment plants (STPs).

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import 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 chemicals in this volume are 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 chemicals in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemicals in the applied soil in 5 and 10 years may be approximately 0.018-0.002 mg/kg and 0.037 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96h LC50 = 1.65 mg/L	Toxic to fish
Daphnia Toxicity	48h EC50 = 1.3 mg/L	Harmful to *Daphnia*
Algal Toxicity	72h E_rC50 = 4.6 mg/L	Harmful to algae
Inhibition of Bacterial Respiration	3h EC50 = 410 mg/L	Not inhibitory to bacterial respiration

Based on the above ecotoxicological data for the isomer mixture, the notified chemicals are expected to be acutely toxic to fish, daphnia and algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemicals are formally classified as "Acute Category 2; Toxic to aquatic life". Based on the ready biodegradability and low bioaccumulation potential, the notified chemicals are not expected to be harmful to aquatic life in the long term, and are therefore not formally classified under the GHS.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) was calculated from the most sensitive endpoint, which in this case is *Daphnia* toxicity. A safety factor of 100 was used as acute endpoints for three tropic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (*Daphnia*, 48h)	1.3	mg/L
Assessment Factor	100	
PNEC:	13	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	13	0.043
Q - Ocean	0.056	13	< 0.01

The risk quotient for discharge of treated effluents containing the notified chemicals to the aquatic environment indicates that the notified chemicals are unlikely to reach ecotoxicologically significant concentrations in surface waters based on its maximum annual importation quantity. The notified chemicals are readily biodegradable, and expected to have a low potential for bioaccumulation. On the basis of the PEC/PNEC ratio and maximum annual importation volume the notified chemicals are not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -20 °C

Method OECD TG 102 Melting Point/Melting Range (1995)
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature (2008)
Remarks The test substance did not freeze under the conditions of the study.
Test Facility Harlan (2014a)

Boiling Point Boils with decomposition from ~237 °C at 102.9 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. After heating to the maximum experimental temperature (450 °C), the test substance had lost approx. 95% of its original weight. A black, solid residue had also formed around the periphery of the crucibles that had initially contained the test item. Based on these observations, the study authors concluded that the test item had decomposed upon boiling.
Test Facility Envigo (2017a)

Density 966 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 Pycnometer method
Test Facility Envigo (2017a)

Vapour Pressure 7×10^{-3} kPa at 25 °C

Method OECD TG 104 Vapour Pressure (2006)
EC Council Regulation No 440/2008 A.4 Vapour Pressure (2008)
Remarks Vapour pressure balance method
Test Facility Harlan (2014b)

Water Solubility 8.17×10^{-2} g/L at 20 °C

Method OECD TG 105 Water Solubility (1995)
EC Council Regulation No 440/2008 A.6 Water Solubility (2008)
Remarks On the basis of the preliminary test, which estimated the water solubility to be greater than 10^{-2} g/L, the flask shake method was performed.
Test Facility Harlan (2014a)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH (2004)
EC Council Regulation No 440/2008 C.7 Abiotic Degradation: Hydrolysis as a Function of pH (2008)

pH	*T (°C)*	*$t_{½}$ days*
4	25	64.4
7	25	54.8
9	25	14.2

Remarks The linearity of the detector response with respect to concentration was assessed over the nominal concentration range of 2.0 to 25 mg/L for each of the three analysis solution matrices used. The results were satisfactory with first order correlation coefficients (r) of ≥ 0.99 being obtained.
Test Facility Envigo (2017b)

Partition Coefficient (n-octanol/water) log Pow = 4.08 – 4.19 at 35 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water) (2004).
EC Council Regulation No 440/2008 A.8 Partition Coefficient (2008).
Remarks The HPLC method was considered to be suitable for the purpose of the study since it showed two main peaks with well-defined and reproducible retention times.
Test Facility Harlan (2014a)

Adsorption/Desorption log K_{oc} = 2.96 at 30 °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 test substance is not ionisable and therefore the study was conducted, at an approximately neutral pH.
Test Facility Envigo (2017b)

Surface Tension 66.5 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. Based on the result of this study, the test item is regarded not to be surface active.
Test Facility Envigo (2017a)

Flash Point 104 ± 2 °C at 101.3 kPa

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

Pyrophoric Properties Non-pyrophoric

Method EC Council Regulation No 440/2008 A.13 Pyrophoric Properties of Solids and Liquids (2008)
Test Facility Envigo (2017b)

Flammability Not flammable in contact with water

Method EC Council Regulation No 440/2008 A.12 Flammability (Contact with Water) (2008)
Remarks No gases were evolved from the test substance during the course of the experiment.
Test Facility Envigo (2017b)

Autoignition Temperature 252 ± 5 °C

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

Explosive Properties No explosive properties

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

Oxidizing Properties No oxidising properties

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids) (2008)

Remarks	Based on chemical structure
Test Facility	Envigo (2017b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 420 Acute Oral Toxicity - Fixed Dose Method (2001) EC Council Regulation No 440/2008 B.1 bis Acute Toxicity (Oral) Fixed Dose Method
Species/Strain	Rat/ Wistar (Rcc:Han)
Vehicle	None for 2000 mg/kg bw Arachis oil BP – 300 mg/kg bw
Remarks - Method	No significant protocol deviations. Animals were dosed by gavage.

RESULTS

Sighting Study

Dose mg/kg bw	*Administered*	*Evident Toxicity*	*Mortality*
300	1F	None	0/1
2000	1F	None	0/1

Signs of Toxicity	No mortalities occurred or clinical signs of toxicity were noted.
Effects in Organs	No abnormalities detected at necroscopy.
Remarks - Results	All animals made expected body weight gains during the study.

Main Study

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

LD50	> 2000 mg/kg bw
Signs of Toxicity	One animal was found dead on day 1 after dosing. No signs of toxicity were recorded for this animal during its previous observation time points. Hunched posture was observed in the remaining rats on day 1 after dosing. No signs of toxicity appeared from day 2 after dosing till the end of the study.
Effects in Organs	The animal that had died during the study presented with haemorrhagic lungs, dark liver and dark kidneys. No abnormalities were detected at necroscopy in the remaining animals.
Remarks - Results	The animal that had died during the study weighed 8 grams less than at the start of the study. All remaining animals had made expected body weight gains during the study. Body weights were not recorded on Day 14 after dosing due to technical error. Based on the results of this study, the LD50 was estimated to be > 2000 mg/kg bw
CONCLUSION	The test substance has low acute oral toxicity
TEST FACILITY	Envigo (2017d)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
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	None
Type of dressing	Semi-occlusive.
Remarks - Method	No deviations from the study protocol 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	All male animals showed expected body weight gains during the study, except the male in Group 1 (weight loss on the second week). All Group 4 females presented with weight loss on the first week of the study, but made expected weight gains during the second week.

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

TEST FACILITY Envigo (2017e)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 403 Acute Inhalation Toxicity (2009)
EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation) (2014)

Species/Strain	Rat/Wistar (Rcc:Han)
Vehicle	Acetonitrile
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	3.11 µm (mean mass aerodynamic diameter)
Remarks - Method	No significant protocol deviations were noted.

RESULTS

`	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5F/5M	17.28	4.78	0/10

LC50	> 4.78 mg/L/4 hours
Signs of Toxicity	During exposure to the test substance, all animals presented with wet fur and decreased respiratory rate. Immediately upon removal from the test chamber, all animals additionally displayed hunched posture and pilo-erection. All effects continued in all animals at one hour post exposure and had regressed from one day post exposure until the end of the study.
Effects in Organs	One male animal presented with dark patches on the lungs and one female animal presented with pale lungs.
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.

Nearly all animals (9/10) showed slight weight loss during the first day of the study, after exposure to the test substance. During the remainder of the recovery period all animals showed expected body weight gains, with the exception of one female animal, which exhibited body weight loss from days 1 to 3 after exposure.

CONCLUSION The test substance is of low acute toxicity via inhalation.

TEST FACILITY Envigo (2017f)

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

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 431 *In vitro* Skin Corrosion - Reconstructed Human Epidermis (RHE) Test Method (2015)
EC Council Regulation No 440/2008 B.40 BIS. *In vitro* Skin Corrosion - Human Skin Model Test (2008)

Vehicle None

Remarks - Method No deviations to the study protocol 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, dark conditions) 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 not able to directly reduce MTT.

Positive and negative controls were run in parallel with the test substance:
- Negative control: Sterile distilled water
- Positive control : 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	1.794	100	1.618	100
Test substance	1.676	93.4	1.787	110.4
Positive control	0.053	3.0	0.039	2.4

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 test substance was non-corrosive to the skin under the conditions of the test.

TEST FACILITY Envigo (2017g)

B.5. Irritation– skin (*in vitro* EPIDERM™ Skin Irritation Test)

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2015)

EC Council Regulation No 440/2008 B.46 *In vitro* Skin Irritation - Reconstructed Human *Epidermis* Test Method (2009)

Vehicle: None

Remarks - Method: No deviations to the study protocol were noted.

The test substance (10 µL) was applied to the tissues in triplicate. Following a 15 minutes expoure at room temperature, the tissues were washed in Dulbecco's PBS (with Ca^{2+} and Mg^{2+}), and then incubated in fresh medium at 37 °C for 42 hours. Each plate (which contained tissues and maintenance medium) was shaken for 15 min and then 1.6mL of maintenance medium from beneath each tissue was transferred to separate tubes and stored in a freezer for potential inflammatory mediator determination. The tissues were transferred to fresh medium containing MTT and incubated at 37 °C for 3 hours to test cell viability. After extraction, optical densities were determined at 562 nm.

Preliminary tests had been conducted, which indicated that the test substance does not directly interfere with MTT.

Positive and negative controls were run in parallel with the test substance:
- Negative control: Dulbecco's PBS with (with Ca^{2+} and Mg^{2+})
- Positive control: sodium dodecyl sulphate (5% w/v - aqueous)

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.731	100	5.7
Test substance	0.842	115.2	9.3
Positive control	0.083	11.4	6.1

OD = optical density; SD = standard deviation

Remarks - Results: The positive and negative controls performed as expected and the standard deviation of the relative mean viability of the test substance-treated tissues was within acceptable range, confirming the validity of the test system.

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

TEST FACILITY: Envigo (2017h)

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

TEST SUBSTANCE: Isomer mixture containing the notified chemicals

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 were noted.

Positive and negative controls were run in parallel with the test substance:
- Negative control: sodium chloride (0.9% w/v)
- Positive control: ethanol

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Negative control	1.7	0.033	2.2
*Test substance**	2.0	0.020	2.3
*Positive control**	32.7	0.845	45.3

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.

The IVIS for the test substance was < 3, indicating that the test substance did not require classification for eye irritation.

CONCLUSION: The test substance was not an eye irritant under the conditions of the test.

TEST FACILITY: Envigo (2017i)

B.7. *In Chemico* Skin Sensitisation (DPRA Test)

TEST SUBSTANCE: Isomer mixture containing the notified chemicals

METHOD: Derived from Gerberick *et al*, 2004, 2007.
Vehicle: DMSO:acetonitrile (1:1) made to a final concentration of 100 mM
Remarks - Method: The test substance was prepared in the vehicle solution. p-benzoquinone was used as a positive control and benzoic acid was used as a negative control. Both controls were set up in the vehicle solution. Vehicle reference controls were setup and run in parallel to the test substance. 0.5 mM solutions of cysteine and lysine peptides were prepared in dimethylformamide and lysine peptide reaction storage buffer, respectively. The test substance was incubated with the peptide solutions for 24 hours at room temperature in the dark. The ratios of test substance to peptides were 1:10 and 1:50 for cysteine and lysine peptides, respectively. Peptide depletion was then monitored by LC/MS/MS.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± CV)*	*Lysine Peptide Depletion (% ± CV)*
Vehicle	0.0 ± 10.0	0.0 ± 13.6
Negative Control	-6.7 ± 5.4	-1.1 ± 9.9
Test Substance	77.1 ± 10.9	6.9 ± 6.3
Positive Control	98.3 ± 10.3	97.9 ± 31.9

CV = Coefficient of Variance

Remarks - Results: The average reactivity of the test substance was calculated as 42%. This result ranked the test substance as moderately reactive overall. However, it should be noted that this average reactivity was just below the cut-off for high reactivity (42.47%).

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

CONCLUSION: The test substance was considered to have moderate to high reactivity (positive for skin sensitisation) under the conditions of the test for peptide depletion, in the first key event of the adverse outcome pathway (AOP) for skin sensitisation.

TEST FACILITY: Cyprotex (2014)

B.8. *In Vitro* Skin Sensitisation (ARE-Nrf2 Luciferase Test)

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	Natsch and Emter 2008; Natsch, Emter and Ellis 2009; Natsch *et al.,*2011
Vehicle	DMSO
Remarks - Method	A 200mM stock solution of test substance was prepared in DMSO. A set of twelve solutions were prepared in DMSO from the stock solution (conc. range 0.98 – 2000 µM). The KeratinoSens cell line was treated with the test substance for 48 hours. DMSO and cinnamic aldehyde were used in parallel with the test substance as vehicle and positive controls respectively. Three independent experiments were conducted with samples tested in triplicate in each experiment. Cell viability was determined for each replicate using the MTT and Neutral Red assays. Skin sensitisation was measured as luciferase induction, calculated from three independent experiments.

RESULTS

Sample	*Cell viability – IC50 (µM) (mean*)*	*Luciferase I_{MAX} (maximum average fold induction of luciferase activity) (mean*)*
Negative control	-	-
Vehicle control	-	-
Test substance	299.20	1.28
Positive control	> 64	-

*: from three independent experiments conducted in triplicate
-: not provided
IC50 = Concentration for 50% reduction in cell viability
I_{MAX} = Maximal fold-gene induction of the reporter gene up to 1000 µM concentration

Remarks - Results	The EC1.5 value (concentration for an induction of 50% above solvent controls) was < 1000 µg/mL in at least 2 out of the 3 independent replicates. As such, the test substance was not considered a potential sensitiser. The positive control performed as expected. No information was given on the performance of the negative and vehicle controls.
CONCLUSION	The test substance was negative under the conditions of the test for the second key event in the AOP for skin sensitisation.
TEST FACILITY	Institute for In Vitro Sciences (2014)

B.9. *In Vitro* Skin Sensitisation (h-CLAT test)

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 442E *In Vitro* Skin Sensitisation: human Cell Line Activation Test (h-CLAT; 2016)
Vehicle	Culture medium was the vehicle for the test substance. DMSO was the vehicle for the positive control. Culture medium: RPMI-1640 supplemented with 10% foetal bovine serum (v/v).
Remarks - Method	No deviations from the study protocol that affected study integrity were noted. Human monocytic leukaemia cell line (THP-1) was used for the assay. Positive control was 1-chloro-2, 4-dinitrobenzene (DNCB) at 2 and 3 µg/mL, solvent control for the test substance was the vehicle and medium control for the test substance was culture medium. Stimuli mediated increase in expression of cell surface markers CD86 and CD54 was measured with fluorescence tagged antibodies.

Two dose finding assays (XTT Test) were conducted to find the test substance concentration at which cell viability is reduced to 75% (CV75) to decide test substance concentrations for CD54 and CD86 expression test. The following test substance concentrations were used: 19.6, 9.1, 78.1, 156.3, 312.5, 625, 1250 and 2500 µg/mL.

Two main tests were conducted to evaluate the ability of the test substance to induce expression of CD54 and CD86. The highest dose of the test substance to be used in the main test was derived from the following formula: CV75 (mean of both assays) × 1.2. The following concentrations of the test substance were used: 193, 231, 278, 333, 400, 480, 576 and 691 µg/mL.

The test substance is considered a sensitiser:
- If the relative fluorescence intensity (RFI) of CD86 is ≥ 150% or;
- If the RFI of CD54 is ≥ 200% in both tests

The two main tests produced opposing results. As such, a third main test had to be performed. In this instance, the test substance is considered a sensitiser:
- If the RFI of CD86 is ≥ 150% at any dose in at least 2 of 3 tests or;
- If the RFI of CD54 is ≥ 200% in at least 2 of 3 tests

RESULTS

Sample	*Concentration (µg/mL)*	*RFI Sensitisation Evaluation*					
		Test 1		*Test 2*		*Test 3*	
		CD54	*CD86*	*CD54*	*CD86*	*CD54*	*CD86*
Medium Control	-	-	-	-	-	-	-
DMSO Control	-	-	-	-	-	-	-
Positive Control	2.0	+	+	+	+	+	+
	3.0	+	+	+	+	+	+
Test Substance	193	-	-	-	-	-	-
	231	-	-	-	-	-	-
	278	-	-	-	+	-	+
	333	-	-	+	+	-	+
	400	-	-	-	+	-	+
	480	-	-	+	+	-	+
	576	-	-	-	+	-	+
	691	-	-	+	+	-	+

RFI: relative fluorescence intensity
+: RFI exceeds sensitisation-positive criteria
−: RFI below sensitisation-positive criteria

Remarks - Results

The dose finding assay and main tests fulfilled the acceptance criteria, confirming the validity of the test systems used in the dose finding assay and main tests.

The RFI of CD86 exceeded the sensitisation-positive criteria (i.e. was ≥ 150%) in 2 out of 3 tests, and at 6 separate doses in each test. The RFI of CD54 exceeded the sensitisation-positive criteria (i.e. was ≥ 200%) in 1 out of 3 tests, at 3 separate doses.

CONCLUSION The test substance was considered a skin sensitiser in the AOP KE3 assay.

TEST FACILITY Envigo CRS GmbH (2017)

B.10. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Isomer mixture containing the notified chemicals

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 deviations from the study protocol or test guideline were noted. A preliminary screening test using the undiluted test substance was conducted to determine dose concentrations for the main study. Based on these results, 100% 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 (DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5F	1859.41	1.00
25	5F	4358.04	2.34
50	5F	7754.98	4.17
100	5F	6009.18	3.23
Positive Control			
25	5F	16628.25	8.94

EC3	34%
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.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.
TEST FACILITY	Envigo (2017j)

B.11. Skin sensitisation – human volunteers

TEST SUBSTANCE	Isomer mixture containing the notified chemicals (1.5% w/w)
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: Patches containing 0.2 mL test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed by the applicants after 24 hours and graded after an additional 24 h (or 48 h for patches applied on Friday). Rest Period: 14 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 72 h post-patch removal.
Study Group	93 F, 20 M; age range 18 - 66 years
Vehicle	Ethanol (24.5% w/w) and diethyl phthalate (74% w/w) were combined with the notified chemical to produce a formulation of the notified chemical at a 1.5% w/w concentration.
Remarks - Method	Occluded. The test substance was spread on a 3.63 cm × 3.63 cm patch.

	The vehicle control was a formulation consisting of distilled water (1.5% w/w), alcohol SD39C (24.5% 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	106/113 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 at 1.5% concentration was non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2014)

B.12. Repeat dose toxicity – 14 Day Preliminary Toxicity Study

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	There are no specific testing guidelines for this study. General regulatory guidelines for toxicity studies were incorporated into the study design.
Species/Strain	Rat/Sprague Dawley
Route of Administration	Oral –diet
Exposure Information	Total exposure days: 14 days Dose regimen: 7 days per week Post-exposure observation period: Nil
Vehicle	None
Remarks - Method	No protocol deviations were noted. No statistical analysis was conducted on the data obtained during the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose ppm (mg/kg bw/day)*	*Mortality*
1	3M/3F	2,500 (173/175)	0/6
2	3M/3F	5,000 (289/341)	0/6
3	3M/3F	10,000 (567/573)	0/6
4	3M/3F	20,000 (899/962)	0/6

Mortality and Time to Death

No unscheduled deaths occurred.

Clinical Observations

The total mean body weight change and total mean food consumption from Day 1 to Day 15 of treatment for males and females treated at 20,000 ppm was noticeably reduced in comparison to animals treated at the lower doses.

Effects in Organs

Males treated at 20,000 ppm had noticeably higher epidydymes and kidney weights compared to males treated at 2,500 ppm (increases of 24% and 20%, respectively). These males also showed lower spleen weight compared to males treated at 2,500 ppm (a decrease of 10%). Females treated at 10,000 and 20,000 pm showed decreased ovaries, spleen and uterus weight compared to females treated at 2,500 ppm (decreases of 28% and 30%, 18% and 8%, and 14% and 19%, respectively).

Remarks – Results

No NOEL or NOAEL was established. Based on this study, the dietary dose levels recommended for

subsequent repeat dose toxicity studies are 0, 2500, 5000 and 10000 ppm.

TEST FACILITY	Huntingdon (2015)

B.13. Repeat dose toxicity (first study)

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (1996)
Species/Strain	Rat/Sprague-Dawley (Crl:CD BR)
Route of Administration	Oral – diet
Exposure Information	Total exposure days: - 35 days for males (2 weeks prior to mating, during mating (up to 2 weeks) and until scheduled necropsy) - Approx. 56 days for females (2 weeks prior to mating, during mating (up to 2 weeks) and until lactation day 6). - Females were given untreated feed on lactation day 6 through scheduled necropsy. Dose regimen: 7 days per week Post-exposure observation period: None
Necropsy	All surviving male animals were euthanised on study day 36. Female animals that produced litters were euthanised on lactation day 7. Female animals with evidence of mating but failed to deliver were euthanised 26 days after evidence of mating. Female animals with no evidence of mating and who failed to deliver a litter were euthanised on gestation day 25. Female animals with total litter loss (all pups found dead prior to scheduled sacrifice) were euthanised on the day of total litter loss.
Vehicle	None
Remarks - Method	Notable protocol deviations include: - Inadvertent fasting of animals for necropsy - Interruption of the 12 hour light/dark cycle on one occasion due to observations being performed after the lights went out. - The male post-mating test substance intake was not measured All protocol deviations were not considered to have affected the integrity of the study. Dose levels were selected as based on the results of a dose-range finding study performed previously (see 'B.12 Repeat dose toxicity – 14 Day Preliminary Toxicity Study').

Results

Group	*Number and Sex of Animals*	*Dose ppm (mg/kg bw/day)*	*Mortality*
control	10M/10F	0	0/20
low dose	10M/10F	2,500 (152/195)	0/20
mid dose	10M/10F	5,000 (296/391)	0/20
high dose	10M/10F	10,000 (567/767)	1/20

Mortality and Time to Death

One female treated at 10,000 ppm died during delivery. During gestation, the clinical parameters of this female were within normal values of the control gestational females. Based on the macroscopic findings during necropsy, this death was considered associated with parturition and not test substance-related.

Clinical Observations

During the gestation and lactation periods, hair loss on the forelimbs occurred across all groups (including controls) in a semi-frequent manner. Study authors considered to be a species-specific effect under laboratory conditions. Males treated at 5,000 ppm and 10,000 ppm showed notable reductions in hindlimb grip strength, compared to controls (decreases of 21 and 22%, respectively).

Males treated at 5,000 ppm showed a noticeable decrease in mean body weight compared to controls (a decrease of 30%) during the first week of treatment (pre-mating period). Males treated at 10,000 ppm showed statistically significant decreases in mean body weights during the first two weeks of treatment (pre-mating period; decreases of 29% and 26%, respectively compared to controls and noticeable decreases in mean body weight during the last two weeks of treatment (post-mating period; decreases of 10% and 16%, respectively in comparison with controls). Over the gestation period (gestation days 0-20), females treated at 10,000 ppm displayed a 15% decrease in mean body weight compared to controls.

Females treated at 2,500, 5,000, and 10,000 ppm presented statistically significant decreases in food consumption during days 4-6 of lactation compared to controls (decreases of 36%, 32% and 30%, respectively). These reductions in food consumption were not considered as reliable results due to cannibalism displayed by several of the dams. Furthermore, these reductions in food consumption were not accompanied by any statistically significant or noticeable changes in body weight.

Laboratory Findings – Clinical Chemistry, Haematology

Red blood cell count was statistically significantly increased and reticulocyte count was noticeably decreased in males treated at 5,000 and 10,000 ppm in comparison to controls (increases of 6% and 9%, and decreases of 19% and 23%, respectively). Males treated at 10,000 ppm also had statistically significantly higher haematocrit concentration (increase of 6%) and a noticeably lower platelet count (reduction of 17%) than controls.

Aspartate aminotransferase activity was statistically significantly reduced in males treated at 5,000 and 10,000 ppm, respectively (decreases of 20% and 31%, respectively). Statistically significant decreases in potassium ion concentrations were observed in males treated at 2,500, 5,000 and 10,000 ppm (decreases of 9%, 12% and 7%, respectively compared with controls). Females treated at 10,000 ppm had a notably lower cholesterol concentration and statistically significant lower triglyceride, total protein, albumin and globulin concentrations in comparison with controls (reductions of 24%, 30%, 11%, 11% and 10%, respectively). Statistically significant decreases in calcium ion concentrations were observed in females treated at 2,500, 5,000 and 10,000 ppm (decreases of 3%, 5% and 7% respectively, compared with controls).

Reproductive/developmental findings

Five female animals failed to litter, including three control females, one female treated at 5,000 ppm and one female treated at 10,000 ppm. It was also reported that two females in the 10,000 ppm group showed total litter loss on postnatal days 2 and 3. The total number of pups found dead or missing by postnatal day 4 was nearly 2.5 times higher in the 10,000 ppm group compared to the control group (an increase of 238%), leading to viability index in the 10,000 ppm group that was 41% lower than the control group. Furthermore, 10,000 ppm group produced heavy decreases in the average number of live pups per litter on postnatal days 4 and 7, compared to the control group (10.8 vs. 5.5 pups, and 10.8 vs. 5.4 pups, respectively). Average pup weight in the 10,000 ppm group was also noticeably decreased on postnatal days 1 and 4, and statistically significantly lower on postnatal day 7 in comparison with the control group (decreases of 11%, 19% and 17%, respectively). These changes were considered as test substance-related as they were greater than historical control values. The higher pup mortality in the 10,000 ppm group was thought to be primarily due to the observed decreases in pup weight at birth, which is often linked with failure to thrive and the subsequent presumed cannibalism by the dams. This reduced failure to thrive was also considered as related to the test substance.

Effects in Organs

No notable effects on absolute or relative organ weights, macroscopic findings or histopathological changes were observed in adult animals and their pups.

CONCLUSION

The findings in this study suggest that there may have been test substance-related reproductive issues occurring in female animals treated at 10,000 ppm. However, the authors of this study note that it was difficult to accurately interpret the findings from the study as there was an abnormally reduced number of pregnant dams that gave birth and had litters that survived until study termination. Hence, the authors of this study recommended the study to be repeated.

TEST FACILITY Envigo CRS Inc. (2017)

B.14. Repeat dose toxicity (second study; repeat of first study)

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (1996)
Species/Strain	Rat/Sprague-Dawley (Crl:CD BR)
Route of Administration	Oral – diet
Exposure Information	Total exposure days: - 35 days for males (2 weeks prior to mating, during mating (up to 2 weeks) and until scheduled necropsy) - Approx. 56 days for females (2 weeks prior to mating, during mating (up to 2 weeks) and until lactation day 6). - Females were given untreated feed on lactation day 6 through scheduled necropsy. Dose regimen: 7 days per week Post-exposure observation period: None
Necropsy	All surviving male animals were euthanised on study day 36. Female animals that produced litters were euthanised on lactation day 7. Female animals which failed to deliver a litter were euthanised on gestation day 25. Female animals with total litter loss (all pups found dead prior to scheduled sacrifice) were euthanised on the day of total litter loss.
Vehicle	None
Remarks - Method	Notable protocol deviations include: - On lactation day 6, female animals were given treated food instead of untreated food. All protocol deviations were not considered to have affected the integrity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Dose ppm (mg/kg bw/day)*	*Mortality*
control	10M/10F	0	0/20
low dose	10M/10F	2,500 (163/198)	0/20
mid dose	10M/10F	5,000 (335/380)	0/20
high dose	10M/10F	10,000 (624/731)	1/20

Mortality and Time to Death

One female treated at 10,000 ppm was found dead on gestational day 22, just after parturition. Based on the macroscopic findings during necropsy, this death was considered associated with parturition and not test substance-related.

Clinical Observations

The mean body weight change for female animals in the 10,000 ppm group during gestation was 15% lower than in the control group. This decrease was not accompanied by any changes in mean food consumption during the gestation period. On lactation day 7, the mean body weight for female animals treated at 5,000 ppm and 10,000 ppm were lower than the control group (decreases of 11 % and 12%, respectively). Average food consumption during day 6-7 of lactation in females treated at 5,000 ppm and 10,000 ppm was also lower than the control group (decreases of 13% and 30%, respectively).

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Female animals treated at 5,000, and 10,000 ppm showed dose-dependent decreases in reticulocyte count compared to controls (decreases of 25% and 34%, respectively). Female animals in the 10,000 ppm group also displayed increased platelet counts and decreased eosinophil counts in comparison with control animals (changes of 23% and 58%, respectively).

All clinical chemistry parameters were comparable between all groups in this study.

Reproductive/developmental findings

One female animal in the 2,500 ppm group failed to litter; this was much lower than in the previous repeat dose toxicity study. However, the number of female animals that had total litter loss was much higher than in the previous repeat dose toxicity study (see 'B.13. Repeat dose toxicity (first study)'). Total litter loss was 5/10,

2/10, 3/10 and 4/9 in the control, 2,500 ppm, 5,000 ppm and 10,000 ppm female groups, respectively. Due to these losses, the study was terminated as this had confounded the evaluation of parameters including post implantation index, live birth index, and viability index.

Effects in Organs

Small spleen was noted in two female animals in the 10,000 ppm group that had total litter loss. However, no other organ weight changes or macroscopic findings were reported.

CONCLUSION

Based on the abnormally high number of animals that experienced total litter loss, it is difficult to interpret the study data.

TEST FACILITY Envigo CRS Inc. (2018)

B.15. Repeat dose toxicity (third study)

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (2016)

Species/Strain Rat/Sprague-Dawley (Crl:CD)

Route of Administration Oral –diet

Exposure Information Total exposure days:

- 6 weeks for main group male animals (2 weeks prior to mating, during mating and until approx. 80% of the females had given birth)
- Approx. 8 weeks for main group female animals (2 weeks prior to mating, during mating and gestation and at least up to post-partum day 14).
- 6 weeks for high dose recovery animals. These animals were not mated during the study; they were exposed to the test substance for an equivalent duration to the main group male animals.

Dose regimen: 7 days per week

Post-exposure observation period: 14 days

Necropsy All surviving male animals were sacrificed after approx. 80% of female had delivered. Female animals that produced litters were sacrificed on post-partum day 15. Female animals which failed to deliver a litter were sacrificed on the 25th day after the last mating day. Recovery group animals were sacrificed 14 days after the first scheduled dam sacrifice.

Vehicle None

Remarks - Method No deviations from the study protocol were noted. The dose levels were selected based on the recommendation of the study sponsor (the notifier of the test substance).

RESULTS

Group	*Number and Sex of Animals*	*Dose ppm (mg/kg bw/day)*	*Mortality*
control	15M/15F	0	0/30
low dose	15M/15F	2,500 (148/175)	0/30
mid dose	15M/15F	5,000 (289/351)	1/30
high dose	15M/15F	10,000 (584/679)	0/30
control recovery	5M/5F	0	0/10
high dose recovery	5M/5F	10,000 (522/694)	0/10

Mortality and Time to Death

One female animal treated at 2,500 ppm was found dead on day 24 of gestation.

Clinical Observations

No clinical signs of toxicity were observed in any of the main group and recovery animals throughout the study.

There were no differences in mean body weights in main test and recovery groups during the study. High dose males had decreased mean body weight gain (statistically significant on Weeks 2 and 7) due to decreased food consumption.

High dose females had statistically significantly lower body weights than control females during day 14 of lactation (a decrease of 8%). The mean body weight gain for females in the 10,000 ppm group was also noticeably lower than controls on days 0-4, 4-7, 7-14 and 0-14 time points during the lactation period (decreases of 39%, 46%, 190% and 69%, respectively), although there were no differences in mean food consumption.

Laboratory Findings –Haematology, Clinical Chemistry, Urinalysis

Main group males and females treated at 10,000 ppm showed increased blood parameters compared to controls (white blood cell, neutrophil, lymphocyte, monocyte, eosinophil and basophil count increases of 32%, 30%, 53%, 39%, 80% and 17%, respectively in males and neutrophil and reticulocyte count increases of 23% and 30%, respectively in females).

Male animals treated at 10,000 ppm showed notable reductions in lactate dehydrogenase and creatinine kinase activity compared to controls (16% reduction in the activity of both enzymes); this trend continued in treated males after recovery (activity reductions of 14% and 13%, respectively). Male animals in the 10,000 ppm group also showed apparent increases in urea, blood urea nitrogen, total bilirubin and bile acid concentration (increases of 13%, 15%, 14% and 37%, respectively); these parameters were more comparable with controls following recovery. Glucose concentration was statistically significantly decreased, and triglyceride concentration was notably decreased in treated recovery male rats compared to controls (decreases of 9% and 37%, respectively). Decreased thyroid stimulating hormone levels were reported in treated males but were only statistically significant at 10,000 ppm (decreases of 28%, 26% and 38%, at 2,500, 5,000, and 10,000 ppm, respectively).

Lactate dehydrogenase and creatinine kinase activity were noticeably decreased in treated females but statistically significant only at 10,000 ppm (decreases of 16% and 17%, 36% and 33%, and 58% and 55%, respectively). These trends were partially reversed after recovery. Alkaline phosphatase activity was decreased in all treated females compared to controls (increases of 78%, 34% and 42% at 2,500, 5,000, and 10,000 ppm, respectively); this trend was partially reversed after recovery. High dose females showed statistically significant increases in urea and blood nitrogen urea concentrations (46% increase for both) and notable increases in total bilirubin and bile acid concentrations (increases of 30% and 18%, respectively); all trends were reversed after. Females treated at 2,500 and 10,000 ppm showed notable increases in thyroid stimulating hormone concentrations compared to controls (increases of 23% and 47%, respectively).

Decreases in urine volume were observed in all treated males compared to controls (decreases of 18%, 46%, and 30% at 2,500, 5,000, and 10,000 ppm, respectively), while females treated at 2,500 ppm and 10,000 ppm showed notable increases in urine volume compared to controls (increases of 27% and 23%, respectively). After recovery, the trend in males was reversed and the trend in females continued. Small amounts of bilirubin were detected in the urine of some males treated at 5,000 pm and 10,000 ppm. After recovery, no urine bilirubin was detected in any of the animals.

Reproductive/developmental findings

Fertility index, parturition index, percentage of pregnant animals, litter size, pup ano-genital distance, mating index and pup mortality were not affected. No consistent signs of toxicity were noted in the pups belonging to the control or treatment groups. The live birth index however, was statistically significantly lower in the 10,000 ppm group compared to the control group (7% decrease).

The combined mean body weight of pups of both sexes on lactation day 14 was statistically significantly lower at 5,000 and 10,000 ppm compared to the control group (reductions of 14% and 26%, respectively). The mean body weight gain over the entire lactation period for pups (both sexes) in these two groups was statistically significantly lower than the control group pups (decreases of 17%, and 32%, respectively).

Effects in Organs

The absolute and relative mean spleen weight for high dose males was elevated compared to controls during the main study (increases of 12% and 15%) and following recovery (16% and 19%). The mean absolute weight of the Cowper's gland in males treated at 2,500 and 10,000 was statistically significantly decreased compared to control animals (decreases of 19% and 17%, respectively) and not reversed during the recovery period.

High dose females showed decreased absolute and relative thymus weight compared to controls (32% and 28%, respectively), but reversed during recovery.

No consistent organ abnormalities were detected during necropsy.

CONCLUSION
The following were established by the study authors:
- The No Observed Adverse Effect Level (NOAEL) for parental toxicity as 2,500 ppm (equivalent to 148 mg/kg bw/day in males and 175 mg/kg bw/day in males);
- The NOAEL for reproductive toxicity as 10,000 ppm (equivalent to 553 mg/kg bw/day in males and 687 mg/kg bw/day in females)
- The NOAEL for developmental (offspring) toxicity as 2,500 ppm.

TEST FACILITY JRF (2018)

B.16. Genotoxicity – bacteria

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1997)
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria (2008)
Test 1: Plate incorporation procedure
Test 2: Pre incubation procedure

Species/Strain *Salmonella typhimurium*: TA1535, TA1537, TA98, TA100
Escherichia coli: WP2uvrA

Metabolic Activation System S9 fraction from phenobarbitone/β-napthoflavone induced rat liver.

Concentration Range in Main Test
Test 1
a) With metabolic activation: 1.5 – 5000 µg/plate
b) Without metabolic activation: 1.5 – 5000 µg/plate

Test 2
a) All *Salmonella* strains (+/- metabolic activation): 0.5 – 500 µg/plate
b) *Escherichia coli* (+/- metabolic activation): 5 - 5000 µg/plate

Vehicle DMSO

Remarks - Method No noted deviations from the study plan. The dose range for Test 1 had been determined prior to the study. The dose range used for Test 2 was determined by the results of Test 1.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 500	-	≥ 5000	Negative
Test 2	-	≥ 150	> 5000	Negative
Present				
Test 1	≥ 500	-	≥ 5000	Negative
Test 2	-	≥ 150	≥ 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 test substance was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY Harlan (2014c)

B.17. Genotoxicity – *in vitro* chromosome aberration test

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
EC Directive 2000/32/EC B.10 Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test (2008)

Species/Strain Human
Cell Type/Cell Line Lymphocytes
Metabolic Activation System S9 fraction from phenobarbitone/β-napthoflavone induced rat liver (S9-mix)
Vehicle DMSO
Remarks - Method No noted deviations from the study plan. A preliminary experiment was conducted to determine the dose range for the main test. The doses used in this experiment were: 7.1, 14.2, 28.5, 56.9, 113.8, 227.6, 455.3, 910.5 and 1821 µg/mL. Test 1 and 2 samples designated for metabolic activation were exposed to S9-mix at 2% and 1% concentration, respectively.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	30, 60*, 90*, 120*, 180, 240	4h	24h
Test 2	3.75, 7.5, 15, 30*, 60*, 90*, 180*	24h	24h
Present			
Test 1	30, 60*, 120*, 240*, 360, 480	4h	24h
Test 2	30, 60, 120*, 240*, 300*, 360*	4h	24h

*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	≥ 56.1	≥ 120	> 240	Negative
Test 2	≥ 14.2	> 180	> 180	Negative
Present				
Test 1	≥ 113.8	≥ 120	> 480	Negative
Test 2	-	≥ 360	> 360	Negative

*Preliminary toxicity test performed using the exposure conditions for Test 1 and Test 2

Remarks - Results In Tests 1 and 2, haemolysis was observed in the presence and absence of metabolic activation at dose concentrations ≥ 60 µg/mL and ≥ 30 µg/mL, respectively.

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 test substance was not clastogenic to human lymphocytes treated *in*

vitro under the conditions of the test.

TEST FACILITY Harlan (2014d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Oxygen Demand (ThOD)
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	61.7	7	72.8
14	68.8	14	70.2
25	76.5	25	-
28	78.8	28	-

Remarks - Results	All validity criteria of the test guideline were satisfied. The percentage degradation of the reference compound (sodium benzoate) surpassed the threshold level of 60% after 14 days (70%). Therefore, the tests indicate the suitability of the inoculums. Oxygen depletion in the inoculum blank was 0.73 mg/L on day 14. The residual concentrations of oxygen in the test bottles were greater than 0.5 mg/L during the test period. The percentage biodegradation in toxicity control at day 14 was 65.8%. The degree of degradation of the test substance after 28 days was 78% and reached the pass level at the end of 10-d window.
CONCLUSION	The test substance is readily biodegradable.
TEST FACILITY	Suzhou Research (2014)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test (1992)
Inoculum	Treated effluent
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. A nominal amount of test substance (50 mg) was dispersed in mineral medium (350 mL) and subjected to high shear mixing for 15 minutes prior to the addition of inoculum (5 mL) and adjusted to a final volume of 500 mL with mineral media to give the test concentration of 100 mg/L.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	43
14	10	14	69
21	17	21	68
28	21	28	67

Remarks – Results

The mean BOD of the inoculated mineral medium (control) was 46.35 mg O2/L after 28 days. The pH of the inoculated test substance vessels on Day 28 ranged from 7.5 to 7.6. The difference between extremes of replicate BOD values at the end of the test was less than 20%.

The study was conducted at 19 to 21 °C. On Day 26 of the test the temperature in the water bath was 19.4 °C. This was a deviation from the study plan which states the test will be conducted at a temperature of between 20 to 24 °C with a maximum deviation of ± 1 ºC. However, this deviation was not considered to have affected the integrity or validity of the study given that all validation criteria were met.

The percentage degradation of the reference compound (Aniline) surpassed the threshold level. Therefore, the tests indicate the suitability of the inoculums. The toxicity control attained 26% biodegradation after 14 days and 43% biodegradation after 28 days thereby confirming that the test item was not toxic to the sewage micro-organisms used in the test. The test substance attained 21% biodegradation after 28 days and therefore cannot be considered to be readily biodegradable under the strict terms and conditions of OECD Guideline 301F.

CONCLUSION The test substance is not readily biodegradable.

TEST FACILITY Envigo (2017k)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi Static (1992)

Species	Zebra fish *(Brachydanio rerio)*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	50 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC)
Remarks – Method	Desired amounts of the test substance were weighed and mixed with the test water to produce test solutions with nominal concentration (loading rates) of 1.0 mg/L, 1.5 mg/L, 2.3 mg/L, 3.4 mg/L, and 5.1 mg/L. The mixed solutions were stirred for about 5 hours. The test solutions were prepared every 24 hours just before use.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	0	10	0	0	0	0
1.0	0.84	10	0	0	0	0
1.5	0.82	10	0	0	0	1

2.3	1.27	10	0	0	1	2
3.4	2.1	10	0	1	2	5
5.1	-*	10	10	10	10	10

*As all fish died in the first 24 hours, the test concentration was not renewed or measured.

LC50 1.68 mg/L at 96 hours (95% confidence limits 1.39-1.65)

Remarks – Results All validity criteria for the test were met. The dissolved oxygen concentration was within the range of 60.6% - 98.3% air saturation value throughout the test.

The measured concentrations of the test solutions ranged from 9.3 % to 84.3 % of the nominal before renewing the test solution, which could not be maintained within 80%-120%. Therefore, the geometric means of the measured concentrations were used for the LC50 calculation.

The 96 h LC50 for fish was determined to be 1.68 mg/L, based on geometrical mean measured concentrations.

CONCLUSION The test substance is toxic to fish

TEST FACILITY Suzhou Research (2015)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 202 *Daphnia sp.* Acute Immobilisation Test – Semi Static

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness 220 mg $CaCO_3$/L
Analytical Monitoring GC-MS
Remarks - Method No significant protocol deviations.

A nominal amount of test substance (1100 mg) was dispersed in 11 litres of test water for 24 hours. Any undissolved test substance was removed by filtration to give a 100% w/v saturated solution. A series of dilutions was made from this saturated solution to give the required test concentrations of 10, 5.6, 3.2, 1.8 and 1.0% v/v saturated solutions.

RESULTS

Concentration (v/v)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
1.0	0.08	20	0	0
1.8	0.13	20	0	0
3.2	0.29	20	0	0
5.6	1.2	20	0	7
10	4.4	20	3	20

EC50 1.3 mg/L at 48 hours

The EC50 value at 48 hours and the slope of the response curve and its standard error were calculated by Probit analysis using Linear Maximum-Likelihood regression. However, this method is not regarded as the most appropriate where there is only one partial toxic response (5.6 mg/L).

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

The dissolved oxygen concentration at the end of the test was equal to or greater than 3 mg/L in the control and test vessels.

Analysis of the freshly prepared test solutions at 0 and 24 h showed measured test concentrations to range from 0.078 to 7.0 mg/L. A decline in measured test concentrations was also observed in the old or expired test solutions at 24 and 48 hours. Given this decline in measured test concentrations it was considered appropriate to calculate the results based on the geometric mean measured test concentration.

CONCLUSION Under the study conditions, the test substance is considered to be toxic to Daphnia on an acute basis.

TEST FACILITY Envigo (2018)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 201 Freshwater 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: 0.1 - 100 mg/L Actual: 0.089 - 71 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	GC-MS
Remarks - Method	No significant protocol deviations.

A nominal amount of test substance (1100 mg) was dispersed in 11 litres of test water for 24 hours. Any undissolved test substance was removed by filtration to give a 100% w/v saturated solution. A series of dilutions was made from this saturated solution to give the required test concentrations of 0.1, 1.0, 10 and 100% w/v saturated solutions.

The definitive test was conducted at 20.8-24.0 °C, outside the 21-24 °C range stated in the protocol. The coefficient of variation for the average specific growth rates in the control cultures between 48 and 72 hours was 37%, just exceeding the 35% criteria stated in the protocol. Neither deviation from protocol was deemed to have had a significant impact on the validity or integrity of the study.

RESULTS

Biomass		*Growth*	
E_bC50	*NOE_bC*	*E_rC50*	*NOE_rC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
1.9*	0.86	4.6 (95% CL 3.5-6.1)	0.86

* It was not possible to calculate 95% confidence limits for the E_yC50 value as the data generated did not fit the models available for the calculation of confidence limits.
CL: Confidence Limits

Remarks - Results Most of the validity criteria for the test were satisfied. The cell concentration of the control cultures increased by a factor of 92 over the test period. The mean of the coefficients of variation of growth rates in the control cultures during the course of the test (days 0-1, 1-2 and 2-3) was 20%. The coefficient of variation of the average specific growth rate in replicate control cultures was 3%. The 72 h E_bC50 and E_rC50 were

	determined to be 1.9 and 4.6 mg/L respectively, based on geometric mean measured concentrations. The 72 h NOEC was determined to be 0.86 mg/L. All statistical analyses were performed using the SAS computer software package (SAS, 1999 - 2001). Analysis of the freshly prepared test solutions at 0 h showed measured test concentrations to range from 0.62 to 77 mg/L. A decline in measured test concentrations was also observed in the old or expired test solutions at 24, 48 and 72 hours. Given this decline in measured test concentrations it was considered appropriate to calculate the results based on the geometric mean measured test concentration
CONCLUSION	Under the study conditions, the test substance is considered to be harmful to algae.
TEST FACILITY	Envigo (2017l)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test (2010)
Inoculum	Aerated activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 100 - 1000 mg/L Actual: Not determined
Remarks – Method	No deviations from the study plan. Following a preliminary range-finding test, activated sewage sludge was exposed to an aqueous dispersion of the test substance at concentrations of 100, 180, 320, 560 and 1000 mg/L for a period of 3 hours at measured temperatures of approximately 21 °C with the addition of a synthetic sewage as a respiratory substrate. 3,5-dichlorophenol was used as the reference control. The respiration rate was determined by measurement of BOD during the test after 3 hours of exposure.
RESULTS	
EC50	410 mg/L (95% confidence limits: 380 - 440 mg/L) at 3 hours.
Remarks – Results	All validity criteria for the test were satisfied. The coefficient of variation of oxygen uptake in the control vessels was 4.2% and the specific respiration rate of the controls was 30.03 mg oxygen per gram dry weight of sludge per hour. The 3-hour EC50 was determined to be 410 mg/L, based on nominal concentrations. The reference substance gave a 3-hour EC50 value of 7.5 mg/L.
CONCLUSION	The test substance is not inhibitory to microbial activity.
TEST FACILITY	Envigo (2017m)

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