File No: LTD/1916
LTD/1917

August 2016

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

LTD/1916: 4,7-Methano-1*H*-inden-5-ol, octahydro-2,4,5-trimethyl-
LTD/1917: 4,7-Methano-1*H*-inden-5-ol, octahydro-3,4,5-trimethyl-

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICALS	INTRODUCTION VOLUME	USE
LTD/1916 LTD/1917	International Flavours and Fragrances (Australia) Pty Ltd	LTD/1916: 4,7-Methano-1*H*-inden-5-ol, octahydro-2,4,5-trimethyl- LTD/1917: 4,7-Methano-1*H*-inden-5-ol, octahydro-3,4,5-trimethyl-	Yes	≤ 1 tonne per annum (each chemical)	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Skin irritation (Category 2)	H315 – Causes skin irritation
Serious eye damage/Irreversible effects on the eye (Category 1)	H318 – Causes serious eye damage
Skin sensitiser (Category 1B)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemicals are recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R41: Risk of serious eye damage
R43: May cause sensitisation by skin contact

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

Hazard classification	*Hazard statement*
Acute (Category 2)	H401 – Toxic to aquatic life
Chronic (Category 2)	H411 – Toxic to aquatic life with long lasting effects

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified 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 and the reported use pattern, the notified chemicals are not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemicals should be classified as follows:
  - Skin irritation (Category 2): H315 – Causes skin irritation
  - Serious eye damage/Irreversible effects on the eye (Category 1) : H318 – Causes serious eye damage
  - Skin sensitiser (Category 1B): H317 – May cause an allergic skin reaction

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

- The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the notified chemicals for listing on the SUSMP.

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
  - Ventilation system including local exhaust ventilation, where possible

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

- 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:
  - Coveralls
  - Impervious gloves
  - Chemical goggles

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

- A copy of the (M)SDS should be easily accessible to employees.

- If products and mixtures containing the notified 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.

Public Health

- Consumer products containing the notified chemicals that have potential to cause eye irritation should be appropriately labelled to warn users about the possible eye irritation effects.

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemicals in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Storage

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

Emergency procedures

- Spills or accidental release of the notified chemicals should be handled by physical containment, collection and subsequent safe disposal.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemicals 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 (each notified chemical);
- the concentration of the isomer mixture containing the notified chemicals exceeds or is intended to exceed 0.66% in fine fragrances, 0.4% in body lotions, 1.25% in hair sprays and air refreshers, 0.1% in deodorants and 0.46% in other cosmetic and 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.

(Material) Safety Data Sheet
The (M)SDS of the notified chemicals and a product containing the notified chemicals provided by the notifier were reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

NOTIFICATION CATEGORY
Limited-small volume (reduced fee notifications): Chemicals other than polymer (1 tonne or less per year) – group assessment (inseparable structural isomers in a reaction mixture)

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

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
China (2014)
Japan (2014)
Philippines (2016)
USA (2013)

2. IDENTITY OF CHEMICALS

The notified chemicals are inseparable structural isomers.

MARKETING NAME
Coolwood (isomer mixture containing notified chemicals)

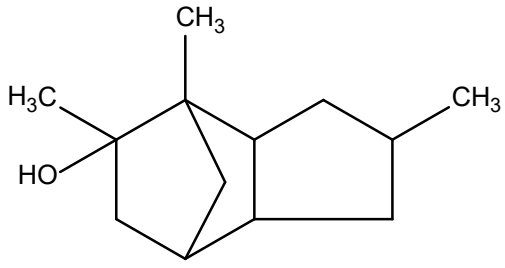
CAS NUMBER
LTD/1916: 1340502-93-3
LTD/1917: 1340502-69-3

CHEMICAL NAME
LTD/1916: 4,7-Methano-1*H*-inden-5-ol, octahydro-2,4,5-trimethyl-
LTD/1917: 4,7-Methano-1*H*-inden-5-ol, octahydro-3,4,5-trimethyl-

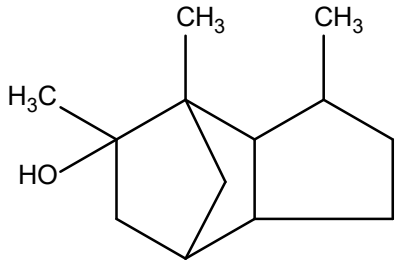
OTHER NAME
FRET 08-0338 (isomer mixture containing notified chemicals)

MOLECULAR FORMULA
LTD/1916 and LTD/1917: 194.31 Da

STRUCTURAL FORMULA
LTD/1916:



LTD/1917:



ANALYTICAL DATA
All analytical data were obtained on the isomer mixture containing the notified chemicals.

METHOD ^{1}H NMR
Remarks Reference spectra were consistent with the structure.
TEST FACILITY IFF (2011)

METHOD IR
Remarks Reference spectra were consistent with the structure.
TEST FACILITY IFF (2011)

METHOD UV
Remarks Maximum absorbance was observed at 202, 203 and 221 nm at neutral, acidic and basic pH, respectively.
TEST FACILITY IFF (2011)

METHOD GC-MS
Remarks Reference spectra were provided. 4,7-Methano-1H-inden-5-ol, octahydro-2,4,5-trimethyl- (LTD/1916) and 4,7-Methano-1H-inden-5-ol, octahydro-3,4,5-trimethyl- (LTD/1917) were detected at 44.74% and 47.75%, respectively. Impurities structurally related to the notified chemicals were also detected.
TEST FACILITY IFF (2011)

3. COMPOSITION

DEGREE OF PURITY
> 90% (isomer mixture)
The notified chemicals are manufactured overseas as an inseparable isomer mixture.

The composition of the notified chemicals in the isomer mixture (Coolwood) is as follows:

Notified chemical	*Weight %*
4,7-Methano-1H-inden-5-ol, octahydro-2,4,5-trimethyl- (LTD/1916)	44.74
4,7-Methano-1H-inden-5-ol, octahydro-3,4,5-trimethyl- (LTD/1917)	47.75

IMPURITIES (> 1% BY WEIGHT)

Chemical Name 4,7-Methano-1*H*-inden-5-ol, octahydro-3,4-dimethyl-
CAS No. 79365-69-8 *Weight %* 1.3

Chemical Name 4,7-Methano-1*H*-inden-5-ol, octahydro-2,4-dimethyl-
CAS No. 79365-68-7 *Weight %* 1.6

ADDITIVES/ADJUVANTS

Chemical Name Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
CAS No. 6386-38-5 *Weight %* 0.1

4. PHYSICAL AND CHEMICAL PROPERTIES

The following physico-chemical properties are for the isomer mixture containing the notified chemicals.

APPEARANCE AT 20 °C AND 101.3 kPa: Clear liquid

Property	Value	Data Source/Justification
Pour Point	0 °C	Measured
Boiling Point	Decomposes above 255 °C before boiling	Measured
Density	1,000 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.003 kPa at 25 °C	Measured
Water Solubility	0.19 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Notified chemicals do not contain hydrolysable functionalities.
Partition Coefficient (n-octanol/water)	log Pow = 4.7 at 20 °C	Measured
Surface Tension	48 mN/m at 20 °C	Measured. The notified chemicals are surface active
Adsorption/Desorption	log K_{oc} = 3.4 at 25 °C	Measured
Dissociation Constant	Not determined	The notified chemicals do not contain any functional groups that are expected to dissociate in water.
Flash Point	114 °C at 101.3 kPa (closed cup)	Measured
Flammability	Combustible liquid*	Estimated based on flash point
Autoignition Temperature	282 °C	Measured
Explosive Properties	Not expected to have explosive properties	Estimated based on chemical structure
Oxidising Properties	Not expected to have oxidising properties	Estimated based on chemical structure

* Based on *Australian Standard AS1940* definitions

DISCUSSION OF PROPERTIES

For full 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 not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

The mixture of the notified chemicals has a flash point of 114 °C. Based on *Australian Standard AS1940* definitions for combustible liquids, a liquid that has a flash point of 150 °C or less is a Class C1 combustible liquid.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICALS (100%) OVER NEXT 5 YEARS

The notified chemicals are constituents of an inseparable isomer mixture, which will be imported as components of finished fragrance oils. The fragrance oils will contain the isomer mixture at 10-92% concentration.

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

LTD/1916

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

LTD/1917

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

PORT OF ENTRY
Melbourne

TRANSPORTATION AND PACKAGING
The notified chemicals will be imported as constituents of finished fragrance oils in 205 L polypropylene-lined steel drums. The imported products containing the notified chemicals will be transported to reformulation sites within Australia by road. The end-use products will be packaged in containers suitable for retail sale.

USE
The notified chemicals will be used as fragrance ingredients. The notified chemicals are manufactured as an inseparable isomer mixture. The inseparable isomer mixture will be imported as a component of finished fragrance oils (at ≤ 92% concentration) and incorporated into a variety of cosmetic and household products in Australia.

The proposed use concentrations of the isomer mixture containing the notified chemicals in finished consumer products are shown below:

Product Type	*Maximum Combined Use Concentration (%)*
Fine fragrances	0.66
Body lotions	0.4
Hair sprays	1.25
Deodorants	0.1
Other leave-on or rinse-off cosmetics	0.46
Household detergents, cleaners or fabric softeners	0.46
Air fresheners	1.25

OPERATION DESCRIPTION
The notified chemicals will not be manufactured within Australia. No reformulation or repackaging of the notified chemicals will occur at the notifier facility. The imported fragrance oils containing the notified chemicals (at ≤ 92% concentration for the isomer mixture) will be stored at the notifier facility until they are sold and distributed to customer facilities for reformulation into end-use products (cosmetic and household products).

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

End use
Cosmetic products
The finished cosmetic products containing the notified chemicals will be used by consumers and professionals such as beauticians and hairdressers. Depending on the nature of the products, applications may be by hand, spray or through the use of applicators.

Household products
Household products containing the notified chemicals may be used by consumers and professional workers such as cleaners. The products may be used in either closed systems with episodes of controlled procedures, for instance automatic washing machine cycles, or open manual processes including spraying, brushing, dipping, wiping and rinsing.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehouse workers	Incidental	Incidental
Plant operators – Compounding	4	250
Plant operators – Drum handling	1	250
Plant operators – Drum cleaning	2	200
Plant operators – Maintenance	2	250
Plant operators – Quality control	1	250

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemicals as components of fragrance oils (at up to 92% concentration for the isomer mixture), only in the unlikely event of accidental rupture of the drum containers.

Reformulation

During reformulation at the consumer product manufacture facilities, dermal, ocular and perhaps inhalation exposure of workers to the notified chemicals (at ≤ 92% concentration for the isomer mixture) may occur during weighing and transfer stages, blending, quality control analysis, and cleaning and maintenance of equipment. The notifier stated in the submission that the exposure is expected to be minimised by the use of engineering controls including local exhaust ventilation and enclosed systems, and by the use of PPE such as coveralls, goggles, impervious gloves and appropriate respiratory protections.

End-use

Exposure to the notified chemicals in end-use products (at ≤ 1.25% concentration for the isomer mixture) may occur in professions where the services provided involve the application of cosmetic products to clients (i.e., hair and beauty salons) or the use of cleaning 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 some PPE to minimise repeated exposure and good hygiene practices are expected to be in place. If appropriate PPE is used, exposure of such workers to the notified chemicals is expected to be of a similar or lesser extent than that experienced by consumers using the same products.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemicals (at ≤ 1.25% concentration for the isomer mixture) through the use of a wide range of cosmetic and household products. The principal routes of exposure will be dermal, while ocular and inhalation exposure (e.g., through the use of spray products) is also possible.

Data on typical use patterns of various types of consumer products in which the isomer mixture containing the notified chemicals may be used are shown in the following tables (SCCS, 2012; Cadby *et al.*, 2002; ACI, 2010; Loretz *et al.,* 2006). For the purposes of exposure assessment via the dermal route, Australian use patterns for various products are assumed to be similar to the consumer use patterns in Europe. In the absence of dermal absorption data and based on the low molecular weight of the notified chemicals (194.31 Da), a dermal absorption (DA) of 100% was assumed (European Commission, 2003). For inhalation exposure estimation of spray products, a 2-zone approach was used (Steiling *et al*., 2014; Rothe *et al*., 2011; Earnest, Jr, 2009) with an adult inhalation rate of 20 m^3/day (enHealth, 2012). It was conservatively assumed that the fraction of the notified chemicals inhaled be 50%, with the remainder ending up on the targets as intended. A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was applied in the calculations.

Cosmetic products (dermal exposure)

Product type	*Amount (mg/day)*	*Chemical concentration (%)*	*Retention Factor (RF)*	*Daily systemic exposure (mg/kg bw/day)*
Body lotion	7820	0.40	1.000	0.4888

Product type	*Amount (mg/day)*	*Chemical concentration (%)*	*Retention Factor (RF)*	*Daily systemic exposure (mg/kg bw/day)*
Face cream	1540	0.46	1.000	0.1107
Hand cream	2160	0.46	1.000	0.1553
Fine fragrance	750	0.66	1.000	0.0773
Deodorant	1430	0.10	1.000	0.0223
Shampoo	10460	0.46	0.010	0.0075
Conditioner	3920	0.46	0.010	0.0028
Shower gel	18670	0.46	0.010	0.0134
Facial cleanser	800	0.46	0.010	0.0006
Hand wash soap	20000	0.46	0.010	0.0144
Hair styling products	4000	0.46	0.100	0.0288
Total				**0.9218**

Daily systemic exposure = (Amount × Chemical concentration × RF × DA)/BW
(RF = retention factor; DA = dermal absorption; BW = body weight)

Household Products (Indirect dermal exposure – from wearing clothes)

Product type	*Amount (g/use)*	*C (%)*	*Product Retained (%)*	*Product Transferred (%)*	*Daily systemic exposure (mg/kg bw/day)*
Laundry liquid	230	0.46	0.95	10	0.0157
Fabric softener	90	0.46	0.95	10	0.0061
Total					**0.0219**

Daily systemic exposure = (Amount × C × PR × PT × DA)/BW
(C = chemical concentration; PR = product retained; PT = product transferred; DA = dermal absorption; BW = body weight)

Household products (Direct dermal exposure)

Product type	*Frequency (use/day)*	*C (%)*	*Contact Area (cm^2)*	*Product Usage (g/cm^3)*	*Film Thickness (cm)*	*Time Scale Factor*	*Daily systemic exposure (mg/kg bw/day)*
Laundry liquid	1.43	0.46	1980	0.01	0.01	0.007	0.0001
Dishwashing liquid	3	0.46	1980	0.009	0.01	0.03	0.0012
All-purpose cleaner	1	0.46	1980	1	0.01	0.007	0.0100
Total							**0.0113**

Daily systemic exposure = Frequency × C × Contact Area × Product Usage × Film Thickness × Time Scale Factor × DA/ BW
(C = chemical concentration; DA = dermal absorption; BW = body weight)

Aerosol products (Inhalation exposure)

Product type	*Amount (g/day)*	*C (%)*	*Exposure Duration Zone 1 (min)*	*Exposure Duration Zone 2 (min)*	*Volume Zone 1 (m^3)*	*Volume Zone 2 (m^3)*	*Daily systemic exposure (mg/kg bw/day)*
Hairspray	9.89	1.25	1	20	1	10	**0.0402**

Daily systemic exposure = [(Amount × C × 20 m^3/day Inhalation Rate × 50% Fraction Inhaled × 0.1) / BW × 1440)] × (Exposure Duration Zone 1/Volume Zone 1 + Exposure Duration Zone 2/Volume Zone 2)
(C = chemical concentration; BW = 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 0.9952 mg/kg bw/day for the isomer mixture. It is acknowledged that inhalation exposure to the notified chemicals from use of other 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 (e.g., air fresheners and deodorants).

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Skin corrosiveness (*in vitro* EpiDerm™ model)	non-corrosive
Skin irritation (*in vitro* EpiSkin™ model)	irritating
Eye irritation (*in vitro* BCOP test)	IVIS = 7.0; no prediction can be made
Rabbit, eye irritation	severely irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Human, skin sensitisation – RIPT (2%)	no evidence of sensitisation
Rat, combined repeated dose toxicity study with the reproduction/developmental toxicity screening test	NOAEL = 93 mg/kg bw/day, for male systemic and reproductive toxicity NOAEL = 104 mg/kg bw/day, for female systemic and reproductive toxicity NOAEL = 274 mg/kg bw/day, for male developmental toxicity NOAEL = 319 mg/kg bw/day, for female developmental toxicity
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vivo* micronucleus test	non genotoxic

Toxicokinetics

No toxicokinetic data was provided for the notified chemicals. Based on the water solubility (0.19 g/L at 20ºC), partition coefficient (log Pow = 4.7 at 20 °C) and the low molecular weight (194.31 Da) of the notified chemicals, passive diffusion across the gastrointestinal (GI) tract and dermal absorption are expected to occur. The notified chemicals may also be absorbed across the respiratory tract.

Acute toxicity

The isomer mixture containing the notified chemicals was found to be of low acute oral toxicity in rats.

No acute dermal or inhalation toxicity data were provided for the notified chemicals. Summary toxicology information provided by the notifier showed that a structurally similar chemical was determined to be of low acute toxicity via the dermal route with LD50 > 5,000 mg/kg bw in rabbit.

Irritation and sensitisation

Two *in vitro* skin corrosion/irritation studies were conducted using reconstructed human epidermis models (EpiDerm™ and EpiSkin™). The skin corrosion study using EpiDerm™ model predicted that the isomer mixture containing the notified chemicals was non-corrosive, whereas the skin irritation study indicated that the isomer mixture resulted in a tissue viability after exposure and post-treatment incubation < 50% (mean tissue viability = 9.6%). Based on the OECD TG 439, the notified chemicals should be considered as a Category 2 skin irritant under GHS.

An *in vitro* bovine corneal opacity and permeability (BCOP) test was conducted on the isomer mixture containing the notified chemicals. The results indicated that the isomer mixture caused an *in vitro* irritancy score (IVIS) of 7.0 (> 3 and < 55) and therefore a prediction could not be made. An eye irritation study in rabbits showed that the isomer mixture causes serious eye damage.

The isomer mixture containing the notified chemicals was found to be sensitising in a Local Lymph Node Assay (LLNA). The EC3 value was calculated to be 29.6%. The sensitising potential of the isomer mixture was also tested in a human repeat insult patch test (HRIPT). The results indicated that the isomer mixture did not cause skin sensitization when tested at 2% concentration.

Repeated dose toxicity

Based on a dose range finding study, a repeated dose oral toxicity study with reproductive/developmental toxicity screening was conducted on the isomer mixture containing the notified chemicals. Among males rats tested, slight to moderate hyaline droplet accumulation was observed in the kidneys in all treated groups, with minimal to slight basophilic tubules in the cortex of the kidney apparent mid and high dose groups. These changes were correlated with an increase in relative kidney weight and an increase in the creatinine in the blood.

However, toxicity in humans through this mechanism is considered to be improbable due to differences between rats and humans. The findings in the kidneys were not considered by the study authors to be relevant to human.

In the same study, minimal to moderate periportal/diffuse hepatocyte vacuolation was evident in the liver of females in mid and high dose groups, which correlated with an increase in liver weight, alanine aminotransferase activity and triglyceride concentrations, a decrease in the total protein and albumin concentrations and an increase in cholesterol concentrations and albumin/globulin ratio. This finding was considered by the study authors to be potentially adverse.

A NOAEL for systemic toxicity was established as 1,500 ppm (equivalent to 93 mg/kg bw/day for males and 104 – 197 mg/kg bw/day for females) in this study, based on potentially adverse liver effects observed in the females at higher dose levels.

Reproductive/developmental toxicity
In the above repeated dose oral toxicity study, reproductive/developmental toxicity of the isomer mixture containing the notified chemicals was screened. At the mid and high dose groups, the mean number of uterine implantation sites was reduced. Potentially adverse reduction in birth weight and subsequent body weight gain of the offspring in the high dose group was also noted. However, within the scope of this study it was not possible to ascertain the aetiology of these potentially adverse findings. The lower number of uterine implantations might also be attributable to subtle effects in the male reproductive tract during the study. The body weight effects in the offspring might possibly be an effect of the notified chemicals by cross-placental in utero exposure followed by post birth exposure in the milk.

A NOAEL for reproductive toxicity was established at 1,500 ppm (equivalent to 93 mg/kg bw/day for males and 104 – 197 mg/kg bw/day for females) in the study, based on reduction of uterine implantations observed in dams at higher doses.

A NOAEL for developmental toxicity was established at 4,500 ppm (equivalent to 274 mg/kg bw/day for males and 319 – 579 mg/kg bw/day for females) in the study, based on reduction of birth weight and subsequent body weight gain of the offspring observed at the highest dose.

Mutagenicity/Genotoxicity
A bacterial reverse mutation test and an *in vitro* mammalian cell micronucleus test were conducted on the isomer mixture containing the notified chemicals. The results did not show evidence of mutagenic or clastogenic properties for the notified chemicals.

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

Hazard classification	***Hazard statement***
Skin irritation (Category 2)	H315 – Causes skin irritation
Serious eye damage/Irreversible effects on the eye (Category 1)	H318 – Causes serious eye damage
Skin sensitiser (Category 1B)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemicals are recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R41: Risk of serious eye damage
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation
The notified chemicals are considered to be skin irritants and skin sensitisers, and may cause serious eye damage. Prolonged or repeated exposure to high concentration of the notified chemicals may also cause adverse liver and reproduction/development effects.

6.3.1. Occupational Health and Safety

Reformulation

Workers may experience dermal, ocular and perhaps inhalation exposure to the notified chemicals up to 92% concentrations for the isomer mixture during reformulation. Cautions should be exercised when handling the notified chemicals during reformulation and quality control processes. The use of enclosed, automated processes and PPE (i.e., coveralls, goggles and impervious gloves) should minimise the potential for exposure. Therefore, provided that adequate control measures are in place to minimise worker exposure, the risk of workers from use of the notified chemicals is not considered to be unreasonable.

End use

Cleaners, hair and beauty care professionals may come into contact with the notified chemicals at ≤ 1.25% concentration for the isomer mixture. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical mixture (for details of the public health risk assessment, see Section 6.3.2).

6.3.2. Public Health

Irritation

The notified chemicals may cause severe eye damage. The main risk of eye irritation will be expected from use of cosmetic products containing the notified chemicals. Given the low proposed use concentration in cosmetics (i.e. ≤ 1.25% for the isomer mixture), significant eye irritation effects are not expected. The eye irritation risk associated with use of the notified chemicals in consumer products may be further minimised by the inclusion of appropriate labelling and directions for use to warn against eye contact.

While the notified chemicals are also considered to be skin irritants, skin irritation effects are not expected from use of the notified chemicals at the proposed use concentrations.

Skin sensitisation

When tested in an LLNA study, the isomer mixture containing the notified chemicals was considered as a skin sensitiser. Proposed methods for the quantitative risk assessment of the dermal sensitisation have been the subject of significant discussion (i.e., Api *et al.,* 2008 and RIVM, 2010). Using fine fragrance as an example product that may contain the notified chemicals (at 0.66% concentration for the isomer mixture), as a worst case scenario, the Consumer Exposure Level (CEL) for the isomer mixture is estimated to be 24.75 μg/cm^2/day (Cadby *et al.,* 2002). Consideration of available information and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 24.69 μg/cm^2/day. In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16), use/time factor (3.16) and database factor (1), giving an overall safety factor of 300.

As the CEL is approximately equivalent to the AEL, considering a conservative safety factor of 300 used in the estimation, the risk to the public of the induction of sensitisation that is associated with the use of fine fragrances (a worst case example of a leave-on cosmetic product) 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 the use of the isomer mixture containing the notified chemicals in cosmetic and household products was estimated to be 0.9952 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 104 mg/kg bw/day derived from a combined repeated dose oral dietary toxicity study with reproductive/developmental toxicity screening, the margin of exposure (MOE) was estimated to be 105. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemicals at ≤ 1.25% concentration for the isomer mixture in cosmetic and household products, is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICALS AT SITE

The notified chemicals will not be manufactured in Australia; therefore there is no release of the notified chemicals to the environment is expected from this activity. Environmental release during importation, transport and distribution may occur as a result of accidental spills. In the event of a spill, the notified chemicals are expected to be contained and collected with an inert absorbent material and disposed of in accordance with local regulations.

Release to the environment during blending of the notified chemicals into end-use products are expected to be minimal as the blending operation will take place in highly automated and fully enclosed/contained environment with local exhaust ventilation. During blending processes, limited release of the notified chemicals is expected from cleaning of equipment as washings will be reused. A total of up to < 1% of the import volume is estimated to be generated as waste from residues in empty containers and spills during reformulation. Empty containers containing the notified chemicals will either be recycled or disposed of through an approved waste management facility.

RELEASE OF CHEMICALS FROM USE

The majority of the notified chemicals are expected to be released to sewers across Australia as a result of their use in cosmetic and domestic products. This includes washed off products from hair and skin of consumers as well as washings of the cleaning activities disposed of to the sewer. A small percentage of up to 3% of the total import volume of the notified chemicals, as residues in empty end use containers, are expected to be disposed of to landfill.

RELEASE OF CHEMICALS FROM DISPOSAL

It is expected that some of the product containing the notified chemicals will remain in end-use containers. The containers are expected to be disposed of through domestic garbage disposal and will enter landfill, or be subjected to recycling processes.

7.1.2. Environmental Fate

Following their use in Australia, the majority of the notified chemicals are expected to enter the sewer before potential release to surface waters on a nationwide basis. The majority of the notified chemicals will enter the sewer system as a result of the use of these chemicals as a fragrance ingredient in cosmetic and household care products. The notified chemicals are not readily biodegradable (0% in 28 days) and, based on their measured adsorption coefficient (log Koc = 3.4), low molecular weight and low water solubility, the notified chemicals are not expected to significantly adsorb to sediment, sludge or soil. The notified chemicals have the potential to be bioaccumulative based on their high partition coefficient (log Pow = 4.7). However, based on modelled data, the BCF is calculated to be 586 L/Kg indicating the notified chemicals are not expected to be bioaccumulative. The notified chemicals are expected to persist in the environment due to lack of ready biodegradability and low water solubility. In surface waters, the notified chemicals are expected to disperse and degrade through biotic and abiotic processes to form water and oxides of oxygen. For the details of the environmental fate study please refer to Appendix C.

The half-life of the notified chemicals in air is calculated to be 10.279 hours based on reactions with hydroxyl radicals (AOPWIN v1.92; US EPA, 2011). Therefore, in the event of release to atmosphere, the notified chemicals are not expected to persist in the atmospheric compartment.

A proportion of notified chemicals may be applied to land when treated sewage effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. Residues of the notified chemicals in landfill and soil are not expected to be very mobile based on their predicted adsorption coefficient, and are eventually expected to degrade to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the Predicted Environmental Concentration (PEC) is summarised in the table below. Based on the reported use in cosmetics and household cleaning products, it is assumed that 100% of the total import volumes of the notified chemicals are released to the sewer. The release is assumed to be nationwide over

365 days per year. It is conservatively assumed that 0% of the notified chemicals will be removed during sewage treatment processes.

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 chemicals 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)	22.613	million
Removal within STP	0%	
Daily effluent production	4,523	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC – River	1.21	µg/L
PEC - Ocean	0.12	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemicals in this volume are assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 1.212 µg/L may potentially result in a soil concentration of approximately 8.077 µg/kg. Assuming accumulation of the notified chemicals in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemicals in the applied soil in 5 and 10 years may be approximately 0.404 µg/kg and 0.808 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish (96 h)	LC50 = 3.965 mg/L	Toxic to fish
Daphnia Toxicity (48 h)	EC50 = 13 mg/L	Harmful to aquatic invertebrates
Algal Toxicity (72 h)	EC50 = 5.40 mg/L	Toxic to algae

Based on the acute ecotoxicity endpoints for the notified chemicals, they are expected to be harmful to daphnia and toxic to fish and algae. Under the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS; United Nations, 2009), the notified chemicals are formally classified as Acute Category 2; Toxic to aquatic life. Based on the acute toxicity and lack of ready biodegradability, the notified chemicals have been formally classified under the GHS as Chronic Category 2; Toxic to aquatic life with long lasting effects.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemicals has been calculated and is presented in the table below. The PNEC is calculated based on the endpoint for the most sensitive species (fish, LC50). Three acute ecotoxicity endpoints for aquatic species from three trophic levels are available. Therefore, an assessment factor of 100 has been used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish).	3.97	mg/L
Assessment Factor	100	
PNEC:	39.65	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated for a worst case discharge scenario based on the predicted PEC and PNEC.

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	1.21	39.65	0.031

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - Ocean:	0.12	39.65	0.003

The risk quotient for discharge of treated effluents containing the notified chemicals to the aquatic environment (Q < 1) indicates that the notified chemicals are unlikely to reach ecotoxicologically significant concentrations in surface waters based on their maximum annual importation quantity. The notified chemicals are not expected to be readily biodegradable and are not expected to be bioaccumulative in the environment.

The risk quotient for discharge of the notified chemicals to the aquatic environment indicates that the notified chemicals are unlikely to reach ecotoxicologically significant concentrations based on their annual importation quantity. Therefore, on the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern in cosmetic and domestic products, the notified chemicals are not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Pour Point 0 °C

Method	OECD TG 102 Melting Point/Melting Range EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	Tested on the mixture of two notified chemicals with a purity of 90.4%.
Test Facility	Huntingdon (2013a)

Boiling Point Decomposes above 255 °C before boiling

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Tested on the mixture of two notified chemicals with a purity of 93.1%. The test substance darkened (indicative of decomposition) at temperatures above approximately 255 °C before boiling. The decomposed sample boiled at approximately 260 °C.
Test Facility	Huntingdon (2015a)

Density 1,000 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	Tested using a pycnometer on the mixture of two notified chemicals with a purity of 93.1%.
Test Facility	Huntingdon (2015a)

Vapour Pressure 0.003 kPa at 25 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Tested using the vapour pressure balance on the mixture of two notified chemicals with a purity of 90.4%.
Test Facility	Huntingdon (2014)

Water Solubility 0.19 g/L at 20 °C

Method	OECD TG 105 Water Solubility EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks	Column Elution Method
Test Facility	Huntingdon (2013a)

Partition Coefficient (n-octanol/water) log Pow = 4.7 at 20 °C

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

Surface Tension 48 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 the mixture of two notified chemicals with a purity of 93.1%. Concentration: 90% saturated aqueous solution.
Test Facility	Huntingdon (2015a)

Adsorption/Desorption log K_{oc} = 3.4 at 25 °C

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge.

Remarks	HPLC Method
Test Facility	Huntingdon (2015a)

Flash Point 114 °C at 101.3 kPa (closed cup)

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Tested using Pensky-Martens closed cup flash point apparatus on the mixture of two notified chemicals with a purity of 93.1%.
Test Facility	Huntingdon (2015a)

Autoignition Temperature 282 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks	Tested on the mixture of two notified chemicals with a purity of 93.1%.
Test Facility	Huntingdon (2015a)

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 notified chemicals.
Test Facility	Huntingdon (2015a)

Oxidizing Properties Not expected to have oxidising properties

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	Estimated from the chemical structure of the notified chemicals.
Test Facility	Huntingdon (2015a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/RccHan®:WIST albino
Vehicle	Corn oil
Remarks - Method	No significant deviations of protocol were noted. The purity of the test substance was reported to be > 93.1%.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	300	0/3
2	3 F	300	0/3
3	3 F	2,000	1/3
4	3 F	2,000	1/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	Two females dosed at 2,000 mg/kg bw were euthanized on Day 1 approximately 5 or 6 hours after dosing. Clinical signs prior to death comprised unsteady gait, decreased activity, piloerection, reduced body tone, abnormally cold to touch, partially closed eyelids, irregular breathing, hunched posture and salivation. A loss in body weight was noted for one decedent. Clinical signs of reaction to treatment seen in the surviving females dosed at 2,000 mg/kg bw comprised unsteady gait and piloerection. Decreased activity, hunched posture, reduced body tone and salivation were also observed. These signs were first noted approximately thirty minutes after dosing. Recovery of surviving animals, as judged by external appearance and behaviour, was observed by Day 4. No clinical signs were seen in animals dosed at 300 mg/kg bw.
Effects in Organs	Macroscopic examination of the animals that were euthanised revealed congestion of the lungs and bronchi. Black/brown fluid contents of the small intestine were seen in both decedents. A thick brown fluid in the stomach and yellow fluid contents of the large intestine were seen in one decedent and clear fluid in the stomach seen in the other. No abnormalities were noted in surviving animals at the macroscopic examination at the end of the study.
Remarks - Results	A low body weight gain was noted for one female dosed at 300 mg/kg bw on Day 15. All other surviving animals were considered to have achieved satisfactory body weight gains throughout the study.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	Huntingdon (2015b)

B.2. Irritation – skin (*in vitro* EpiDerm™ model)

TEST SUBSTANCE	Isomer mixture containing the notified chemicals

METHOD OECD TG 431 *In vitro* Skin Corrosion – Reconstructed human Epidermis (RHE) Test Method

Vehicle None

Remarks - Method No significant deviations of protocol were noted. The purity of the test substance was reported to be 93.1%. The test substance was applied for 3 minutes and 1 hour to the EpiDerm™ three dimensional human skin model.

RESULTS

Three minute treatment

Test material	*Mean OD_{570} of duplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean Viability*
Negative control	1.907	100	0.755
Test substance	1.999	104.8	0.617
Positive control	0.254	13.3	1.896

OD = optical density; SD = standard deviation

One hour treatment

Test material	*Mean OD_{570} of duplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	1.784	100	2.158
Test substance	1.628	91.2	0.981
Positive control	0.040	2.3	0.103

OD = optical density; SD = standard deviation

Remarks - Results The test substance was shown not to reduce MTT.

CONCLUSION The test substance was predicted to be non-corrosive to the skin under the conditions of the test.

TEST FACILITY Huntingdon (2015c)

B.3. Irritation – skin (*in vitro* EpiSkin™ model)

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method
EpiSkin™ Skin Irritation Test (15 min – 42 hr)

Vehicle None

Remarks - Method No significant deviations of protocol were noted. The purity of the test substance was reported to be 93.1%. The test substance was applied to the EpiSkin™ human epidermis for 15 minutes and incubated for 42 hour before MTT assays.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.884	100	14.3
Test substance	0.085	9.6	3.1
Positive control	0.130	14.7	1.1

OD = optical density; SD = standard deviation

Remarks - Results The test substance was shown not to reduce MTT. It resulted in a mean tissue viability of < 50% (i.e. 9.6%), and therefore was predicted to be an irritant to the skin (Category 2 under GHS).

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

TEST FACILITY Huntingdon (2015d)

B.4. Irritation – eye (*in vitro* BCOP test)

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants

Vehicle None

Remarks - Method No significant deviations of protocol were noted. The purity of the test substance was reported to be 93.1%. Ethanol was used as a positive control and negative control was 0.9% saline.

RESULTS

Test material	*Mean opacities of triplicate tissues (SD)*	*Mean permeabilities of triplicate tissues (SD)*	*IVIS (SD)*
Negative control	2.333 (± 0.577)	0.014 (± 0.011)	-
*Test substance**	4.667 (± 1.000)	0.157 (± 0.115)	7.0 (± 2.6)
*Positive control**	18.667 (± 1.000)	0.824 (± 0.074)	31.0 (± 0.1)

SD = Standard deviation; IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results The test substance resulted in an IVIS of 7.0. Under the study guidelines, no prediction can be made for scores > 3 and ≤ 55.

CONCLUSION No prediction could be made for the test substance under the conditions of the test.

TEST FACILITY Huntingdon (2015e)

B.5. Irritation – eye

TEST SUBSTANCE Isomer mixture containing the notified chemicals

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

Species/Strain Rabbit/New Zealand White

Number of Animals 1

Observation Period 7 days

Remarks - Method A single rabbit was tested in the study. The presence of a severe effect in the animal prevented further animals being committed to the study.

The purity of the test substance was reported to be > 93.1%.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	2.0	2	> 7 days	2
Conjunctiva: chemosis	0.3	2	< 48 h	0
Conjunctiva: discharge	0.0	2	< 24 h	0
Corneal opacity	1.0	2	> 7 days	2
Iridial inflammation	0.3	1	> 7 days	1

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

Remarks - Results A crimson-red conjunctival appearance was evident throughout the study and slight chemosis was apparent during the first 24 hours with discharge

evident at 1 hour after instillation. Iritis was evident during the first 24 hours after instillation and also 7 days later. Scattered or diffuse areas of opacity covering up to the entire corneal surface were apparent throughout the first 72 hours after instillation. An easily discernible translucent area of opacity covering approximately one quarter of the corneal surface and an area of scattered or diffuse opacity covering approximately half the corneal surface were evident 7 days after instillation. In addition, two areas of pannus formation (corneal neovascularisation) were apparent. Pannus formation was considered to be an irreversible effect and the animal was humanely killed immediately after this observation.

Instillation of the test material did not give rise to initial pain response.

CONCLUSION The test substance causes serious eye damage.

TEST FACILITY Huntingdon (2015f)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Commission Regulation No 400/2008 B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain	Mouse/CBA/Ca
Vehicle	Acetone:olive oil (4:1 v/v)
Preliminary study	Yes (tested at 50% and 100%)
Positive control	25% (v/v) hexylcinnamic aldehyde
Remarks - Method	No significant deviations of protocol were noted. The purity of the test substance was reported to be > 93.1%. Based on the results of the preliminary investigations, the test substance was tested at concentrations of 10%, 25% and 50% (v/v) in the main study.

RESULTS

Concentration (% v/v)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5 F	331.5	1.0
10	5 F	422.2	1.3
25	5 F	844.8	2.5
50	5 F	1734.5	5.2
Positive Control			
25	5 F	3257.6	9.8

EC3	29.6%
Remarks - Results	No deaths and signs of toxicity were noted during this study. No signs of dermal irritation were seen on the ear during the main study.

CONCLUSION There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.

TEST FACILITY Huntingdon (2015g)

B.7. Skin sensitisation – human volunteers

TEST SUBSTANCE Isomer mixture containing the notified chemicals (tested at 2%)

METHOD Repeated insult patch test with challenge (modified Shelanski – Shelanski human patch test method, H.A. Shelanski and M.V. Shelanski, Proc. Sci.

	Sect. Toilet Goods Assoc. 19:46, 1953)
Study Design	Induction Procedure: The test substance at 2% concentration was applied to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of 24 hours in occlusive condition. Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications. Rest Period: approximately 2 weeks Challenge Procedure: The challenge patches were applied to previously untreated test sites on the back. After 24 hours, the patches were removed and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 and 72 hours.
Study Group	93 F, 21 M; age range 18 – 68 years
Vehicle	Ethanol:diethyl phthalate (1:3)
Remarks - Method	Occluded. The test substance (0.2 mL) was spread on a 3.63 cm^2 patch.

RESULTS

Remarks - Results	This study was initiated with 114 subjects. Ten subjects (8 F and 2 M) discontinued for reasons unrelated to the test substance. A total of 104 subjects completed the study. No adverse events were reported during the study.

CONCLUSION	The test substance at 2% concentration was non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2014)

B.8. Repeat dose oral toxicity – dose range finding

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	Dose range finding study for subsequent OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 14 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Diet
Remarks - Method	The purity of the test substance was reported to be 94.8%. The test substance was tested at dietary concentrations of 7500, 11000 and 15000 ppm. Control group received untreated diet.

RESULTS

Group	*Number and Sex of Animals*	*Dose Concentration (ppm)*	*Mortality*
Control	3 F/3 M	0	0/6
Low dose	3 F/3 M	7,500	0/6
Mid dose	3 F/3 M	11,000	0/6
High dose	3 F/3 M	15,000	0/6

Mortality and Time to Death

There were no premature deaths.

Clinical Observations

There were no treatment-related changes in clinical condition.

Effects in Organs

There were no treatment-related macroscopic abnormalities detected at scheduled termination. Treatment-related effects on relative organ weight were recorded for increase of liver weight in males and females given 11,000 or 15,000 ppm with no apparent dose response.

Remarks – Results

The administration of the test substance was associated with a non-dose dependent reduction in food consumption in all treated groups that persisted for 1-3 days in males and 3 days in females. This reduction in food intake was accompanied by a dose-dependent body weight loss between Days 1 and 4 in males and females given 11,000 or 15,000 ppm. From Days 4 to 15, the body weight performance of individual animals was similar to Control. The body weight performance of males and females given 7,500 ppm was unaffected.

CONCLUSION

The dietary level of 15,000 ppm was selected for use as the high dose level in the subsequent OECD TG 422 study (see Appendix B.9).

TEST FACILITY Huntingdon (2015h)

B.9. Repeat dose oral toxicity

TEST SUBSTANCE Isomer mixture containing the notified chemicals

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

Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – diet
Exposure Information	Total exposure: at least 5 weeks in males and approximately 7 weeks in females (until Day 7 of lactation) Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Diet
Remarks - Method	No significant deviations of protocol were noted. The purity of the test substance was reported to be 94.8%.

RESULTS

Group	*Number and Sex of Animals*	*Dose (ppm)*	*Mean Achieved Dose (mg/kg bw/day)*				*Mortality*
			Male	*Female*			
			Week 1-5	*Prior Pairing*	*Gestation*	*Lactation*	
Control	10 F/10 M	0	0	0	0	0	0/20
Low dose	10 F/10 M	1,500	93	104	116	197	0/20
Mid dose	10 F/10 M	4,500	274	319	372	579	0/20
High dose	10 F/10 M	15,000	941	957	1,140	1,805	0/20

Mortality and Time to Death

There were no premature deaths during the study.

Clinical Observations

It was noted that the forelimb grip strength of males and females given 4,500 or 15,000 ppm was slightly lower than Control in the absence of a clear dose response relationship with no statistical significance attained. The majority of group mean high and low beam activity scores, including total scores, for all treated males and females were slightly high compared with Controls, with occasional scores for individual attaining statistical significance. All scores attained were within the historical control data (HCD) range for animals of this strain and age.

The above observations were not considered by the study authors to be treatment-related.

Effects on food consumption were evident during Days 1 to 4 of treatment for females given 4,500 ppm and for males and females given 15,000 ppm. These effects indicated that the test diets were slightly unpalatable and resulted in slightly reduced weight gain/weight loss during Week 1 of the study. During the remainder of the study, food consumption effects were limited to females given 15,000 ppm showing slightly low food intake

during Days 0 to 2 and 6 to 12 of gestation, and throughout Days 1 to 6 of lactation, resulting in low mean body weight gain during these periods. These effects were considered by the study authors to be non-specific and non-adverse.

Laboratory Findings – Clinical Chemistry, Haematology

Blood chemistry

Biochemical changes in the plasma at the end of the treatment period (Week 6 for males and Day 8 of lactation for females) revealed the following differences from Control:

- Alanine aminotransferase activity was slightly high in females given 15,000 ppm and was above the 5-95% confidence limits HCD range.
- Bilirubin concentrations were marginally low in males given 15,000 ppm.
- Bile acid concentrations were low for males given 4,500 or 15,000 ppm with no dose response.
- Creatinine concentrations were slightly high for males given 15,000 ppm but slightly low for all groups of treated females.
- Glucose concentrations were slightly low for all groups of treated males without a dose response.
- Cholesterol concentrations were slightly elevated in all groups of treated females with a dose response apparent (ranging from 31% to 140% higher than Control) and values for females given 15,000 ppm were above the HCD range.
- Triglyceride concentrations were high in males given 15,000 ppm and in females given 4,500 or 15,000 ppm with the values for females exceeding the HCD range.
- Calcium concentrations were marginally increased in females given 15,000 ppm and exceeded the HCD range.
- Total protein and albumin concentrations were slightly low in females given 4,500 or 15,000 ppm and albumin/globulin ratio was slightly low in all groups of treated females.

Haematology

During Week 6 of treatment, males given 15,000 ppm showed statistically significantly low total white blood cell counts when compared to Controls, and were below the HCD range. This difference was attributable to statistically significantly low neutrophil, lymphocyte, basophil and large unstained cell counts. Males given 4,500 ppm also showed statistically significantly low total white blood cell counts, predominantly due to low lymphocyte counts; however, all mean and individual values in this group of males were within the HCD range. There were no changes in leucocytic parameters for males given 1,500 ppm. Erythrocytic parameters were unaffected in all groups of treated males.

Haematological investigations conducted for females on Day 8 of lactation did not reveal any treatment-related changes when compared to Controls.

Effects in Organs

At scheduled termination, mean liver weights were increased in a dose-related manner in males given 4,500 or 15,000 ppm with statistical significance. Liver weights in females were also increased with statistical significance attained in the high dose group. The kidney and epididymides weights of males given 4,500 or 15,000 ppm and the spleen weight of females in these groups were statistically significantly high with no apparent dose response. For females given 15,000 ppm, brain weights were marginally but statistically significantly high with no effect on absolute brain weights, indicating that the effect was due to the slightly lower terminal body weight of these females. Mean uterus/cervix/oviduct weights were slightly low for females given 15,000 ppm. This effect was considered by the study authors likely to reflect the stage of oestrus at the time of necropsy and was unrelated to the treatment.

The macroscopic examination performed at scheduled termination revealed no test substance related lesions. The incidence and distribution of all findings were consistent with the common background seen in rats.

Histopathological examinations revealed treatment-related changes in the kidneys (males) and the liver (females). Slight to moderate hyaline droplets in the cortical tubules of the kidneys was seen in all groups of treated males. Minimal to slight cortical basophilic tubules were seen in males given 4,500 or 15,000 ppm. Minimal to moderate vacuolation of periportal/diffuse hepatocytes was observed in the liver of females given 4,500 or 15,000 ppm. Other histological changes were not considered by the study authors to be treatment-related.

Effects on Dams

Mating performance was considered by the study authors to be unaffected by the treatment, with all pairs mating at the first oestrus opportunity. There was no evidence of dystocia, all females were pregnant and all successfully gave birth to live young, indicating that fertility was unaffected by the treatment. Gestation length for all females was within the expected range of 22 to 23 days, and gestation index was 100% in all groups.

Seminiferous tubules were evaluated with respect to their stage in spermatogenic cycle and the integrity of the cell types present within different stages. No cell or stage specific abnormalities were noted.

Effects on Foetus

There were no treatment-related clinical signs seen among the offspring. There were also no macroscopic abnormities detected prior to or at the scheduled termination indicative of an adverse effect of the parental treatment.

Litter size, sex ratio and survival indices

Among females given 4,500 or 15,000 ppm, mean implantation counts were slightly, but statistically significantly lower, with 5/10 and 7/10 females having fewer implantation sites than the lowest concurrent Control. The mean values of the implantation sites were outside the HCD range. As a consequence, mean litter size in these 2 groups on Day 1 of lactation was lower than Control. The mean number of implantation sites and mean litter size were unaffected at 1,500 ppm. The mean post-partum corpora lutea count recorded on Day 8 of lactation was similar in all groups of females.

Sex ratio and offspring survival was unaffected by the treatment.

Offspring body weight

On Day 1 of age, the mean absolute body weight of offspring in litters derived from parent animals given 15,000 ppm was 7% and 8% lower than Control for males and females respectively, with differences attaining statistical significance. The mean body weight gain of these offspring was 30-31% lower than Control throughout Days 1 to 7 of age, such that mean absolute body weight on Day 7 of age was 20% lower than Control in both sexes. At 4,500 ppm, mean offspring body weights on Day 1 of age were essentially similar to Control. Mean body weight gain between Days 1 and 7 of age was 7-9% lower than Control such that mean absolute body weight on Day 7 of age was 4-5% lower than Control. However, none of these differences attained statistical significance. The body weight and body weight gain of offspring in the 1,500 ppm group was considered unaffected by the parental treatment.

Remarks – Results

Among males, slight to moderate hyaline droplet accumulation was observed in the kidneys in all treated groups, with minimal to slight basophilic tubules in the cortex of the kidney apparent at 4,500 and 15,000 ppm. These changes correlated with an increase in body weight adjusted kidney weight in the groups and an increase in the creatinine in the blood in males given 15,000 ppm. Hyaline droplets contain α2u globulin and their prolonged accumulation is associated with chronic cell damage and increased cell turnover. The appearance of basophilic tubules in the kidneys of the test animals most likely reflected this change. However, toxicity in humans through this mechanism is considered to be improbable as little or no α2u globulin is present in humans. Therefore the findings in the male rat kidney were not considered by the study authors to be relevant to human.

Minimal to moderate periportal/diffuse hepatocyte vacuolation was evident in the liver of females given 4,500 or 15,000 ppm, which correlated with an increase in liver weight, alanine aminotransferase activity and triglyceride concentrations, a decrease in the total protein and albumin concentrations and an increase in cholesterol concentrations and albumin/globulin ratio. The incidence of this lesion was considered by the study authors likely to be an exacerbation of spontaneous change due to metabolism of the test substance. This effect potentially was adverse.

At 4,500 or 15,000 ppm, the mean number of uterine implantation sites was lower than Control but within the scope of this study it was not possible to ascertain the aetiology of the potentially adverse finding. Although testes weights were unaffected and the histopathological evaluation of the testes did not reveal any abnormalities, these assessments were unable to ascertain functional reproductive capacity. The lower number of uterine implantations might also have been attributable to subtle effects in the male reproductive tract.

Within the scope of this study it was not possible to establish the aetiology of the potentially adverse reduction

in birth weight and subsequent body weight gain of the offspring in the 15,000 ppm group. This might have been a result of the parent females being smaller and consuming less food, or a direct effect of the test substance by cross-placental in utero exposure followed by post birth exposure in the milk.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) for systemic toxicity and reproductive toxicity was established as 1,500 ppm (equivalent to 93 mg/kg bw/day for males and 104 – 197 mg/kg bw/day for females) in this study, based on potentially adverse liver effects in females and reduction of uterine implantations in dams observed at higher doses.

The NOAEL for developmental toxicity was established as 4,500 ppm (equivalent to 274 mg/kg bw/day for males and 319 – 579 mg/kg bw/day for females) in this study, based on reduction of birth weight and subsequent body weight gain of the offspring derived from parent animals given higher dose.

TEST FACILITY Envigo (2016)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Commission Regulation No. 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation and Pre incubation procedures

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA (pKM101)
Metabolic Activation System	S9 mix prepared from the liver of phenobarbital sodium and 5,6-benzoflavone induced male rat
Concentration Range in Main Test	a) With metabolic activation: 5,000 µg/plate b) Without metabolic activation: 1,500 µg/plate
Vehicle	Dimethyl sulphoxide (DMSO)
Remarks - Method	No significant deviations of protocol were noted. The purity of the test substance was reported to be 99.3%.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1 (Plate incorporation)	≥ 1,500	> 5,000	Negative
Test 2 (Pre incubation)	≥ 1,500	> 1,500	Negative
Present			
Test 1 (Plate incorporation)	≥ 1,500	> 5,000	Negative
Test 2 (Pre incubation)	≥ 1,500	> 1,500	Negative

Remarks - Results Positive, negative and sterility controls showed expected results.

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

TEST FACILITY Huntingdon (2013b)

B.11. Genotoxicity – *in vitro* mammalian cell micronucleus test

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 487 *In vitro* Mammalian Cell Micronucleus Test

Species/Strain	Human lymphocytes
Cell Type/Cell Line	Human lymphocytes in whole blood culture
Metabolic Activation System	S9 mix prepared from the liver of phenobarbital sodium and 5,6-

	benzoflavone induced male rat
Vehicle	Dimethyl sulphoxide (DMSO)
Remarks - Method	No significant deviations of protocol were noted. The purity of the test substance was reported to be 99.3%.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	20, 50, 60, 70 and 90	3 h	20 h
Test 2	4, 6, 8, 10 and 13	20 h	20 h
Present			
Test 1	10, 60, 120, 140 and 145	3 h	20 h

All cultures were selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 90	> 90	Negative
Test 2	≥ 13	> 13	Negative
Present			
Test 1	≥ 145	> 145	Negative

Remarks - Results	Positive and negative controls showed expected results.
CONCLUSION	The test substance was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Huntingdon (2013c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

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

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	0	4	51
7	0	7	70
14	0	14	84
21	0	21	90
28	0	28	95

Remarks - Results

After 28 days, the percent degradation for the notified chemicals was 0%. The percent degradation calculated in the reference item replicate (procedure control) up to day 28 was 95%.

There was no blank corrected mean oxygen consumption in mixtures containing the notified chemicals throughout the 28 days of the test. Substances are considered to be readily biodegradable in this type of test if oxygen consumption is equal to or greater than 60% of the theoretical oxygen demand (ThOD) of the test mixtures within ten days of the consumption achieving 10%. Therefore, the notified chemicals were not considered to be readily biodegradable under the conditions of this test.

CONCLUSION The notified chemicals are not readily biodegradable.

TEST FACILITY Huntingdon (2015i)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 203 Fish, Acute Toxicity Test -semi-static. EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - semi-static.
Species	Zebrafish *(Brachydanio* rerio*)*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	None
Analytical Monitoring	High performance liquid chromatography using UV detection (HPLC-UV)
Remarks – Method	The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed.

RESULTS

Concentration (mg/L) *Nominal*	*Number of Fish*	*Mortality (%)* *24 h*	*48 h*	*72 h*	*96 h*
2.20	10	0	0	0	0
2.66	10	0	0	0	0
3.22	10	0	0	0	10
3.90	10	0	0	0	40
4.72	10	0	0	0	90
5.71	10	0	0	20	100

LC50 3.96 mg/L at 96 hours (95% confidence limits of 3.636 - 4.325 mg/L).

Remarks – Results All validity criteria were within acceptable limits and therefore the study is considered valid.

CONCLUSION The notified chemicals are toxic to fish.

TEST FACILITY Suzhou Research (2014)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Isomer mixture containing the notified chemicals

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - static.
EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia - static

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
Water Hardness None
Analytical Monitoring High performance liquid chromatography using UV detection (HPLC-UV)
Remarks - Method The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed

Twenty animals in test group and control group, divided into 4 replicates (5 animals / replicate) were exposed to an aqueous solution of test substance at 21 - 22 °C under static conditions.

A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 90 mg/L was obtained from a saturate solution method of preparation indicating this to be the limit of water solubility of this test item under test conditions.

RESULTS

Concentration (mg/L) *Nominal*	*Number of D. magna*	*Number Immobilised* *24 h [acute]*	*48 h [acute]*
1.0	20	0	0
3.2	20	0	0
10	20	0	0
32	20	20	20
100	20	20	20

EC50 13 mg/L at 48 hours
NOEC 7.3 mg/L at 48 hours
Remarks - Results All validity criteria were within acceptable limits and therefore the study is considered valid.

CONCLUSION	The notified chemicals are harmful to aquatic invertebrates
TEST FACILITY	Envigo (2015a)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Isomer mixture containing the notified chemicals
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Council Regulation No 440/2008 C.3 Algal Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 3.2,10, 32 and100 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	High performance liquid chromatography using UV detection (HPLC-UV)
Remarks - Method	The test was conducted in accordance with the test guideline without significant deviations. Good Laboratory Practice (GLP) was followed. A preliminary media preparation trial indicated that a dissolved test item concentration of approximately 90 mg/L was obtained from a saturate solution method of preparation indicating this to be the limit of water solubility of this test item under test conditions.

RESULTS

Biomass		*Growth*	
EC50 (mg/L at 72h)	*NOEC (mg/L at 72h)*	*EC50 (mg/L at 72 h)*	*NOEC (mg/L at 72h)*
3.1*	0.86	5.4	2.8

* 95% Confidence limits (2.8 - 3.5)

Remarks - Results	All validity criteria were within acceptable limits, no mortality and therefore the study is considered valid.
CONCLUSION	The notified chemicals are toxic to algae
TEST FACILITY	Envigo (2015b)

BIBLIOGRAPHY

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

Cadby et al. (2002) Consumer Exposure to fragrance Ingredients: Providing Estimates for Safety Evaluation. Regulatory Toxicology and Pharmacology 36: 246- 252.

CRL (2014) Repeated insult Patch Test (RIPT) – Shelanski Method (CRL Study Numbers: CRL81214-1 and CRL81214-2, December, 2014) USA, Clinical Research Laboratories, Inc. (Unpublished report submitted by the notifier).

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

enHealth (2012) Australian Exposure Factor Guidance. Guideline for Determining Human Health Risks from Environmental Hazards, Commonwealth of Australia.

Enovigo (2015a) FRET 08-0338: Daphania sp., 48-Hour Acute Immobilization Test (Study number: 41501247, December, 2015). UK, Envigo Research Limited, Shardlow Business Park (Unpublished report submitted by the notifier).

Enovigo (2015b) FRET 08-0338: Algal Growth Inhibition Test (Study number: 41501241, November, 2015). UK, Envigo Research Limited, Shardlow Business Park (Unpublished report submitted by the notifier).

Enovigo (2016) FRET 08-0338: Combined Repeated Dose Toxicity Study and Reproductive/Developmental Toxicity Screening Study in the Rat (Envigo study number: JMN0068, February, 2016). UK, Envigo CRS Limited (Unpublished report submitted by the notifier).

European Commission (2003) Technical Guidance Document on Risk Assessment (in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances, Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances, Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market – Part II). Institute for Health and Consumer Protection, European Chemicals Bureau.

Huntingdon (2013a) FRET 08-0338 Physicochemical Properties (HLS study number: JMN0038, August, 2013). UK, Huntingdon Life Sciences, Eye Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2013b) FRET 08-0338 Bacterial Reverse Mutation Test (HLS study number: JMN0035, June, 2013). UK, Huntingdon Life Sciences, Eye Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2013c) FRET 08-0338 *In Vitro* Micronucleus Test in Human Lymphocytes (HLS study number: JMN0036, July, 2013). UK, Huntingdon Life Sciences, Eye Research Centre and Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2014) FRET 08-0338 Vapour Pressure (HLS study number: JMN0042, June, 2014). UK, Huntingdon Life Sciences, Eye Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015a) FRET 08-0338 Physicochemical Properties (HLS study number: JMN0071, April, 2015). UK, Huntingdon Life Sciences, Eye Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015b) FRET 08-0338: Acute Oral Toxicity to the Rat (Acute Toxic Class Method) (HLS study number: JMN0072, February, 2015). UK, Huntingdon Life Sciences, Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015c) FRET 08-0338 *In Vitro* Skin Corrosivity Test Using the EpiDerm™ Humen Skin Model (HLS study number: JMN0073, April, 2015). UK, Huntingdon Life Sciences, Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015d) FRET 08-0338 EpiSkin™ Skin Irritation Test $^{15\ min\ –\ 42\ hours}$ (HLS study number: JMN0074, April, 2015). UK, Huntingdon Life Sciences, Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015e) FRET 08-0338 The Bovine Corneal Opacity and Permeability Assay (BCOP) (HLS study number: JMN0075, January, 2015). UK, Huntingdon Life Sciences, Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015f) FRET 08-0338: Eye Irritation to the Rabbit (HLS study number: JMN0088, September, 2015). UK, Huntingdon Life Sciences, Eye Research Centre and Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015g) FRET 08-0338: Assessment of Skin Sensitization Potential Using the Local Lymph Node Assay in the Mouse (Individual Animal Approach) (HLS study number: JMN0079, April, 2015). UK, Huntingdon Life Sciences, Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015h) FRET 08-0338: Preliminary Toxicity Study by Dietary Administration to Crl:CD(SD) Rats for 14 Days (HLS study number: JMN0069, June, 2015). UK, Huntingdon Life Sciences, Eye Research Centre and Huntingdon Research Centre (Unpublished report submitted by the notifier).

Huntingdon (2015i) FRET 08-0338 Assessment of Ready Biodegradability by Respirometry (HLS study number: JMN0078, June, 2015). UK, Huntingdon Life Sciences, Eye Research Centre (Unpublished report submitted by the notifier).

Loretz *et al*., (2006) Exposure Data for Personal Care Products: Hairspray, Spray Perfume, Liquid Foundation, Shampoo, Body Wash and Solid Antiperspirant. Food and Chemical Toxicology 44: 2008-2018.

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

Rothe *et al*., (2011) Special Aspects of Cosmetic Spray Safety Evaluations: Principles on Inhalation Risk Assessment. Toxicology Letters 205: 97-104.

Steiling *et al*., (2014) Principle Considerations for the Risk Assessment of Sprayed Consumer Products. Toxicology Letters 227: 41-49.

Suzhou Research (2014) Acute Toxicity of FRET 08-0338 on Zebrafish (Study number: 2013-182-01-01, March, 2014). China, Suzhou Xishan Zhongke Drug R & D Co., Ltd. (Unpublished report submitted by the notifier).

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, http://www.safeworkaustralia.gov.au/sites/swa/about/publications/pages/managing-risks-of-hazardous-chemicals-in-the-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 >.