File No.: LTD/2111
LTD/2112

February 2020

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

LTD/2111: 1,5-Octadiene, 2-methoxy-6-methyl-
LTD/2112: 2,5-Octadiene, 2-methoxy-6-methyl-

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 Agriculture, Water and the Environment.

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

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

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2111	International Flavours and Fragrances (Australia) Pty Ltd	1,5-Octadiene, 2-methoxy-6-methyl-	Yes	≤ 1 tonne per annum	Fragrance ingredient
LTD/2112	International Flavours and Fragrances (Australia) Pty Ltd	2,5-Octadiene, 2-methoxy-6-methyl-	Yes	≤ 1 tonne per annum	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 hazard classification applicable to the notified chemicals is presented in the following table.

Hazard Classification	*Hazard Statement*
Flammable liquids (Category 4)	H227 – Combustible liquid
Skin corrosion/irritation (Category 2)	H315 – Causes skin irritation
Sensitisation, Skin (Category 1)	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*
Chronic Aquatic (Category 2)	H411 – Toxic to aquatic life with long lasting effects

Human Health Risk Assessment
Under the conditions of the occupational settings described, the notified 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:
  - Flammable liquids (Category 4): H227 – Combustible liquid
  - Skin corrosion/irritation (Category 2): H315 – Causes skin irritation
  - Sensitisation, Skin (Category 1): 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:
  - Enclosed/automated processes, where possible
  - Local exhaust ventilation

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

- 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:
  - Impervious gloves
  - Protective clothing
  - Respiratory protection if aerosols or mists are expected to be generated

  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.

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 collected using an inert absorbent material and appropriately sealed in labelled drums.

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.

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 chemicals, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemicals are 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 final use concentration of the isomer mixture containing the notified chemicals exceeds 0.45% in body lotions, 0.095% in face creams, 0.1% in hand creams, 0.07% in fine fragrances, 0.035% in deodorants, 0.67% in hair styling products, and 1% in other cosmetic products or household products;

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 notified chemicals provided by the notifier was reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for hydrolysis as a function of pH, water solubility and dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
European Union (2019)
China (2019)

2. IDENTITY OF CHEMICAL

MARKETING NAME
PG-RAW-0004 (isomer mixture containing the notified chemicals at 95.5% concentration)

CAS NUMBER
LTD/2111: 2109705-93-1
LTD/2112: 2111193-86-1

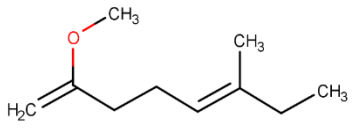
CHEMICAL NAME
LTD/2111: 1,5-Octadiene, 2-methoxy-6-methyl-
LTD/2112: 2,5-Octadiene, 2-methoxy-6-methyl-

OTHER NAME
None

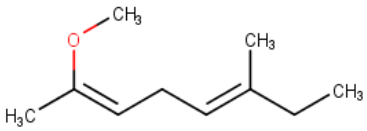
MOLECULAR FORMULA
LTD/2111: $C_{10}H_{18}O$
LTD/2112: $C_{10}H_{18}O$

STRUCTURAL FORMULA

LTD/2111:



LTD/2112:



MOLECULAR WEIGHT
LTD/2111: 154.25 g/mol
LTD/2112: 154.25 g/mol

ANALYTICAL DATA
Reference NMR, IR, GC, GC-MS, UV spectra were provided. All analytical data were obtained on the isomer mixture containing the notified chemicals.

3. COMPOSITION

DEGREE OF PURITY
95.5% (for the isomer mixture)

The notified chemicals are manufactured overseas as an isomer mixture. The composition of the notified chemicals in the isomer mixture is as follows (with double bond geometries).

	Notified chemical	*Weight %*
LTD/2111	1,5-Octadiene, 2-methoxy-6-methyl-, (5*Z*)-	22.4
	1,5-Octadiene, 2-methoxy-6-methyl-, (5*E*)-	35.2
LTD/2112	2,5-Octadiene, 2-methoxy-6-methyl-, (2*Z*,5*Z*)-	3.9
	2,5-Octadiene, 2-methoxy-6-methyl-, (2*Z*,5*E*)-	4.9
	2,5-Octadiene, 2-methoxy-6-methyl-, (2*E*,5*Z*)-	12.6
	2,5-Octadiene, 2-methoxy-6-methyl-, (2*E*,5*E*)-	16.7

IDENTIFIED IMPURITIES

Chemical Name	5-Octen-2-one, 6-methyl-, (*E*)-		
CAS No.	18437-34-8	*Weight %*	≤ 5
Hazardous Properties	Unknown		

Chemical Name	5-Octen-2-one, 6-methyl-, (5*Z*)-		
CAS No.	18437-35-9	*Weight %*	≤ 5
Hazardous Properties	Unknown		

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear colourless liquid (neat isomer mixture)

Property	***Value***	***Data Source/Justification***
Freezing Point*	< -20 °C	Measured
Boiling Point*	188 °C at 102 kPa	Measured
Density*	841 kg/m^3 at 20 °C	Measured
Vapour Pressure*	3.84×10^{-2} kPa at 25 °C	Measured
Water Solubility	5.18×10^{-3} g/L at 20 °C	QSAR (US EPA 2012)
Hydrolysis as a Function of pH	Not determined	Contains functionality that will hydrolyse at environmental pH 4-9.
Partition Coefficient (n-octanol/water)*	log Pow = 2.75 – 4.86 at 20 °C	Measured
Surface Tension*	56.5 mN/m at 20 °C	Measured
Adsorption/Desorption*	log K_{oc} = 2.16 – 3.18	Measured
Dissociation Constant	Not determined	Does not contain dissociable functionality
Flash Point*	64 °C at 101.2 kPa	Measured
Flammability	Combustible liquid	Based on flash point
Autoignition Temperature*	240 °C	Measured
Explosive Properties	Predicted negative	Based on chemical structure
Oxidising Properties	Predicted negative	Based on chemical structure

* Property of the isomer mixture containing the notified chemicals

DISCUSSION OF PROPERTIES
For details of tests on physical and chemical properties of the isomer mixture containing the notified chemicals, 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 notified chemicals are recommended for physical hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard Classification	***Hazard Statement***
Flammable liquids (Category 4)	H227 – Combustible liquid

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemicals are constituents of an inseparable isomer mixture, which will be imported as components of fragrance oils. The fragrance oils will contain the isomer mixture at ≤ 10% concentration.

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

LTD/2111

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

LTD/2112:

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

PORT OF ENTRY
Melbourne

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

TRANSPORTATION AND PACKAGING
The notified chemicals will be imported as constituents of fragrance oils (containing the isomer mixture at ≤ 10% concentration) in 208.2 L polypropylene-lined steel drums. The imported products containing the notified chemicals will be transported to the notifier's warehouse for storage and then distributed to reformulation sites within Australia by road. The reformulated end-use products containing the isomer mixture of the notified chemicals at ≤ 1% concentration will be packaged in containers suitable for retail sale.

USE
The notified chemicals will be used as fragrance ingredients. The notified chemicals are manufactured overseas as an inseparable isomer mixture. The inseparable isomer mixture will be imported as a component of fragrance oils at ≤ 10% concentration and reformulated into a variety of cosmetic and household products in Australia.

The proposed use concentrations of the isomer mixture of the notified chemicals in finished consumer products are shown in 6.1.2.

OPERATION DESCRIPTION
Reformulation of fragrance oil formulations containing the notified chemicals (at ≤ 10% concentration for the isomer mixture) into finished consumer goods may vary depending on the type of product and may involve both automated and manual transfer steps. Typically, reformulation processes may incorporate blending operations that

are highly automated and occur in a fully enclosed/contained environment, followed by automated filling of the reformulated end-use products into containers of various sizes.

End-use products containing the notified chemicals (at ≤ 1% concentration for the isomer mixture) will be used by consumers and professionals such as hairdressers, beauticians or cleaners. Depending on the nature of the product, these could be applied in a number of ways, such as by hand, using an applicator or sprayed.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	Unknown	Unknown
Mixing	4	250
Drum handling	1	250
Drum cleaning	2	200
Equipment Maintenance	2	250
Quality Control	1	250
Professional users - hairdressers, cleaners etc.	8	250

EXPOSURE DETAILS

Transport and storage
Transport and storage workers may come into contact with the notified chemicals as components of fragrance oils or as components of end-use products only in the unlikely event of accidental rupture of containers.

Reformulation
During reformulation, dermal and ocular exposure of workers to the notified chemicals (at ≤ 10% concentration for the isomer mixture) may occur during weighing and transfer stages, blending, quality control analysis, and cleaning and maintenance of equipment. Due to the low vapour pressure of the notified chemicals (3.84×10^{-2} kPa at 25 °C for the isomer mixture), inhalation exposure is not expected, unless aerosols or mists are formed.

The notifier states that exposure is expected to be minimised through the use of local exhaust ventilation and/or enclosed systems, and through workers using personal protective equipment (PPE) such as protective clothing, goggles, impervious gloves and respiratory protection (in cases where there is inadequate ventilation).

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

Data on typical use patterns of cosmetic and household cleaning product categories (SCCS, 2012; Cadby *et al*., 2002; ACI, 2010; Loretz *et al*., 2006), in which the isomer mixture of the notified chemicals may be used are shown in the following tables. For the purposes of exposure assessment via the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal

absorption data, a dermal absorption (DA) of 100% was assumed for the notified chemicals (ECHA, 2017). For inhalation exposure assessment, taking hairspray as a typical example, a 2-zone approach was used (Steiling *et al*., 2014; Rothe *et al*., 2011; Earnest, Jr., 2009). An adult inhalation rate of 20 m^3/day (enHealth, 2012) was applied and it was conservatively assumed that the fraction of the notified chemicals inhaled is 50% of the amount sprayed, with remaining fraction ending up on the hair as intended. 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	0.450	1	0.5498
Face cream	1540	0.095	1	0.0229
Hand cream	2160	0.1	1	0.0338
Fine fragrances	750	0.07	1	0.0082
Deodorant (spray)	1430	0.035	1	0.0078
Deodorant (non-spray)	1500	0.035	1	0.0082
Shampoo	10460	1	0.01	0.0163
Hair conditioner	3920	1	0.01	0.0061
Shower gel	18670	1	0.01	0.0292
Hand wash soap	20000	1	0.01	0.0313
Hair styling products	4000	0.67	0.1	0.0419
Facial cleanser	800	1	0.01	0.0013
Total				**0.7567**

C = concentration (%); RF = Retention Factor
Daily systemic exposure = (Amount × C × RF × dermal absorption) / body weight

Hair spray (inhalation exposure)

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

Total daily systemic exposure = daily systemic exposure in zone 1 [(amount × C × inhalation rate × exposure duration (zone 1) × fraction inhaled) / (volume (zone 1) × body weight)] + daily systemic exposure in zone 2 [(amount × C × inhalation rate × exposure duration (zone 2) × fraction inhaled) / (volume (zone 2) × body weight)]

Household products (Indirect dermal exposure – from wearing clothes)

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

Daily systemic exposure = (amount × C × PR × PT) / body weight

Household products (Direct dermal exposure)

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

Daily systemic exposure = (frequency × C × contact area × product use concentration × film thickness on skin × time scale factor × dermal absorption) / body weight

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemicals. This would result in a combined internal dose of 0.8609 mg/kg bw/day for the isomer mixture. It is acknowledged that inhalation exposure to the isomer

mixture of the notified chemicals from use of other spray cosmetic and household products in addition to hair spray may occur. However it is considered that the combination of the conservative (screening level) 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 lower exposure factors.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the neat isomer mixture containing the notified chemicals are summarised in the following table. For details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Acute oral toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Skin corrosion – *in vitro* EpiDerm™ Reconstructed Human Epidermis Model	non-corrosive
Skin irritation – *in vitro* EpiSkin™ Reconstituted Human Epidermis Model	non-irritating
Skin irritation – rabbit	irritating
Eye irritation – *in vitro* Bovine Corneal Opacity and Permeability Assay	not corrosive or irritating (no classification required)
Eye irritation – *in vitro* EpiOcularTM Human Cornea Model Test	non-irritating (no classification required)
Skin sensitisation – HRIPT (2.5% notified chemicals)	no evidence of sensitisation
Skin sensitisation – *in chemico* DPRA test	positive
Skin sensitisation – *in vitro* KeratinoSens luciferase test	positive
Skin sensitisation – *in vitro* h-CLAT test	positive
Repeat dose oral toxicity – rat, 28 days	NOAEL = 300 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test in human lymphocytes	non genotoxic

Toxicokinetics

No toxicokinetic data were provided for the notified chemicals. Given the low molecular weight of the notified chemicals (154.25 g/mol) and partition coefficient (log Pow = 2.75 – 4.86 at 20 °C), absorption across biological membranes may occur.

Acute Toxicity

The isomer mixture containing the notified chemicals is expected to have low acute oral toxicity based on a study conducted in rats. No dermal or inhalation toxicity data were provided for the notified chemicals.

Irritation

Two *in vitro* studies were performed using reconstructed human epidermis models. Under the conditions of the EpiDerm™ Model assay, the isomer mixture containing the notified chemicals was not considered to be corrosive to the skin. Under the conditions of the EpiSkin™ Model assay, the isomer mixture was not considered as a skin irritant. However, the isomer mixture was found to be irritating to the skin when tested in one female rabbit. The skin reactions were not fully recovered at the end of the study period (14 days). Based on the results of this study, the notified chemicals warrant hazard classification for skin irritation according to the GHS criteria.

Sensitisation

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 the isomer mixture containing the notified chemicals.

The *in chemico* direct peptide reactivity assay (DPRA) aims to address the first key event (molecular initiation) of the AOP by measuring the interaction of the notified chemicals with cysteine and lysine, small synthetic peptides representing the nucleophilic centres in skin proteins. The ARE-Nrf2 Luciferase Assay aims to address the second key event (keratinocyte activation) of the AOP by measuring the expression of a report luciferase gene under the

control of a promoter from the antioxidant response element (ARE), a responding gene known to be upregulated by contact sensitisers. The *in vitro* h-CLAT assay aims to address the third key event (dendritic cell activation) of the AOP by measuring the expression of cell surface markers (such as CD54 and CD86) in human monocyte leukaemia cells (THP-1) upon stimulation with the isomer mixture containing the notified chemicals.

The isomer mixture showed positive responses in all three key event assays of the AOP (DPRA assay, KeratinoSens test and h-CLAT test), confirming its skin sensitisation potential. Based on these results, the notified chemicals warrant hazard classification for skin sensitisation.

The isomer mixture did not induce skin sensitisation effects in a human repeat insult patch test (HRIPT) conducted at 2.5% concentration in 106 subjects.

Repeated Dose Toxicity
In a 28-day repeated dose oral toxicity study in rats with a 14-day recovery period, the isomer mixture containing the notified chemicals was administered daily by oral gavage at dose levels of 0, 100, 300 and 1,000 mg/kg bw/day. All animals made the expected body weight gains.

Statistically significant increases in mean liver weight were observed in males and females in the mid- and high-dose groups when compared to control animals. Microscopically, minimal to mild degree of focal and/or diffuse hepatocellular hypertrophy of liver was observed in males and females in the mid- and high-dose groups and bile duct proliferation was observed in male and females of the high-dose group. Microscopic changes of liver observed in the high-dose animals were considered by the study authors as test substance-related adverse effects as they were associated with increases in liver weight (mild), hepatic enzymes (ALT/ALP), total bilirubin and bile acids. However, the mid-dose animals did not show any evidence of altered hepatic function except for minimal increase in liver weights in correlation to hepatocellular hypertrophy, and hence were considered by the study authors as test substance-related non-adverse changes and adaptive response to metabolic change. Therefore, the No Observed Adverse Effect Level (NOAEL) was established as 300 mg/kg/day.

Mutagenicity/Genotoxicity
The isomer mixture containing the notified chemicals was non-mutagenic in a bacterial reverse mutation assay and non-genotoxic in an *in vitro* mammalian chromosome aberration test in cultured human lymphocytes.

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

Hazard Classification	***Hazard Statement***
Skin corrosion/irritation (Category 2)	H315 – Causes skin irritation
Sensitisation, Skin (Category 1)	H317 – May cause an allergic skin reaction

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available toxicological information, the isomer mixture containing the notified chemicals is a skin irritant and a skin sensitiser.

Reformulation
Exposure of workers to the notified chemicals (at ≤ 10% concentration for the isomer mixture) may occur during reformulation. The use of local exhaust ventilation, enclosed/automated processes and PPE (i.e. protective clothing, impervious gloves, goggles and respiratory protection (if inhalation exposure may occur) are expected to minimise the potential for exposure

Therefore, provided control measures are in place to minimise worker exposure, the risk to workers from use of the notified chemicals is not considered to be unreasonable.

End-use
Cleaners and beauty care professionals will be exposed to the notified chemicals (at ≤ 1 % concentration for the isomer mixture) in end use products, similar to the public. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. Therefore the risk to workers who use products containing the notified chemicals is expected to be of a similar or lesser extent than consumers who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health

Members of the public may experience repeated exposure to the notified chemicals (at ≤ 1% concentration for the isomer mixture) in individual products through the use of cosmetic and household products. The main route of exposure is expected to be dermal, while ocular and inhalation exposure are also expected where products are applied by spray.

Irritation
The isomer mixture containing the notified chemicals is a skin irritant. However, irritant effects are not expected from the use of products containing the isomer mixture at the proposed low use concentration of ≤ 1% in cosmetic and household products.

Skin sensitisation
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 shown in the table below, the Consumer Exposure level (CEL) from use of the isomer mixture containing the notified chemicals in a number of different cosmetic products may be estimated (SCCS, 2012 and Cadby *et al,* 2002).

The isomer mixture is considered to be a skin sensitiser based on the results of a DPRA assay, a KeratinoSens test and a h-CLAT test. When tested in a human repeat insult patch study, the isomer mixture containing the notified chemicals at 2.5% concentration was determined as non-sensitising. Consideration of the details of the study, and application of appropriate safety factors, allowed the derivation of an Acceptable Exposure Level (AEL) of 2.66 μg/cm^2/day. In this instance, the factors employed included an intraspecies factor (10), a matrix factor (3.16), a use and time factor (3.16) and a database uncertainty factor (3.16), giving an overall safety factor of ~300.

Product type	Proposed usage concentration (%)	CEL chemicals (μg/cm^2)	AEL chemicals (μg/cm^2)	Recommended usage concentration (%)
Body lotion	≤ 0.45	2.25	2.66	≤ 0.533
Fine fragrances	≤ 0.07	2.63	2.66	≤ 0.071
Face cream	≤ 0.095	2.59	2.66	≤ 0.098
Hand cream	≤ 0.1	2.51	2.66	≤ 0.106
Hair styling products	≤ 0.67	2.65	2.66	≤ 0.672
Deodorant (spray)	≤ 0.035	2.50	2.66	≤ 0.037
Deodorant (non-spray)	≤ 0.035	2.63	2.66	≤ 0.035
Rinse-off cosmetics (assumed: hand wash soap)	≤ 1	2.33	2.66	≤ 1.144

As the AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of the isomer mixture at a concentration of ≤ 0.45% in body lotion, ≤ 0.07% in fine fragrances, ≤ 0.095% in face cream, ≤ 0.1% in hand cream, ≤ 0.035% in deodorant, ≤ 0.67% in hair styling products and ≤ 1% in rinse-off cosmetic products (using hand wash soap as a worst case example) is not considered to be unreasonable. Based on lower expected exposure level from other cosmetic products and household products, by inference, the risk of induction of sensitisation associated with the use of these products is also not considered to be unreasonable. However, it is acknowledged that consumers may be exposed to multiple products containing the notified chemicals, and a quantitative assessment based on aggregate exposure has not been conducted.

Repeat dose toxicity
The potential systemic exposure to the public from use of the notified chemicals as an isomer mixture in cosmetic and household products was estimated to be 0.8609 mg/kg bw/day. Using a NOAEL of 300 mg/kg bw/day, which was derived from a 28-day repeated dose toxicity study on the notified chemicals as an isomer mixture, the margin of exposure (MOE) was estimated to be 348. A MOE value greater than or equal to 100 is considered acceptable

to account for intra- and inter-species differences, and to account for long-term exposure. Therefore the MOE is considered to be acceptable.

Overall, based on the information available, the risk to the public associated with the use of the notified chemicals as an isomer mixture at ≤ 0.45% in body lotions, ≤ 0.095% in face creams, ≤ 0.1% in hand creams, ≤ 0.07% in fine fragrances, ≤ 0.035% in deodorants, ≤ 0.67% in hair styling products, and ≤ 1% in other cosmetic products or household products, is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemicals will not be manufactured in Australia. The notified chemicals will be imported into Australia as part of an inseparable mixture in fragrance oils for reformulation into cosmetics, detergents and other household cleaning products. In general, the reformulation process involves automatic blending operations in closed systems, followed by automatic filling of the reformulated products into end-use containers. Accidental spills of the notified chemicals during import, storage, transport, reformulation and equipment washings are to be collected and disposed of either to an on-site wastewater treatment plant or a licenced waste disposal contractor.

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemicals will be washed down the sewer from their use as fragrances in cosmetics, detergents and other household cleaning products.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemicals may remain in the end use product containers (estimated at 1%) which will be collected for recycling. Wash water from the recycling process containing the notified chemicals are expected to be released to surface waterways. Some of the notified chemicals are also expected to be disposed of to landfill through the disposal of empty containers.

7.1.2. Environmental Fate

Following their use as fragrances in cosmetics and cleaning agents, the majority of the notified chemicals are expected to be released into the sewer system and treated at sewage treatment plants (STP) before potential release to surface waters nationwide. The notified chemicals are moderately volatile, therefore it is expected that a significant proportion of the notified chemicals will volatilise to air. The half-life of the notified chemicals in air is calculated to be 0.812 h based on reactions with hydroxyl radicals (US EPA, 2012; AOPWIN v1.92). Therefore, the notified chemicals are not expected to persist in the air compartment.

The notified chemicals are poorly soluble but readily hydrolysed in water. Two biodegradation studies indicated the isomer mixture of the notified chemicals were readily biodegradable. In another biodegradation study the isomer mixture of the notified chemicals were found to be not readily biodegradable however a significant amount of biodegradation (46%) had occurred after 28 days. The studies were likely complicated by the hydrolysability and high volatility of the notified chemicals. On the weight of evidence the notified chemicals are considered to be readily biodegradable. For details on the environmental fate studies see Appendix C.

Due to their log Pow and Koc, another significant proportion of the notified chemicals are expected to sorb to sludge in STPs. In surface waters, the notified chemicals are expected to partition to suspended solids and organic matter. A proportion of the notified chemicals are expected to bioaccumulate based on their partition coefficient range (log Pow = 2.75 – 4.86). However, the notified chemicals are not expected to be significantly bioavailable due to insignificant release to surface waters. The sewage sludge containing notified chemicals may be sent to landfill or applied to soils for land remediation. The notified chemicals are expected to ultimately degrade biotically and abiotically to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The use pattern will result in most of the notified chemicals being washed into the sewer. The predicted environmental concentration (PEC) has been calculated based on the realistic scenario with 100% release of the notified chemicals into sewer systems nationwide over 365 days per annum. The extent to which the notified chemicals are removed from the effluent in STP processes is based on the physico-chemical properties and its

ready biodegradability, modelled by SimpleTreat 3.0 (Struijs, 1996), is estimated as 95%. The PEC in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume*	2,000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	2,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	5.48	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	95	%
Daily effluent production:	4,877	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC – River:	0.06	µg/L
PEC – Ocean:	0.01	µg/L

* Combined volume for LTD/2111 and LTD/2112

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 0.337 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemicals may approximate 0.002 mg/kg in applied soil. This assumes that degradation of the notified chemicals occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 0.01 mg/kg and 0.02 mg/kg, respectively.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the neat isomer mixture of 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	96 h LC50 = 3.8 mg/L	The test substance is toxic to fish
Daphnia Toxicity	48 h EC50 = 2.8 mg/L	The test substance is toxic to aquatic invertebrates
Algal Toxicity	72 h ErC50 = 1.3 mg/L	The test substance is toxic to algae
Earthworm Toxicity	14 d EC50 = 492 mg/kg	The test substance is slightly toxic to earthworms

A worst case scenario for release of the notified chemicals to soil from STP biosolids indicates that the notified chemicals will reach concentrations of 0.002 mg/kg, over 1 year. Even using the most conservative assessment factor of 1,000, the worst case 1 year soil concentration is well below the PNEC of 0.492 mg/kg derived from the earthworm toxicity (14 d LC50). It also does not take into account any degradation or dissipation of the notified chemicals from soil and hence is a conservative estimate.

Based on the above ecotoxicological endpoints for the notified chemicals, they are expected to be acutely toxic to aquatic organisms and slightly toxic to terrestrial life. Therefore, the notified chemicals are classified as "Acute Category 2; Toxic to aquatic life" according to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). Since one chronic endpoint is available (algae NOEC) the long-term hazard is determined based on the most stringent outcome determined from both the acute and chronic data. Therefore, the notified chemicals are formally classified as "Chronic Category 2; Toxic to aquatic life with long lasting effects" under the GHS for its long-term hazard.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) of the notified chemical was calculated using the most sensitive ecotoxicity endpoint provided (72 h algae ErC50 = 1.30 mg/L). An assessment factor of 100 was used as three aquatic endpoints encompassing three trophic levels were available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
ErC50 (algae)	1.30	mg/L
Assessment Factor	100	

Mitigation Factor	1.00	
PNEC	13.0	µg/L

7.3. Environmental Risk Assessment

The risk quotient is calculated as the ratio of the PEC to PNEC:

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q – River	0.06	13	**0.004**
Q – Ocean	0.01	13	**0.001**

The risk quotient has been calculated based on the assumption of release of 100% of the notified chemicals introduced into Australia will go into the sewers. Since the Q value determined was much less than 1 for both river and ocean compartments, the notified chemicals are unlikely to reach ecotoxicologically significant concentrations based on the proposed annual importation and use patterns. A majority of the notified chemicals is expected to partition to air and break down due to hydrolysis and biodegradation. On the basis of the PEC/PNEC ratio, the notified chemicals are not considered 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 EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	Tested on a mixture of the two notified chemicals with a purity of 95.5%. The test substance had not solidified at -21 °C.
Test Facility	Envigo (2018a)

Boiling Point 188 °C at 102.0 kPa

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Differential scanning calorimeter (DSC) procedure. Tested on a mixture of the two notified chemicals with a purity of 95.5%.
Test Facility	Envigo (2019a)

Density 841 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Pycnometer procedure. Tested on a mixture of the two notified chemicals with a purity of 95.5%.
Test Facility	Envigo (2019a)

Vapour Pressure 0.0384 kPa at 25 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Tested on a mixture of the two notified chemicals with a purity of 95.5%. Isoteniscope procedure.
Test Facility	Envigo (2018b)

Water Solubility Could not be determined.

Method	OECD TG 105 Water Solubility
Remarks	Column Elution Method. The test substance underwent significant hydrolysis or transformation in solution.
Test Facility	Envigo (2018a)

Partition Coefficient (n-octanol/water) log Pow = 2.75 – 4.86 (5 peaks) at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method/Flask Method
Test Facility	Envigo (2018a)

Surface Tension 56.5 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions EC Council Regulation No 440/2008 A.5 Surface Tension
Remarks	Tested on a mixture of the two notified chemicals with a purity of 95.5%. Concentration: 90% saturation concentration. Ring procedure. Test substance is considered to be surface-active.
Test Facility	Envigo (2019a)

Adsorption/Desorption – screening test $\log K_{oc} = 2.16 – 3.18$ (3 Peaks)

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks	HPLC Method
Test Facility	Envigo (2019b)

Flash Point 64 °C at 101.2 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Tested on a mixture of the two notified chemicals with a purity of 95.5%. Closed cup procedure.
Test Facility	Envigo (2019c)

Autoignition Temperature 240 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases) EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids
Remarks	Tested on a mixture of the two notified chemicals with a purity of 95.5%. Flask heater procedure.
Test Facility	Envigo (2019c)

Explosive Properties Not expected to have explosive properties

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Estimated from the chemical structure of the two notified chemicals which form the mixture.
Test Facility	Envigo (2019c)

Oxidising Properties Not expected to have oxidising properties

Method	EC Council Regulation No 440/2008 A.21 Oxidising Properties.
Remarks	Estimated for the chemical structure of the two notified chemicals which form the mixture.
Test Facility	Envigo (2019c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat, Fixed Dose

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	Corn oil
Remarks – Method	No significant protocol deviations

RESULTS

Sighting Study

Dose (mg/kg bw)	*Administered*	*Evident Toxicity*	*Mortality*
300	1 F	Nil	0/1
2,000	1 F	Clinical effects	0/1

Signs of Toxicity	Piloerection, hunched posture, underactive behaviour, partially closed eyelids and unsteady gait were observed from 2 - 3 hours following treatment at 2,000 mg/kg bw. Recovery was observed on Day 2. No clinical signs were observed in the animal treated at 300 mg/kg bw.
Effects in Organs	No abnormalities were observed during necropsy.

Main Study

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	4 F	2,000	0/4

Discriminating Dose	> 2,000 mg/kg bw
Signs of Toxicity	Piloerection, hunched posture, underactive behaviour, partially closed eyelids and unsteady gait were observed from 1 - 4 hours following treatment. Recovery was complete by Day 2.
Effects in Organs	No abnormalities were observed during necropsy.
Remarks – Results	All animals showed expected body weight gains during the study period.
CONCLUSION	The test substance is of low acute toxicity via the oral route.
TEST FACILITY	Envigo (2019d)

B.2. Skin Corrosion – *In Vitro* EpiDerm™ Skin Corrosion Test

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 431 *In vitro* Skin Corrosion – Human Skin Model Test EpiDerm™ Reconstructed Human Epidermis Model
Vehicle	None
Remarks – Method	No significant protocol deviations. In a preliminary test the test substance was shown to directly reduce MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide]. Therefore, the study was performed in parallel on viable and freeze-killed tissues. Negative control: Sterile distilled water Positive control: 8.0 N Potassium hydroxide

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*		*Relative Mean Viability (%)*	
	3 min	*60 min*	*3 min*	*60 min*
Negative control	1.981 ± 0.018	1.864 ± 0.050	100	100

Test substance	2.042 ± 0.085	1.684 ± 0.001	103.1	90.3
Positive control	0.078 ± 0.012	0.066 ± 0.003	3.9	3.5

OD = optical density

Remarks – Results

The results from the additional procedure using freeze-killed tissues showed interference due to the test substance's ability to directly reduce MTT. It was considered by the study authors to be negligible and unnecessary to use the results from the additional procedure for quantitative correction. The data for the MTT reduction potential and the additional procedure were not included in the study report.

Based on the mean tissue viability of ≥ 50% after 3 min exposure and ≥ 15% after 60 min exposure, the test substance is not predicted to be corrosive according to the test guidelines, using GHS criteria.

Positive and negative controls performed as expected.

CONCLUSION

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

TEST FACILITY

Envigo (2019e)

B.3. Skin Irritation – *In Vitro* EpiSkin™ Reconstituted Human Epidermis Test

TEST SUBSTANCE

Isomer mixture containing the notified chemicals at 95.5% purity

METHOD

OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method
EpiSkin™ Reconstituted Human Epidermis Model

Vehicle

None

Remarks – Method

No significant protocol deviations. In a preliminary test the test substance was shown to possibly reduce MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide] directly. The test solution did not turn blue/purple, but a dark yellow colour. Therefore, the study was performed in parallel on viable and water-killed tissues.

Negative control: Dulbecco's Phosphate Buffered Saline with Ca^{++} and Mg^{++}
Positive Control: Sodium dodecyl sulphate

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.808	100	2.6
Test substance	0.606	75	18.3
Positive control	0.075	9.3	9.3

OD = optical density; SD = standard deviation

Remarks – Results

The results from the additional procedure using water-killed tissues showed no interference due to the test substance's possible ability to directly reduce MTT. Therefore, it was considered by the study authors to be unnecessary to use the results from the additional procedure for quantitative correction.

Based on the mean tissue viability of > 50%, the test substance is not classified as a skin irritant according to the test guidelines, using GHS criteria.

Positive and negative controls performed as expected. The standard deviation of the relative mean variability values from the three test item treated tissues was slightly greater (18.3%) than the upper limit of the assay acceptance criteria (≤ 18%). The acceptance criteria were not satisfied. However, as the relative mean viability results from the exposed tissues were negative for skin irritation effects, the study authors did not consider the slightly higher standard deviation to have affected the integrity or validity of the study.

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

TEST FACILITY Envigo (2019f)

B.4. Skin Irritation – Rabbit

TEST SUBSTANCE Isomer mixture containing the notified chemicals at 95.5% purity

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion

Species/Strain	Rabbit/New Zealand White
Number of Animals	One
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks – Method	Only one female rabbit was tested.

RESULTS

3 minutes exposure

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1			
Erythema/Eschar	0.3	2	> 14 days	2
Oedema	0	0	-	0

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

1 hour exposure

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1			
Erythema/Eschar	1	1	> 14 days	1
Oedema	0	0	-	0

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

4 hours exposure

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1			
Erythema/Eschar	4	4	> 14 days	2
Oedema	1	4	< 48 hours	2

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

Remarks – Results No mortality or signs of systemic toxicity were observed.

The test site exposed to the test substance for 3 minutes exhibited very slight erythema after 24 hours (when bandage was removed) with recovery indicated on Day 2. However, very slight erythema was again observed on

Day 8, persisting until Day 12 before increasing in severity (well-defined erythema) on Days 13 and 14.

The test site exposed to the test substance for 1 hour exhibited very slight erythema after 24 hours (when bandage was removed) which persisted over the course of the study with no change in the severity of the reaction. Slight fissuring at the test site was observed on Day 11 with no indication of recovery at the end of the observation period (Day 14).

The test site exposed to the test substance for 4 hours exhibited well-defined erythema with severe oedema 1 hour after bandage removal. Twenty-four hours after the bandage was removed, there was an increase in severity of erythema (from well-defined to severe) a decrease in oedema (severe to moderate) and a loss of flexibility.

Recovery from oedema was indicated at the 48 hour observation. However, very slight oedema was recorded on Days 8, 9 and 10, becoming severe on Day 11 before showing signs of recovery on Days 12 and 13 (moderate), and Day 14 (slight). Severe erythema persisted to Day 5, with recovery indicated over the remainder of the study period (slight to moderate erythema on Days 6 – 10, and slight erythema on Days 11 – 14)

Loss of flexibility persisted to the end of the study period. Dark areas were observed 72 hours after removal of the bandage and on days 5 – 10 and 12 – 13. A thickening of the skin was observed on Day 7 – 10 and pale areas were observed on Days 8 – 10. Fissuring was recorded on Day 11 and the effect persisted to the end of the study period. Desquamation of the test site was observed on the last day of the study period (Day 14).

At the end of the study period, the test site and associated control site associated with the 4 hour exposure test was examined. The skin at the site exposed to the test substance was approximately twice as thick as that of the control site. No trauma was observed on the underside of the skin or in the underlying musculature.

CONCLUSION	The test substance is irritating to the skin.
TEST FACILITY	Covance (2019)

B.5. Eye Irritation – *In Vitro* Bovine Corneal Opacity and Permeability Test

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
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
Vehicle	None
Remarks – Method	No significant protocol deviations Negative control: Sodium chloride (0.9% w/v in water) Positive control: Ethanol

RESULTS

Test Material	*Mean Opacities of Triplicate Tissues*	*Mean Permeabilities of Triplicate Tissues*	*IVIS*
Vehicle control	1.0	0.004	1.1
*Test substance**	1.7	0.014	1.9
*Positive control**	38.0	1.031	53.5

IVIS = *in vitro* irritancy score

*Corrected for background values

Remarks – Results	Based on the *in vitro* irritancy score (IVIS) of ≤ 3 calculated for the test substance, it does not require GHS classification for eye irritation. Positive and negative controls performed as expected.
CONCLUSION	The test substance was not considered irritating to the eye under the conditions of the test.
TEST FACILITY	Envigo (2019g)

B.6. Eye Irritation – *In Vitro* Human Corneal Model Test

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 492 Reconstructed Human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage.
Vehicle	None
Remarks – Method	No significant protocol deviations The test substance was an MTT reducer and additional tests using freeze-killed tissues were performed. The viability values obtained using the freeze-killed tissues were used to correct those values obtained in the viable test. Negative control: Deionised water Positive control: Methyl acetate

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*	*Relative Mean Viability (%)*
Negative Control	2.158	100.0
Negative Control – freeze-killed tissues	0.076	3.53
Test Substance	1.571	72.82
Test Substance – freeze-killed tissues	0.063	2.94
Positive Control	0.925	42.88

OD = optical density

Remarks – Results	The relative mean viability after correction for the effects of MTT reduction was 73.40% (> 60%) for the test substance, it does not require GHS classification for eye irritation or serious eye damage according to the test guidelines. The positive and negative controls performed as expected.
CONCLUSION	The test substance was considered non-irritating to the eye under the conditions of the test.
TEST FACILITY	Envigo (2019h)

B.7. Skin Sensitisation – *In Chemico* DPRA Test

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (2015)

Vehicle	Acetonitrile
Remarks – Method	No significant protocol deviations

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Vehicle	0.00*	0.00*
Test substance	99.6 ± 0.21	23.4 ± 6.81
Positive control - cinnamic aldehyde	71.1 ± 0.38	58.9 ± 0.31

* Normalised
SD = Standard Deviation

Remarks – Results	The reactivity of the test substance with the peptides measured as mean depletion of cysteine and lysine peptides was 61.5%, indicating high reactivity. Positive and negative controls performed as expected. All quality criteria were met. The individual and mean concentrations of the cysteine peptide concentration of the positive control was outside the historical control range. However, the study authors did not consider that this had an impact on the result as the individual and mean depletion values were within the test guideline recommended acceptance criteria of 60.8% - 100% depletion.
CONCLUSION	The test substance was predicted as positive for the first key event (molecular initiating) of the adverse outcome pathway (AOP) for skin sensitisation.
TEST FACILITY	Envigo (2018c)

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

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 442d *In Vitro* Skin Sensitisation Assays Addressing the AOP Key Event on Keratinocyte Activation (2015) - The ARE-Nrf2 luciferase KeratinoSens™ test method (Appendix IA)
Vehicle	1% Dimethylsulphoxide in cell culture medium
Remarks – Method	No significant protocol deviations

RESULTS

Sample	*Concentration (μM)*	*Mean Cell viability (% ± SD)*	*Mean Luciferase Induction (% ± SD)*
Test substance	0.977	103.393 ± 12.763	0.974 ± 0.157
	1.953	94.467 ± 12.083	1.058 ± 0.138
	3.906	94.150 ± 3.326	1.153 ± 0.103
	7.813	100.147 ± 3.297	1.310 ± 0.110
	15.625	97.633 ± 3.050	1.419 ± 0.062
	31.250	99.460 ± 4.266	1.560 ± 0.316
	62.5	106.740 ± 4.356	2.390 ± 0.929
	125	121.630 ± 11.539	3.873 ± 2.575
	250	137.440 ± 13.193	11.413 ± 12.793
	500	153.973 ± 15.494	119.034 ± 190.469
	1000	175.627 ± 30.497	823.016 ± 1330.456
	2000	123.450 ± 115.432	646.541 ± 1089.229
Positive control - Cinnamic Aldehyde	32	>100	2.11

SD = Standard Deviation

Sample	*$EC_{1.5}$ (µM)*	*IC_{50} (µM)*	*I_{max}*
Test substance - Repetitions 1/2/3	32.22/53.74/16.63	> 2000/> 2000/> 1687	1904.107/35.506/2358.35

EC1.5 - concentration for induction of luciferase activity above 50% of the vehicle control
IC50 - concentration leading to 50% reduction in cell viability compared to the vehicle control
I_{max} – maximal induction
^ Mean of three repetitions

Remarks – Results	The 3 repetitions met the determination criteria for skin sensitisation potential, including at least one concentration of the test substance induce luciferase activity ≥ 1.5 fold, the first concentration inducing luciferase activity above 1.5 have a viability above 70%, $EC_{1.5}$ value occur at a concentration < 1,000 µM and the test substance induce the luciferase in a dose-dependent manner. The study was reported to have passed the assay acceptance criteria.
CONCLUSION	The test substance was positive for the second key event (keratinocytes response) of the adverse outcome pathway (AOP) for skin sensitisation.
TEST FACILITY	XCELLR8 (2018)

B.9. Skin Sensitisation – *In Vitro* Human Cell Line Activation Test (h-CLAT)

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 442e *In Vitro* Skin Sensitisation Assays Addressing the Key Event on Activation of Dendritic Cells on the Adverse Outcome Pathway for Skin Sensitisation *In Vitro* Skin Sensitisation (2015) - Human Cell Line Activation Test (h-CLAT)
Vehicle	Dimethylsulphoxide (DMSO)
Remarks – Method	The concentration selection for main experiments was based on the cytotoxic effects in a pre-test performed at up to 5000 µg/mL. This study deviated from the OECD test guideline by using XTT test instead of flow cytometry for measuring and estimating the CV75 values of the dose finding assay. Negative control: Culture medium Vehicle control: DMSO in culture medium Positive control: 2,4-dinitrochlorobenzene

RESULTS

Sample	*Concentration (µg/mL)*	*Mean RFI* CD86 (%)*	*Mean RFI* CD54 (%)*	*Mean Relative Viability (%)*
Vehicle Control	-	100.0	100.0	100.0
Negative Control	-	100.0	100.0	100.0
Test substance				
	160	222.3	223.7	83.6
	192	230.7	242.2	83.0
	231	276.3	305.0	72.5
	277	326.7	361.9	78.6
	332	311.2	336.6	75.1
	399	357.6	261.3	56.1
	478^	388.4	312.8	58.9
	574^	333.3	224.2	53.1
Positive Control				
	2.0	537.0	221.5	70.1
	3.0	512.5	244.1	72.1

* RFI = relative fluorescence intensity
^ The cell viability at the concentrations in the second run were below 50% and were excluded from the evaluation as they didn't meet the quality criteria.

Remarks – Results	As the RFI of CD86 and CD54 was greater than 150% and 200% respectively in more than one of the concentrations in both repetitions, the test substance was predicted to be positive under the conditions of this test. Positive and negative controls performed as expected.
CONCLUSION	The test substance was considered positive in the third key event (dendritic cell activation) of the adverse outcome pathway (AOP) for skin sensitisation.
TEST FACILITY	Envigo (2018d)

B.10. Skin Sensitisation – Human Volunteers

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 2.5% concentration
METHOD	Repeated insult patch test
Study Design	Induction procedure: patches infused with 0.15 mL test substance were applied to the upper back 3 times per week on Mondays, Wednesdays and Fridays for a total of 9 applications. Patches were removed after 24 h and graded after an additional 24 h (or 48 h for patches applied on Friday). Rest period: 10 - 21 days Challenge procedure: identical patches were applied to a naïve site (lower back). Patches remained in place for 24 h. Sites were graded 24 and 72 h post-patch removal.
Study Group	88 F, 24 M; age range 21 - 70 years
Vehicle	Distilled water
Remarks – Method	Occluded. The test substance (0.15 mL) was spread on a 3.63 cm × 3.63 cm patch.
RESULTS	
Remarks – Results	106/112 subjects completed the study. No withdrawals were related to the application of the test substance. No skin reactions were noted throughout the study.
CONCLUSION	The test substance (with the notified chemicals at 2.5% concentration) was non-sensitising under the conditions of the test.
TEST FACILITY	Eurofins (2018a)

B.11. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Rat/Wistar (HanTac: WH)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Corn oil
Remarks – Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	6 M, 6 F	0	0/10
Low Dose	6 M, 6 F	100	0/10
Mid Dose	6 M, 6 F	300	0/10
High Dose	6 M, 6 F	1,000	0/10
Control Recovery	6 M, 6 F	0	0/10
High Dose Recovery	6 M, 6 F	1,000	0/10

Mortality and Time to Death

There were no unscheduled deaths.

Clinical Observations

No adverse clinical findings were recorded.

All animals made the expected body weight gains. Males in the high-dose group showed statistically significantly lower mean absolute body weight gains compared to the control group during days 1 – 8, 22 – 28 and over the total exposure period (days 1 – 28), while males in the high-dose recovery group showed statistically significantly higher absolute body weight gains during days 35 - 42. Statistically significantly higher mean absolute body weight gains compared to the control animals were observed in females in the low- and mid-dose groups during days 1 – 8, and in the mid-dose group over the total exposure period (days 1 – 28). The total percentage weight gain for females in the mid-dose group was statistically significantly higher than that observed in the control animals. These findings were not considered to be toxicologically relevant by the study authors as no dose-response was observed, the total percentage weight gain (except for females in the mid-dose group) was not statistically significantly different to that recorded for control animals, and the increase or decrease in absolute body weight was not consistently seen in animals in the high-dose recovery group.

Food consumption was statistically significantly lower (compared to relevant control animals) in males in the high-dose group (days 1 – 8) and statistically significantly higher (compared to relevant control animals) in males in the high-dose recovery (days 35 – 42) groups. These findings were not considered by the study authors to be related to the test substance as the effect was not observed in both sexes exposed to the highest dose studied, no dose-response relationship was observed, and animals in the high-dose recovery group were no longer being exposed to the test substance during the recovery period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Alterations in hepatic parameters were recorded. Statistically significantly higher level of alanine aminotransferase, alkaline phosphatase and total bilirubin were observed in high-dose males and females (dose-response relationship observed with increasing alkaline phosphatase levels across males in the low-, mid- and high-dose groups). Statistically significantly higher levels of bile acids was also observed in females in the high-dose group. Statistically significantly lower levels of alkaline phosphatase was observed in males in the high-dose recovery group.

No other toxicologically significant changes in haematology, coagulation and urinalysis parameters were recorded. Statistically significant changes recorded but not considered toxicologically relevant by the study authors as they were either within the range of historical controls, did not show a strong dose-response relationship, were not consistent between the test-period and recovery-period groups, were observed in one sex only, or could not be correlated with effects in organs. These changes included lower levels of mean corpuscular haemoglobin (MCH) (males in the low-dose and high-dose recovery groups), mean corpuscular haemoglobin concentration (MCHC) (high-dose recovery males), prothrombin time (females in all dose groups and males in the mid-dose group), mean corpuscular volume (females in the high-dose recovery group), calcium levels (high-dose males and females), glucose and inorganic phosphorous (high-dose recovery group males), lower activated partial thromboplastin time (APTT), higher absolute lymphocyte levels and white blood cells (males in the high-dose recovery group), platelet levels and prothrombin time (females in the high-dose recovery group), glucose levels and total cholesterol (high-dose females).

Effects in Organs

No toxicologoically significant gross lesions were observed at the end of treatment or during the recovery period.

Statistically significant increases in mean liver weight were observed in males and females in the mid- and high-dose groups when compared to control animals. Males in the high-dose groups exhibited statistically significant lower mean prostrate, seminal vesicle and coagulating gland weights, while females in the high-dose group exhibited statistically significantly lower mean thymus weights when compared to animals in the control group. The changes observed in the liver, thymus and accessory sex glands returned to normal at recovery. No histopathological findings were noted in the male recovery groups. Reductions in weights of prostate, seminal vesicle and coagulating gland in males and thymus in females were considered by the study authors to be test substance-related non-adverse changes as there were no microscopic changes observed in these organs.

Microscopically, minimal to mild degree of focal and/or diffuse hepatocellular hypertrophy of liver was observed in males and females in the mid- and high-dose groups and bile duct proliferation was observed in male and females of the high-dose group. These histopathologic findings were not present in the recovery groups. Microscopic changes of liver observed in the high-dose animals were considered by the study authors as test substance-related adverse effects as they were associated with increases in liver weight (mild), hepatic enzymes (ALT/ALP), total bilirubin and bile acids. However, the mid-dose animals did not show any evidence of altered hepatic function except for minimal increase in liver weights in correlation to hepatocellular hypertrophy, and hence were considered by the study authors as test substance-related non-adverse changes and adaptive response to metabolic change.

Non-glandular mucosa- epithelial hyperplasia/hyperkeratosis was observed in the stomach of males and females in the high-dose group and was reversible during recovery.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 300 mg/kg bw/day in this study, based on test substance-related adverse changes observed in the liver in male and female animals treated at 1000 mg/kg bw/day.

TEST FACILITY	Eurofins (2018b)

B.12. Genotoxicity – Bacteria

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Experiment 1: Plate incorporation procedure Experiment 2: Pre incubation procedure
Species/Strain	*Salmonella typhimurium* strains: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Experiment 1	a) With metabolic activation: 1.5, 5, 15, 50, 150, 500, 1,500, 5,000 µg/plate b) Without metabolic activation: 1.5, 5, 15, 50, 150, 500, 1,500, 5,000 µg/plate
Concentration Range in Experiment 2	a) With metabolic activation: 5, 15, 50, 150, 500, 1,500, 5,000 µg/plate b) Without metabolic activation: TA98 and WP2uvrA: 5, 15, 50, 150, 500, 1,500, 5,000 µg/plate TA100, TA1535, TA1537: 0.05, 0.15, 0.5, 1.5, 5, 15, 150 µg/plate
Vehicle	Dimethylsulphoxide
Remarks – Method	No significant protocol deviations. No preliminary toxicity test performed. In the second experiment, a concentration range of 5 – 5,000 µg/plate was originally tested based on the results obtained from Experiment 1. However, an insufficient number of non-toxic concentrations were obtained for strains TA100, TA1537 and TA1535 in the absence of metabolic activation. These strains were repeated (in the absence of metabolic activation) using the concentration range 0.05, 0.15, 0.5, 1.5, 5, 15, 150 µg/plate. Positive controls:

- With metabolic activation: 2-Aminoanthracene (TA100, TA1535, TA1537, Wp2*uvrA*), Benzo(a)pyrene (TA98)
- Without metabolic activation: N-ethyl-N'-nitro-N-nitrosoguanidine (Wp2*uvrA*, TA100, TA1535), 9-Aminoacridine (TA1537), 4-Nitroquinoline-1-oxide (TA98)

RESULTS

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

Remarks – Results

In Experiment 1, a visible reduction in the bacterial lawn was observed at the highest dose tested (5,000 µg/plate) in the presence (TA100) and absence (TA100, TA1535, TA1537) of metabolic activation. In Experiment 2, a weakened growth of bacterial lawn at 5 µg/plate (TA100), 15 µg/plate (TA1535, TA1537) and 500 µg/plate (TA98, Wp2*uvrA*) was observed in the absence of metabolic activation and at 500 µg/plate (TA100, TA1537), 1,500 µg/plate (TA98, TA1535) and 500 µg/plate (TA98, Wp2*uvrA*) in the presence of metabolic activation.

No biologically relevant increases in the frequency of revertant colonies were observed for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation.

Positive and negative controls performed as expected.

CONCLUSION

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

TEST FACILITY

Envigo (2018e)

B.13. Genotoxicity – *In Vitro* Chromosome Aberration Test

TEST SUBSTANCE

Isomer mixture containing the notified chemicals at 95.5% purity

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test

Species/Strain

Human

Cell Type/Cell Line

Lymphocytes

Metabolic Activation System

S9 fraction from phenobarbital/β-naphthoflavone induced rat liver.

Vehicle

Dimethyl sulphoxide

Remarks – Method

No significant protocol deviations. The dose selection for the main experiments was based on toxicity observed in a preliminary toxicity test performed at 6.01 – 1540 µg/mL.

Positive controls: presence of metabolic activation: Cyclophosphamide; without metabolic activation: Mitomycin C.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period (hours)*	*Harvest Time (hours)*
Absent			
Test 1	0*, 8, 12, 16, 24, 32*, 48*, 64*	4	24
Test 2	0*, 8, 12, 16, 24, 32*, 48*, 64*	24	24
Present			
Test 1	0*, 12, 16, 24, 32*, 48*, 64*, 96	4	24

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 48.13	> 64	> 64	negative
Test 2	≥ 24.06	> 64	> 64	negative
Present				
Test 1	≥ 96.25	> 64	> 96	negative

Remarks – Results

The test substance did not cause a statistically significant increase in the proportion of cells with chromosomal aberrations at any concentration in the presence or absence of metabolic activation.

No statistically significant increase in the number of polyploid cells was observed following exposure to the test substance at any concentration in the presence or absence of metabolic activation.

Positive and negative controls performed as expected in both tests.

CONCLUSION

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

TEST FACILITY

Envigo (2018f)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability (Study 1)

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated Sewage Sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD
Remarks – Method	The study was carried out in accordance with the test guidelines and GLP where no deviations were reported.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Mean Degradation*	*Day*	*% Degradation*
1	0.0	1	53.5
4	15.8	4	69.1
10	50.9	10	85.6
14	62.9	14	90.0
21	73.9	21	99.8
28	76.3	28	102.6

Remarks – Results	The test met all validity criteria. The standard reference material reached more than ≥ 60% after 3 days, the difference between replicate values of % degradation was < 20% (7%), the average oxygen uptake in the control blank did not exceed 60 mg/L (16.2 mg/L) and the toxicity control reached ≥ 25% (68.8%) degradation after 3 days which indicated the test item had no toxic inhibition to the inoculum. The test substance passed the 10 day window with degradation of 15.8% on day four to 62.9% on day 14 whilst also satisfying the pass level of ready biodegradability (> 60%).
CONCLUSION	The test substance is readily biodegradable.
TEST FACILITY	CTI (2018a)

C.1.2. Ready Biodegradability (Study 2)

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)
Inoculum	Activated Sewage Sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	TOC analyser and GC-FID
Remarks – Method	The study was carried out in accordance with the test guidelines and GLP where no deviations were reported.

RESULTS

Test Substance		*Aniline*	
Day	*% Mean Degradation (BOD)*	*Day*	*% Degradation*
7	33.5	7	74
14	71	14	89
21	75.5	21	93
28	79	28	94

Remarks – Results: The test met all validity criteria. The standard reference material reached more than ≥ 60% within 7 days; the difference between replicate values of CO_2 production was < 20% and the average oxygen uptake in the control blank did not exceed 60 mg/L. Biodegradation by DOC was not calculated because it was unmeasurable. In the test solution (water + test substance) the GC results showed that all of the main components of the test substance transformed into subcomponents and methanol after 28 days.

CONCLUSION: The test substance completely biodegraded under the test conditions.

TEST FACILITY: CERI (2019)

C.1.3. Ready Biodegradability (Study 3)

TEST SUBSTANCE: Isomer mixture containing the notified chemicals at 95.5% purity

METHOD: OECD TG 310 Ready Biodegradability – CO_2 in sealed vessels (Headspace Test)

Inoculum: Activated Sewage Sludge
Exposure Period: 28 days
Auxiliary Solvent: None
Analytical Monitoring: TOC analyser and GC-FID
Remarks – Method: The study was carried out in accordance with the test guidelines and GLP with one deviation. A test concentration of 20 mg carbon/L was to be employed following the test guidelines, however in error, the test concentration employed was equivalent to 16.35 mg C/L. This was considered to have no effect on the integrity of the test. The degradation values for the test item and toxicity control vessels were re-calculated using the revised carbon concentration. The test was run in parallel to controls and the reference material, aniline.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
2	0	2	61
6	28	6	77
8	38	8	78
10	31	10	88
14	35	14	83
16	39	16	89
21	42	21	88
28	46	28	87

Remarks – Results: The test met all validity criteria. The standard reference material reached more than ≥ 60% within 2 days; the TIC produced from the control bottles at the end of the test was ≤ 15% of the TOC added initially and the toxicity control reached ≥ 25% degradation within 8 days. The result for the toxicity

control indicated the test item had no toxic inhibition to the inoculum. The test substance attained 46% degradation after 28 days and therefore cannot be considered to be readily biodegradable.

CONCLUSION The test substance is not readily biodegradable.

TEST FACILITY Envigo (2018g)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE Isomer mixture containing the notified chemicals at 95.5% purity

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi-Static
Species *Gobiocypris rarus* (Rare Minnow)
Exposure Period 96 hours
Auxiliary Solvent None
Water Hardness 150 and 170mg $CaCO_3$/L (Tests 1 and 2)
Analytical Monitoring GC-FID
Remarks – Method The study was carried out in accordance with the test guidelines and GLP. Following a preliminary range-finding test, two definitive tests were conducted with different ranges of nominal concentrations of the test substance (4.5 – 8.1 mg/L and 11 – 50 mg/L) under semi-static conditions (renewal every 24 hours). A positive control was run with potassium dichromate.

RESULTS (TESTS 1 AND 2)

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Measured (Geometric Mean)*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Blank Control	Blank Control	10	0	0	0	0	0
4.5	1.7	10	0	0	0	0	0
6.0	2.2	10	0	0	0	0	0
8.1	3.1	10	0	0	0	0	0
11	4.7	10	0	0	0	4	10
15	6.1	10	0	0	2	3	10
20	8.2	10	0	5	8	8	10
27	13	10	8	10	10	10	10
37	11	10	4	10	10	10	10
50	17	10	10	10	10	10	10

LC50 3.8 mg/L at 96 hours (based on geometric mean of measured concentrations)
NOEC 3.1 mg/L at 96 hours (based on geometric mean of measured concentrations)
Remarks – Results All but one of the validity criteria for the test were satisfied. The mortality in the control was 0%, the dissolved oxygen concentration was in the range of 60.4-100.7% and held between pH 7.23-7.91 at approximately 23.3-24.7°C during the test. The measured concentrations of the test substance were maintained in the range of 70-109% (outside of 80-120%) of those in new solutions, therefore the concentration was based on the geometric mean. The result from the positive control (96 h LC50 = 205 mg/L) was within the range of the lab ring test.

CONCLUSION The test substance is toxic to fish.

TEST FACILITY CTI (2018b)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Semi-Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not measured
Analytical Monitoring	GC-FID
Remarks – Method	The study was carried out in accordance with the test guidelines and GLP. Following preliminary range-finding tests, 20 daphnids were exposed to a water soluble fraction (WSF) of the test item. The test solution was prepared by shaking 100 mg/L of the test item for 24 h, then filtering to produce a 100% v/v saturated solution. Lower test concentrations were obtained by further dilution of the 100% v/v saturated solution. A positive control was run with potassium dichromate.

RESULTS

Concentration		*Number of D. magna*	*Number Immobilised*	
Nominal (% v/v Saturated Solution)	*Measured (Geometric Mean, mg/L)*		*24 h*	*48 h*
Control	Control	20	0	0
10	0.96	20	0	0
18	2.1	20	0	0
32	3.8	20	20	20
56	6.9	20	20	20
100	13	20	20	20

EC50	2.8 mg/L at 48 hours (based on geometric mean of measured concentrations)
NOEC	2.1 mg/L at 48 hours (based on geometric mean of measured concentrations)
Remarks – Results	The validity criteria of the test were met. No daphnids in the control group showed immobilisation or signs of disease. The dissolved O_2 was maintained between 7.8-9.2 mg/L, pH between 7.8-8.2 and the temperature between 20-21 °C. Analysis of the old test preparations showed measured test concentrations had declined (0.8 – 11 mg/L) therefore the results were calculated based on the geometric means. The NOEC was calculated using the Fisher's Exact Binomial Test with Bonferroni correction and the EC50 was calculated using the binomial method. The results from the positive control with potassium dichromate were within the normal range for the reference item.
CONCLUSION	The test substance is toxic to aquatic invertebrates.
TEST FACILITY	Envigo (2019i)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0 – 100% v/v saturated solution

	Measured (Geometric Means):0.032 – 8.6 mg/L
Auxiliary Solvent	None
Water Hardness	Not measured
Analytical Monitoring	GC-FID
Remarks – Method	The study was carried out in accordance with the test guidelines and GLP where no deviations were recorded. Following preliminary range-finding tests, algae were exposed to a water soluble fraction (WSF) of the test item. The test solution was prepared by shaking 100 mg/L of the test item for 24 h, then filtering to produce a 100% v/v saturated solution. Lower test concentrations were obtained by further dilution of the 100% v/v saturated solution. Testing was conducted in completely filled, stoppered test vessels in order to minimise possible losses due to volatilisation. Sodium bicarbonate was added to prevent inhibition of growth due to the restriction in gaseous exchange associated with testing in an enclosed system. A positive control was run with potassium dichromate.

RESULTS

Biomass		*Growth*	
EyC50 mg/L at 72 h	*NOEC mg/L*	*ErC50 mg/L at 72 h*	*NOEC mg/L*
0.61	0.16	1.3	0.16

Remarks – Results	All validity criteria for the test were satisfied. The mean cell density in the control increased 80 (> 16) times after 72 hours. The mean coefficient of variation for section specific growth rate for the control culture was 13% (< 35%) and the average specific growth rate for the control was 5% (< 7%). One way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control was carried out on the growth rate and yield data after 72 hours for the control and all test concentrations to determine any statistically significant differences between the test and control groups.
CONCLUSION	The test substance is toxic to algae.
TEST FACILITY	Envigo (2018h)

C.2.4. Acute Toxicity to Earthworms

TEST SUBSTANCE	Isomer mixture containing the notified chemicals at 95.5% purity
METHOD	OECD TG 207 Earthworm, Acute Toxicity Test
Species	*Eisenia foetida*
Exposure Period	14 days
Concentration Range	100 – 1,000 mg/kg
Remarks - Method	The study was carried out in accordance with the test guidelines with no deviations recorded. Following preliminary tests, earthworms (4 × 10) were exposed to the test substance of nominal concentrations up to 1000 mg/kg. A positive control was conducted with 2-chloracetamide.

RESULTS

Concentration (mg/kg)	*Total number of test earthworms*	*Cumulative mortality (%)*	
		7 d	*14 d*
Blank Control	40	5	5
Solvent Control	40	5	5
100	40	8	8

Concentration (mg/kg)	*Total number of test earthworms*	*Cumulative mortality (%)*	
		7 d	*14 d*
147	40	5	8
215	40	5	13
316	40	15	23
464	40	28	40
681	40	50	75
1000	40	100	100

LC50	492 mg/kg at 14 days
Remarks – Results	The earthworm mortality of the blank and solvent control groups was 5% which satisfied the validity of the test. The result of the positive control (14 d LC50 = 58.7 mg/kg) was within the recommended range. The LC50 was calculated using the Trimmed Spearman-Karber method.
CONCLUSION	The test substance is slightly toxic to earthworms.
TEST FACILITY	CTI (2018c)

BIBLIOGRAPHY

ACI (2010) Consumer Product Ingredient Safety, Exposure and risk screening methods for consumer product ingredients, 2nd Edition, American Cleaning Institute, Washington DC.

Api *et al* (2008) Api, A.M., Basketter, D.A., Cadby, P.A., Cano, M.F., Ellis, G., Gerberick, G.F., Griem, P., McNamee, P.M., Ryan, C.A., Safford, R.; Dermal Sensitisation Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Regulatory Toxicology and Pharmacology 52 (2008) 3-23.

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

CERI (2019) Biodegradation study of PG-RAW-0004 (Study No. 16613, June, 2019). Kurume, Japan, Chemicals Evaluation and Research Institute (Unpublished report submitted by the notifier).

Covance (2019) PG-RAW-0004: Skin Irritation to the Rabbit (Study No. RW40QS, July, 2019). Suffolk, United Kingdom, Covance CRS Limited (Unpublished report submitted by the notifier).

CTI (2018a) Ready Biodegradability Test of PG-RAW-0004 (Manometric Respiratory Test) (Study No. G17301045A, March, 2018). Suzhou, China, CTI-Safety Evaluation Technical Service Co., Ltd (Unpublished report submitted by the notifier).

CTI (2018b) PG-RAW-0004: Fish Acute Toxicity Test (Rare Minnow) (Study No. G17203043A, September, 2018). Suzhou, China, CTI-Safety Evaluation Technical Service Co., Ltd (Unpublished report submitted by the notifier).

CTI (2018c) PG-RAW-0004: Earthworm Acute Toxicity Test (Study No. G17207036A, May, 2018). Suzhou, China, CTI-Safety Evaluation Technical Service Co., Ltd (Unpublished report submitted by the notifier).

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

ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance, June 2017, version 3.0. European Chemicals Agency, https://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf

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

Envigo (2018a) PG-RAW-0004: Determination of Melting/Freezing Temperature, Water Solubility and Partition Coefficient (n-Octanol/Water) (Study No. MT85TS, July, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2018b) PG-RAW-0004: Determination of Vapour Pressure. (Study No. WG82PF, May, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2018c) PG-RAW-0004: Direct Peptide Reactivity Assay (Study No. SJ16PK, July, 2018). Cambridgeshire, United Kingdom, Envigo CRS Limited (Unpublished report submitted by the notifier).

Envigo (2018d) PG-RAW-0004: *In vitro* Skin Sensitisation Test – Human Cell Line Activation Test (h-CLAT). (Study No. 1868600, March, 2018). Rossdorf, Germany, Envigo CRS GmbH (Unpublished report submitted by the notifier).

Envigo (2018e) PG-RAW-0004: Reverse Mutation Assay 'Ames Test' using *salmonella typhimurium* and *Escherichia coli* (Study No. SJ94FR, February, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2018f) PG-RAW-0004: Chromosome Aberration Test in Human Lymphocytes *in Vitro*. (Study No. MV48SN, March, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2018g) PG-RAW-0004: Assessment of Ready Biodegradability; CO_2 in Sealed Vessels (CO_2 Headspace Test) (Study No. XJ78XR, May, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2018h) PG-RAW-0004: Algal Growth Inhibition Test (Study No. FM19RD, July, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2019a) PG-RAW-0004: Determination of Boiling Temperature, Density and Surface Tension (Study No. JT60JL, April, 2019). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2019b) PG-RAW-0004: Determination of Adsorption Coefficient (Study No. NK96GT, March, 2019). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2019c) PG-RAW-0004: Determination of Hazardous Physico-Chemical Properties (Study No. LR36PV, April, 2019). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2019d) PG-RAW-0004: Acute Oral Toxicity Study in the Rat – Fixed Dose Method (Study No. MY58QS, April, 2019). Cambridgeshire, United Kingdom, Envigo CRS Limited (Unpublished report submitted by the notifier).

Envigo (2019e) PG-RAW-0004: *In vitro* EPIDERM™ Skin Corrosion Test (Study No. DG42NT, February, 2019). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2019f) PG-RAW-0004: Determination of Skin Irritation Potential using the EPISKIN™ Reconstructed Human Epidermis Model (Study No. VT80QN, March, 2019). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2019g) PG-RAW-0004: The Bovine Corneal Opacity and Permeability (BCOP) Assay (Study No. BL83WX, April, 2019). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2019h) PG-RAW-0004: *In vitro* Eye Irritation Test: Human Cornea Model Test – OECD 492 (Study No. 1925400, January, 2019). Rossdorf, Germany, Envigo CRS GmbH (Unpublished report submitted by the notifier).

Envigo (2019i) PG-RAW-0004: *Daphnia* sp., 48-Hour Acute Immobilization Test (Study No. GM44SP, April, 2019). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Eurofins (2018a) Repeated Insult Patch Test (RIPT) – Shelanski Method (Study No. CRL2018-1142, December, 2018). New Jersey, United States of America, Eurofins CRL, Inc (Unpublished report submitted by the notifier).

Eurofins (2018b) PG-RAW-0004: 28 Day Repeat Dose Oral Toxicity Study by Oral Gavage in Wistar Rats with 14-Day Recovery Period (Study No. G16003, July, 2018). Bengaluru, India, Eurofins Advinus Limited (Unpublished report submitted by the notifier).

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

OECD (2016) Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation, Series on Testing and Assessment No. 256, Organisation for Economic Co-Operation and Development (OECD).

RIVM (2010) Observations of the Methodology for Quantitative Risk Assessment of Dermal Allergens, Report 320015003/2010, National Institute of Public Health and the Environment, Netherlands.

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

SCCS (2012) Notes of Guidance for testing of Cosmetic Ingredients and Their Safety Evaluation (7th revision) European Commission - Scientific Committee on Consumer Safety.

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

Struijs J (1996). SimpleTreat 3.0: A Model to Predict the Distribution and Elimination of Chemicals by Sewage Treatment Plants. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, https://www.safeworkaustralia.gov.au/doc/model-code-practice-managing-risks-hazardous-chemicals-workplace

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

US EPA (2012) Estimation Programs Interface (EPI) Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency. Washington DC, USA. Available at http://www.epa.gov.

XCellR8 (2018) *In vitro* Assessment of the Skin Sensitisation Potential of PG-RAW-0004 According to OECD Test Guideline 442D (the KeratinoSens™ Test Method) (Study No. 17ENV11, February, 2018). Cheshire, United Kingdom, XCellR8 Ltd (Unpublished report submitted by the notifier).