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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2*H*-Pyran, 3,6-dihydro-4,6-dimethyl-2-(1-phenylethyl)-

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2091	International Flavours and Fragrances (Australia) Pty Ltd	2*H*-Pyran, 3,6-dihydro-4,6-dimethyl-2-(1-phenylethyl)-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard Classification
Based on the available information, the notified chemical is a 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 chemical is presented in the following table.

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

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

Hazard Classification	*Hazard Statement*
Chronic Aquatic Toxicity (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 chemical is not considered to pose an unreasonable risk to the health of workers.

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin irritation (Category 2): H315 – Causes skin irritation
  - Skin Sensitisation (Category 1B): H317 – May cause an allergic skin reaction

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

Health Surveillance

- As the notified chemical is a skin sensitiser, 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 chemical during reformulation:
  - Enclosed/automated processes
  - 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 chemical for reformulation:
  - Avoid contact with 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 chemical during reformulation:
  - Impervious gloves
  - Protective clothing
  - Respiratory protection if inhalation exposure may occur

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Storage

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

Emergency procedures

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

Disposal

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify

NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the final use concentration of the notified chemical exceeds at 0.076% in deodorants, 0.49% in face and hand creams, 0.85% in fine fragrances, 0.94% in body lotions and 3% in other cosmetic and household products;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a fragrance ingredient, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

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

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

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
China (2017), EU (2018)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Vertonic

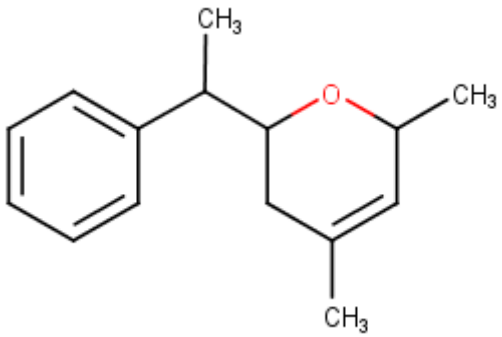
CAS NUMBER
1945993-03-2

CHEMICAL NAME
2*H*-Pyran, 3,6-dihydro-4,6-dimethyl-2-(1-phenylethyl)-

OTHER NAME
FRET 12-0492

MOLECULAR FORMULA
$C_{15}H_{20}O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
216.32 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 75% (isomeric mixture)

The notified chemical is manufactured overseas as an inseparable isomeric mixture.

The composition of the isomeric mixture is as follows:

Chemical Name	*CAS No.*	*Weight %*
2*H*-Pyran, 3,6-dihydro-4,6-dimethyl-2-[(1*R*)-1-phenylethyl]-, (2*R*,6*S*)-*rel*-	Not assigned	44.67
2*H*-Pyran, 3,6-dihydro-4,6-dimethyl-2-[(1*S*)-1-phenylethyl]-, (2*R*,6*S*)-*rel*-	Not assigned	26.58
2*H*-Pyran, 3,6-dihydro-4,6-dimethyl-2-[(1*R*)-1-phenylethyl]-, (2*R*,6*R*)-*rel*-	1971064-69-3	5.08
2*H*-Pyran, 3,6-dihydro-4,6-dimethyl-2-[(1*S*)-1-phenylethyl]-, (2*R*,6*R*)-*rel*-	1971064-68-2	2.21

IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)

Chemical Name *rel*-(2*R*,3*S*)-2,3,5-Trimethyl-2-phenylhex-4-enal
CAS No. Not assigned *Weight %* 6.77

Chemical Name *rel*-(2*R*,3*R*)-2,3,5-Trimethyl-2-phenylhex-4-enal
CAS No. Not assigned *Weight %* 6.56

Chemical Name *rel*-(2*R*,6*S*)-2-Methyl-4-methylidene-6-[(1*S*)-1-phenylethyl]tetrahydro-2*H*-pyran
CAS No. Not assigned *Weight %* 1.29

Chemical Name *rel*-(2*R*,6*S*)-2-Methyl-4-methylidene-6-[(1*R*)-1-phenylethyl]tetrahydro-2*H*-pyran
CAS No. Not assigned *Weight %* 1.07

Chemical Name Unidentified impurities (~32 minor peaks on GC chromatogram)
CAS No. Not assigned *Weight %* 5.77 (total)

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: pale yellow liquid

Property	*Value*	*Data Source/Justification*
Freezing Point	< -20 °C	Measured
Boiling Point	174-176 °C at 5.33 kPa	Measured
Density	970.6 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.8 x 10^{-2} kPa at 20 °C	Measured
Water Solubility	0.018 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains no hydrolysable functional groups in the environmentally relevant pH range (4-9)
Partition Coefficient (n-octanol/water)	log Pow = 3.9 at 20 °C	Measured
Adsorption/Desorption	log Koc = 3.38 – 3.70 at 30 °C	Measured
Dissociation Constant	Not determined	Contains no dissociable functionality
Flash Point	150 °C at 101.3 kPa	Measured
Flammability	Not flammable	Estimated based on flash point
Autoignition Temperature	220 °C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Measured

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

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical Hazard Classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

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

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

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

PORT OF ENTRY
Melbourne

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

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of fragrance oil formulations in 210 L polypropylene lined steel drums. Within Australia the drums will be transported mainly by road to the warehouse for storage and later distributed to the formulators by road for reformulation. Finished consumer products containing the notified chemical will be transported primarily by road to retail stores in packages suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient in cosmetic and household products. The proposed maximum use concentration of the notified chemical in various consumer products will be:

Finished consumer product	***Maximum proposed use concentration (%)***
Fine fragrance	0.85
Body lotion	0.94
Face cream and hand cream	0.49
Deodorant	0.076
Other cosmetic (such as shampoo and facial cleanser) and household products	3.0

OPERATION DESCRIPTION
Reformulation of fragrance oil formulations containing the notified chemical at ≤ 10% concentration 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 chemical at ≤ 3% concentration 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	None	Incidental
Mixers	4	250
Drum handlers	1	250
Drum cleaners	2	250
Equipment cleaners	2	250
Quality control	1	250
Professional end users	1-8	250

EXPOSURE DETAILS

Transport and storage
Transport, storage and warehouse workers may come into contact with the notified chemical as a component of fragrance formulations at ≤ 10% concentration, only in the unlikely event of accidental rupture of containers.

Reformulation
During reformulation, dermal and ocular exposure of workers to the notified chemical at ≤ 10% concentration may occur during weighing and transfer stages, blending, quality control analysis, and cleaning and maintenance of equipment. Due to the notified chemical's low vapour pressure (0.028 kPa at 20 °C), inhalation exposure is not expected, unless aerosols or mists are formed.

The notifier stated that exposure is expected to be minimised through the use of local exhaust ventilation and/or enclosed systems, and through the use of 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 chemical in end-use products at ≤ 3% concentration 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 cleaning products in the cleaning industry. The principal route of exposure will be dermal, while ocular exposure and inhalation exposure are also possible. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using the products containing the notified chemical.

6.1.2. Public Exposure
There will be widespread and repeated exposure to the notified chemical at ≤ 3% concentration through the use of a wide range of cosmetic and household products. The main route of exposure will be dermal, while ocular and inhalation exposure are also possible, particularly if products are applied by spray.

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

Cosmetic products (Dermal exposure):

Product type	Amount (mg/day)	C (%)	RF (unitless)	Daily systemic exposure (mg/kg bw/day)
Body lotion	7820	0.940	1	1.1486
Face cream	1540	0.490	1	0.1179
Hand cream	2160	0.490	1	0.1654
Fine fragrances	750	0.850	1	0.0996
Deodorant spray	1430	0.076	1	0.0178
Shampoo	10460	3.000	0.01	0.0490
Conditioner	3920	3.000	0.01	0.0184
Shower gel	18670	3.000	0.01	0.0875
Hand soap	20000	3.000	0.01	0.0938
Hair styling products	4000	3.000	0.1	0.1875
Facial cleanser	800	3.000	0.01	0.0038
Total				**1.9892**

C = maximum intended concentration of notified chemical; RF = retention factor.
Daily systemic exposure = (Amount × C × RF × DA)/BW

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	3.0	0.25	10	0.1024
Fabric softener	90	3.0	0.25	10	0.0401
Total					**0.1425**

C = maximum intended concentration of notified chemical
Daily systemic exposure = (Amount × C × PR × PT × DA)/BW

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	3.0	1980	0.01	0.01	0.007	0.0009
Dishwashing liquid	3	3.0	1980	0.0093	0.01	0.03	0.0075
All-purpose cleaner	1	3.0	1980	1	0.01	0.007	0.0650
Total							**0.0734**

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

Hairspray (Inhalation exposure):

Product type	Amount (g/use)	C (%)	Inhalation rate (m^3/day)	Exposure duration zone 1 (min)	Exposure duration zone 2 (min)	Fraction inhaled (%)	Volume zone 1 (m^3)	Volume zone 2 (m^3)	Daily systemic exposure (mg/kg bw/day)
Hairspray	20	3.0	20	15	20	50	1	10	**0.0966**

C = maximum intended concentration of notified chemical
Total daily systemic exposure = Daily systemic exposure in Zone 1 [(amount × C × inhalation rate × exposure duration (zone 1) × fraction inhaled)/(volume (zone 1) × body weight)] + Daily systemic exposure in Zone 2 [(amount × C × inhalation rate × exposure duration (zone 2) × fraction inhaled)/(volume (zone 2) × body weight)]

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical at the maximum intended concentrations specified by the notifier in various product types. This would result in a combined internal dose of 2.3017 mg/kg bw/day for the notified chemical. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products (in addition to hair spray) may occur. However, it is considered that the combination of the conservative hair spray inhalation exposure assessment parameters, and the aggregate exposure from use of the dermally applied products, which assumes a conservative 100% dermal absorption, is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray cosmetic and household products with low exposure factors (e.g. air fresheners).

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For 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
Acute inhalation toxicity – rat	LC50 > 5.25 mg/L/4 hour; low toxicity
Skin corrosion – *in vitro* EPIDERM™ human skin model	non-corrosive
Skin irritation – *in vitro* EPISKIN™ reconstructed human epidermis model	irritating
Eye irritation – *in vitro* bovine corneal opacity and permeability (BCOP) test	not irritating
Eye irritation – *in vitro* human cornea model	not irritating
Eye irritation – rabbit	slightly irritating
Skin sensitisation – mouse local lymph node assay	evidence of sensitisation (EC3 = 39.8%)
Skin sensitisation – *in chemico* DPRA test	negative
Skin sensitisation – *in vitro* ARE-Nrf2 luciferase test	positive
Skin sensitisation – HRIPT	no evidence of sensitisation at 0.8%
Skin sensitisation – HRIPT	no evidence of sensitisation at 5%
Repeat dose oral toxicity – rat, 28 days	NOAEL = 340.5/311.9 mg/kg bw/day (m/f)*
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosomal aberration test in human lymphocytes	non genotoxic

* established by the study authors

Toxicokinetics
Given the low molecular weight (216.32 g/mol), the notified chemical may be absorbed through the skin. However, based on its low water solubility (0.018 g/L at 20 °C) and high partition coefficient (Pow = 3.9 at 20-25 °C) the notified chemical has a reasonably high lipophilicity. Hence percutaneous absorption is expected to be limited.

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

No acute dermal toxicity study was provided for the notified chemical.

Irritation
In *in vitro* skin irritation/corrosion studies, the notified chemical was found to be irritating but non-corrosive. Based on the results of the *in vitro* studies, the notified chemical should be classified as a category 2 skin irritant according to the GHS criteria.

In two *in vitro* eye irritation tests, the notified chemical was determined to not require classification for eye irritation.

The notified chemical is slightly irritating to eyes based on a study conducted in two rabbits. Moderate conjunctival irritation was observed in both treated animals at the 1 hour observation, reducing to slight conjunctival irritation at the 24 and 48-hour observations. All signs of irritation were resolved at the 72-hour observation.

Sensitisation
The notified chemical was found to be a weak skin sensitiser in a mouse Local Lymph Node Assay (LLNA) with stimulation indices of 2.02, 3.68 and 4.0 at 25, 50 and 100% concentrations, respectively. The EC3 value was calculated to be 39.8%.

One *in chemico* and one *in vitro* cell based assays were conducted to evaluate the skin sensitisation potential of the notified chemical. 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 notified chemical along with other supporting information.

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 chemical 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.

In the DPRA assay, the notified chemical showed a positive response (low reactivity). However, given the result (8.5%) was within a range close to the cut-off (6.38%) for a negative prediction, the test substance was re-tested. In three additional tests, the test substance showed a negative response (minimal reactivity). Based on the weight of evidence approach, the study authors have therefore considered the test substance to have minimal reactivity for peptide depletion. However, in the ARE-Nrf2 luciferase assay, the notified chemical gave a positive response.

In two human repeated insult patch tests (HRIPT), the notified chemical at 0.8% and 5% concentrations did not elicit a positive response when tested in over 100 human volunteers.

Overall, based on the results from the LLNA study, the notified chemical is considered a weak skin sensitiser (GHS category 1B).

Repeated Dose Toxicity
A repeated dose oral (diet) toxicity study on the notified chemical was conducted in rats, in which the test substance was administered at 1,500, 4,500 and 9,000 ppm (equivalent to 114.5/114.1, 340.5/311.9 and 676..1/615.4 mg/kg bw/day in males/females) for 28 consecutive days, with a 14-day recovery period at the high dose. The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 4,500 ppm (equivalent to 340.5 mg/kg bw/day for males and 311.9 mg/kg bw/day for females) based on adverse microscopic changes (mixed cellular inflammation and reactive hyperplasia of the caecal mucosa) observed in the caecum in both sexes at the highest dose tested and 1/5 low dose males and females also showed caecum changes. The study authors note that the toxicological significance of this change is unknown and may be related to a secondary change in the gut flora, pH, or an irritant effect of the test substance at high concentrations. There were other statistically significant changes (compared to controls) reported in low and mid dose animals such as decreased thyroid weights and decreased prostrate and seminal vehicle weights. However, these changes were considered as adaptive by the study authors. Those changes were not observed in recovery group animals.

Mutagenicity/Genotoxicity
The notified chemical tested negative both in a bacterial reverse mutation study and in an *in vitro* chromosomal aberration test in human lymphocytes.

Health Hazard Classification
Based on the available information, the notified chemical is a 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 chemical is presented in the following table.

Hazard Classification	***Hazard Statement***
Skin irritation (Category 2)	H315 – Causes skin irritation
Skin sensitisation (Category 1B)	H317 – May cause an allergic skin reaction

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the toxicological information provided, the notified chemical is a skin irritant and a weak skin sensitiser.

Reformulation
Workers may experience dermal, ocular and perhaps inhalation exposure to the notified chemical at ≤ 10% concentration during reformulation. Given the notified chemical is a skin sensitiser caution should be exercised when handling the notified chemical during reformulation processes. The use of local ventilation, enclosed/automated processes and PPE (i.e. protective clothing, goggles, impervious gloves and respiratory protection, if inhalation exposure may occur), as stated by the notifier, should 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 chemical is not considered to be unreasonable.

End-use
Cleaners and beauty care professionals will handle the notified chemical at ≤ 3% concentration, similar to public use. 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 chemical 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 below.

6.3.2. Public Health

Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic and household products containing the notified chemical at ≤ 3% concentration.

Irritation
The notified chemical is a skin irritant. However, effects are not expected from the use of products containing the notified chemical at the proposed low use concentrations in cosmetic and household products.

Sensitisation
Based on the results of an LLNA study, the notified chemical is considered to be a weak skin sensitiser (EC3 = 39.8%). As shown in the table below, the Consumer Exposure Level (CEL) from use of the notified chemical in cosmetic products was estimated using various references (SCCS, 2012 Cadby *et al.*, 2002). Consideration of available information and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 32.19 µg/cm^2/day was estimated for the notified chemical. 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.

Product type	***Proposed maximum use concentration (%)***	***CEL (µg/cm^2)***	***AEL (µg/cm^2)***
Deodorant	0.076	5.7	32.19
Body lotion	0.94	4.69	32.19
Other leave-on cosmetics (assumed: fine fragrances)	0.85	31.88	32.19
Rinse-off cosmetics (assumed: hand wash soap)	3	6.98	32.19

As the AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of the notified chemical at ≤ 0.076% in deodorants, ≤ 0.94% concentration in body lotion, ≤ 0.85% in other leave-on cosmetic products (using fine fragrances as a worst case example) and at ≤ 3% in rinse-off cosmetic products (using hand wash soap as a worst case example), is not considered to be unreasonable.

Based on the expected low exposure from household products, by inference, the risk of induction of sensitisation associated with the use of these products is also not considered to be unreasonable. It is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on the aggregate exposure has not been conducted.

Systemic Effects
The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario from use of multiple products by an individual with total exposure of 2.3017 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 311.9 mg/kg bw/day derived from a 28 day repeated dose oral toxicity study in rats on the chemical, the margin of exposure (MoE) was estimated to be 136. A MoE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences.

Overall, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 0.076% in deodorants, ≤ 0.49% in face and hand creams, ≤ 0.85% in fine fragrances, ≤ 0.94% in body lotion and at ≤ 3% in other cosmetic and household products, is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of fragrance formulations for reformulation into finished cosmetic and household products. In general, the reformulation processes are expected to involve automated blending operations in an enclosed environment, followed by automated filling of the finished products into end-use containers. Wastes containing the notified chemical generated during reformulation include equipment wash water, residues in empty import containers and spilt materials. These wastes will either be released to sewers or disposed of to landfill according to local government regulations. Release of the notified chemical to the environment in the event of accidental spills or leaks during reformulation, storage and transport is expected to be collected for disposal, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemical is expected to be released to sewers across Australia as a result of its use in cosmetic and household products, which will be washed off the hair and skin of consumers as well as from cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical in empty import and end-use containers are likely to either share the fate of the containers and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

The majority of the notified chemical will enter sewers and be subsequently treated at sewage treatment plants (STPs) following its use in products available to the general public (e.g. shampoo, fabric softener, detergents and cleaning products).

The notified chemical is expected to be efficiently removed from effluent in STPs by partitioning to sludge (16 %) and volatilisation (77 %; Struijs *et al*., 1991). After treatment at STPs, approximately 7% of the notified chemical is expected to be released to surface waters. Based on its moderate water solubility (18 mg/L) and partition coefficient (log Pow = 3.9), when released to surface waters, the notified chemical is expected to partition between water and sediment. The measured vapour pressure (28 Pa at 20 °C) indicates that the notified chemical is volatile and will partition to air during its lifecycle, especially during STP treatment. The predicted half-life of the notified chemical in air due to reaction with hydroxyl radicals is 1.0 h assuming 12 h of sunlight per day and hydroxyl radical concentration of 1.5×10^6 molecules/cm^3 (AOPWIN v1.92; US EPA, 2012). Ozone is also predicted to react with the notified chemical facilitating a second atmospheric degradation pathway; this reaction has an estimated half-life of 38 minutes assuming 12 h of sunlight per day (AOPWIN v1.92; US EPA, 2012). The notified chemical is therefore not expected to be persistent in air.

Based on the results of a ready biodegradability study, the notified chemical was demonstrated to be not readily biodegradable by microorganisms (0% in 28 days). For details of the environmental fate study, refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

The Predicted Environmental Concentration (PEC) for a worst case scenario has been calculated on the assumption that 100% of the annual import quantity of the notified chemical is released to the sewer over 365 days/year. It is also assumed under the worst-case scenario that there is no removal of the notified chemical by STP processes (volatilisation, partitioning to solids and biodegradation). The resulting PEC in receiving waters is reported in the table below. This is the worst-case scenario for aquatic release.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	1000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day

Water use	200.0	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC – River:	0.56	µg/L
PEC – Ocean:	0.06	µg/L

The worst-case scenario for terrestrial release was predicted separately by considering the removal of the notified chemical in STPs and the application of biosolids to agricultural soils. A predicted concentration of the notified chemical in soils was calculated using worst-case SimpleTreat STP modelling (Struijs *et al*., 1991) which assumes a 93% removal rate during sewage treatment (16% partitioning to sludge; 77% volatilisation), based on the physical and chemical properties of the notified chemical. Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 0.843 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 chemical may approximate 0.006 mg/kg in applied soil. This assumes that degradation of the notified chemical 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 0.03 mg/kg and 0.06 mg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 1.01 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 3.1 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	72 h EC50 = 3.5 mg/L	Toxic to algae
Acute Earthworm Toxicity	14 day LC50 = 39.4 mg/kg	Toxic to soil dwelling macro-organisms
Inhibition of bacterial respiration	3 h EC50 > 1,000 mg/L	Not inhibitory to microorganisms

Based on the above ecotoxicological endpoints for the notified chemical, it is expected to be acutely toxic to aquatic life. However, as the notified chemical is not biodegradable the effects are expected to be long lasting. Therefore, the notified chemical is formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) as Chronic Category 2.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) has been calculated from the Acute Fish Test. A safety factor of 100 was used given acute endpoints for three trophic levels are available and the endpoints are similar in magnitude.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish, 96 h)	1.01	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC	10.1	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q – River	0.56	10.1	0.06
Q – Ocean	0.06	10.1	< 0.01

The risk quotient (Q=PEC/PNEC) has been calculated based on the assumption of complete release into the waterways. With a Q value much less than 1 for both river and ocean compartments it is highly unlikely that the notified chemical will reach ecotoxicologically significant concentrations based on the proposed annual importation and use patterns.

A worst case scenario for release to soil from STP sludge indicates that the notified chemical will reach concentrations of 0.03 mg/kg and 0.06 mg/kg over 5 and 10 years respectively. These concentrations are approximately 700 times below the measured LC50 for earthworms (39.4 mg/kg). Even with a conservative safety factor of 1000, the worst case soil concentration, which does not consider degradation or dissipation of the notified chemical from soil, only just exceeds the PNEC by 0.04 mg/kg. Therefore the notified chemical is not expected to pose an unreasonable risk to organisms in soil.

On the basis of the PEC/PNEC ratio, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -20 °C

Method EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Test Facility CTL (2015)

Boiling Point 174-176 °C at 5.33 kPa

Method OECD TG 103 Boiling Point (1995)
Remarks Determined by Siwoloboff method.
Test Facility JRF (2017a)

Density 970.6 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids (2012)
Remarks Determined using a Pycnometer.
Test Facility JRF (2017b)

Vapour Pressure 2.8 x 10^{-2} kPa at 20 °C

Method EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks Determined using static method apparatus.
Test Facility CTL (2015)

Water Solubility 0.018 g/L at 20 °C

Method EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks Flask Method
Variation between the three independent trials is > 15%. However, given the low solubility of the test substance, the difference between the measured concentrations is virtually negligible (0.017, 0.016 and 0.020 g/L, respectively). Therefore the test is considered valid.
Test Facility CTL (2015)

Partition Coefficient (n-octanol/water) log Pow = 3.9.at 20 °C

Method EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks Shake Flask Method
Concentrations determined by HPLC analysis and interpolation from a standard concentration curve.
Test Facility CTL (2015)

Adsorption/Desorption log Koc = 3.38 - 3.70 at 30 °C

Method OECD TG 12: Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks Up to five peaks are observed in the chromatogram with different retention times, but peak 1 has > 98% of the normalised area (*i.e.* 98 % of the substance elutes in peak 1). Within this peak there are three sub-peaks which can be resolved. The log Koc for these three sub-peaks range from 3.38 - 3.70.
Test Facility ERL (2018a)

Flash Point 150 °C at 101.3 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point
Remarks Determined by Pensky Martens method.
Test Facility JRF (2017c)

Autoignition Temperature 220 °C

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

Explosive Properties Not explosive

Method	US EPA Product Properties Test Guidelines OCSPP 830.6316 "Explodability" (1996)
Remarks	Determined using Differential Scanning Calorimetry (DSC). No exothermic decomposition peak was observed at up to 430 °C.
Test Facility	JRF (2017e)

Oxidizing Properties Not oxidising

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks	The mean pressure rise time for the test mixture (cellulose and test substance) and the reference mixture (cellulose and 65% nitric acid) was 18.7 seconds and 1.1 seconds, respectively.
Test Facility	JRF (2018)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure (2001)

Species/Strain	Rat/Wistar (RccHan™)
Vehicle	Arachis oil BP for 300 mg/kg bw and none for 2000 mg/kg bw
Remarks – Method	The test substance was administered via gavage. Clinical observations were made 30 minutes, 1, 2 and 4 hours following dosing and then daily for 14 days.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	1 F	300	0/1
2	5 F	2000	0/5

LD50	> 2000 mg/kg bw
Signs of Toxicity	No signs of toxicity were observed.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks – Results	The animals showed expected body weight gains during the 14 day observation period.

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

TEST FACILITY: ERL (2018b)

B.2. Acute Inhalation Toxicity – Rat

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 403 Acute Inhalation Toxicity (2009)

Species/Strain	RccHan™/Wistar
Vehicle	Nil
Method of Exposure	Nose only
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	2.39 µm
Remarks – Method	No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 M/5 F	16.82	5.25	0/10

LC50	> 5.25 mg/L/4 hours
Signs of Toxicity	Common clinical observations included decreased respiratory rate, hunched posture, pilo-erection and wet fur. Noisy respiration was also noted in 2 animals. All appeared normal at the day 2 observation.
Effects in Organs	Dark patches of the lungs were observed in 3 males and all 5 females, however, no abnormalities were detected during necropsy in the upper respiratory tract. In a private communication, the study author stated that "as these types of lung findings were observed amongst control animals from studies where the route of exposure may not be via inhalation, it confirms that this type of macroscopic finding is not always due to test item exposure to the lung, and may be due to other factors such as a non-

	specific post mortem change resulting from incomplete exsanguination of animals".
Remarks – Results	Except for one female, all exposed animals showed a body weight loss on day 1 post-exposure. In addition, a body weight loss was noted in one female from days 1-3 post-exposure and in another two females from days 7-14 post-exposure. No body weight gain was noted in one female from days 3-7 post-exposure. Normal body weight gains were observed in all other animals during the remainder of the recovery period.
CONCLUSION	The notified chemical is of low acute toxicity via inhalation.
TEST FACILITY	ERL (2018c)

B.3. Skin Corrosion – *In Vitro* Human Skin Model

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 431 *In vitro* Skin Corrosion – Human Skin Model Test (July, 2016)
Vehicle	Nil
Remarks – Method	The EpiDerm test system was used. Positive (8N potassium hydroxide) and negative (deionised water) controls were run in parallel with the test substance. The MTT tetrazolium salt [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to determine cell viability. The optical densities were determined at 570 nm.

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*		*Relative Mean Viability (%)*		*SD of Relative Mean Viability*	
	3 minute exposure	60 minute exposure	3 minute exposure	60 minute exposure	3 minute exposure	60 minute exposure
Negative control	1.502	1.469	100.0	100.0	0.2	3.5
Test substance	1.626	1.590	108.2	108.3	0.8	1.0
Positive control	0.339	0.047	22.6	3.2	3.3	8.1

OD = optical density; SD = standard deviation

Remarks – Results	The preliminary test indicated that the test substance did not directly reduce MTT. The relative mean viabilities of the test substance treated tissues were 108.2% and 108.3% after 3 and 60 minute exposure periods, respectively. A mean tissue viability of ≥ 50% (for 3 minute exposure) and ≥ 15% (for 60 minute exposure) is considered as non-corrosive. The positive and negative controls gave satisfactory results, confirming the validity of the test system.
CONCLUSION	The notified chemical was considered as non-corrosive to the skin under the conditions of the test.
TEST FACILITY	ECRS (2017a)

B.4. Skin Irritation – *In Vitro* Human Skin Model

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (July, 2015)
Vehicle	Nil
Remarks – Method	Phosphate buffered saline and 5% sodium lauryl sulphate were used as negative control and positive control, respectively. The MTT tetrazolium salt assay was used to determine cell viability. The optical densities were determined at 570 nm.

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	1.387	100	3.2
Test substance	0.428	30.9	0.7
Positive control	0.017	1.2	0.3

OD = optical density; SD = standard deviation

Remarks – Results	The preliminary test indicated that the test substance did not directly reduce MTT. The relative mean tissue viability for the test substance as compared to the negative control was 30.9%. As the relative mean tissue viability for the test substance was below 50%, it is considered as an irritant. The positive and negative controls gave satisfactory results, confirming the validity of the test.
CONCLUSION	Based on the mean tissue viability of < 50%, the notified chemical should be classified as a skin irritant (Category 2) according to the GHS criteria.
TEST FACILITY	ECRS (2017b)

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

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage (July, 2013)
Vehicle	Nil
Remarks – Method	Positive (2-ethoxyethanol) and negative (saline – 0.9% NaCl in deionised water) controls were run in parallel with the test substance.

RESULTS

Test Material	*Mean Opacities of Triplicate Tissues*	*Mean Permeabilities of Triplicate Tissues*	*IVIS (SD)*
Vehicle control	0.0	0.05	0.75
*Test substance**	1.3	0.045	2.02
*Positive control**	62.7	0.89	76.0

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

Remarks – Results	The IVIS of the test substance was 2.02. An IVIS ≤ 3 is considered as not requiring classification for eye irritation. The controls gave satisfactory results confirming the validity of the test system.
CONCLUSION	The notified chemical was not considered an eye irritant under the conditions of the test.
TEST FACILITY	ECRS (2017c)

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

TEST SUBSTANCE	Notified chemical
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 (July, 2015)
Vehicle	Nil
Remarks – Method	The EpiOcular test system was used. Positive (methyl acetate) and negative (deionised water) controls were run in parallel with the test substance. The MTT tetrazolium salt [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to determine cell viability. The optical densities were determined at 570 nm.

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*	*Relative Mean Viability (%)*
Negative Control	1.846	100.0
Test Substance	1.540	83.4
Positive Control	0.379	20.6

OD = optical density

Remarks – Results	The test substance was shown not to directly reduce MTT. The relative mean viability for the test substance as compared to the negative control was 83.4%. As the relative mean tissue viability for the test substance was above 60%, it is considered a non-irritant. The positive and negative controls gave satisfactory results, confirming the validity of the test
CONCLUSION	The notified chemical was not considered an eye irritant under the conditions of the test.
TEST FACILITY	ECRS (2017d)

B.7. Eye Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	2F
Observation Period	72 hours
Remarks – Method	Study was conducted on 2 animals only.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva – Redness	0.7	0.7	2	< 72 h	0
Conjunctiva – Chemosis	0.3	0.3	1	< 48 h	0
Conjunctiva– Discharge	0.3	0.3	2	< 48 h	0
Corneal Opacity	0.0	0.0	0	n/a	0
Iridial Inflammation	0.0	0.0	0	n/a	0

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

Remarks – Results

Moderate conjunctival irritation was observed in both animals at the 1 hour observation reducing to slight conjunctival irritation at the 24 and 48-hour observations. All signs of irritation were resolved at the 72-hour observation.

No corneal or iridial effects were noted.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

ERL (2017a)

B.8. Skin Sensitisation – LLNA

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2010)

Species/Strain: Mouse/CBA/Ca

Vehicle: Acetone:olive oil (4:1)

Preliminary study: Yes

Positive control: Conducted in parallel with the test substance using α-hexylcinnamaldehyde (85%).

Remarks – Method: A preliminary test was conducted using undiluted test substance to justify the concentrations for the main study.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (DPM/lymph node)*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5 F	2504.97	-
25	5 F	5058.56	2.02
50	5 F	9220.09	3.68
100	5 F	10018.86	4.00
Positive Control			
25	5 F	21342.58	8.52

EC3: 39.8%

Remarks – Results

No unscheduled mortalities or signs of systemic toxicity were observed during the study period.

The stimulation index was > 3 in mid and high dose test groups, indicating a sensitising response. The EC3 was calculated to be 39.8%.

Slight reduction in bodyweight gain was observed in a vehicle control animal, one low-dose animal and one positive control animal.

CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	ERL (2018d)

B.9. Skin Sensitisation – *In Chemico* DPRA Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) (2015)
Vehicle	Acetonitrile (phosphate or ammonium acetate buffer)
Remarks – Method	No significant deviations from the OECD test guideline were reported.

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*	*Mean Cysteine + Lysine Peptide Depletion (%)*
Test 1	11.8 (± 1.63)	5.2 (± 1.64)	8.5
Test 2	5.8 (± 2.13)	0.2 (± 0.38)	3.0
Test 3	9.0 (± 2.3)	1.9 (± 2.3)	5.5
Test 4	11.4 (± 0.59)	0.2 (± 0.29)	5.8

SD = Standard Deviation

Remarks – Results	In the first test, the test substance gave a mean cysteine and lysine peptide depletion of 8.5% (low reactivity class) which is above the threshold of 6.38% for a negative prediction. Given the result was within a range close to the cut-off, the test substance was re-tested. In three additional tests, the test substance showed a negative response (minimal reactivity). Based on the weight of evidence approach, the study authors have therefore considered the test substance as a non-sensitiser. The positive controls fulfilled all quality criteria in each study confirming the validity of the test.
CONCLUSION	The test substance was considered to have minimal reactivity for peptide depletion under the conditions of the test, showing negative results in the first key event (molecular initiating) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.
TEST FACILITY	IIVSI (2015a)

B.10. Skin Sensitisation – *In Vitro* ARE-Nrf2 Luciferase Test

TEST SUBSTANCE	Notified chemical
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	Dimethylsulfoxide (DMSO)
Remarks – Method	The test substance was dissolved in DMSO (final concentration 1%) and tested according to the standard operating procedure of the KeratinoSens assay at 12 concentrations (range of 0.978-2,000 µM). DMSO and cinnamic aldehyde (4-64 µM) were used as negative and positive controls, respectively. Three independent assays were conducted. Each assay included a set of 4 plates (3 for gene induction, 1 for cytotoxicity assessment).

A test substance is predicted to have sensitisation potential if:
- the EC1.5 value is < 1,000 µM in at least 2 of 3 repetitions,
- cellular viability is > 70% at the lowest concentration with a luciferase gene induction > 1.5, and
- there is an apparent overall dose response which was similar between repetitions.

The mean values for cell viability and luciferase induction were provided. Individual values from the replicate experiments were not included in the report.

RESULTS

Sample	*Mean EC1.5 (µM)*	*Mean IC50 (µM)*	I_{max}
Test substance	35.59	101.12	2.49
Positive Control	8.92	> 64	not determined

EC1.5 - concentration for an induction of luciferase activity 50% above vehicle control
IC50 - concentration leading to 50% cell viability compared to vehicle control
I_{max} – maximal induction

Remarks – Results

Two tests were conducted.

In the first test, induction of the luciferase above the threshold of 1.5 was noted in all 3 repetitions; however, this was only statistically significant in 1/3 repetitions. Given the luciferase induction was occurring at the same concentration range where cytotoxicity was occurring, a second test was performed.

In the second test, an induction of the luciferase above the threshold of 1.5 was noted in all 3 repetitions and was statistically significant for 2/3 repetitions. The EC1.5 value presented in the table is the average of these 2 repetitions. In 1/3 repetitions, there was induction above threshold in some replicates (2/3) but it was not statistically significant. Based on these results, the study authors classified the notified chemical as a potential sensitiser.

The positive control performed as expected.

CONCLUSION

The test substance was positive in the second key event (keratinocytes response) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.

TEST FACILITY

IIVSI (2015b)

B.11. Skin Sensitisation – Human Volunteers

TEST SUBSTANCE

Notified chemical (0.8%)

METHOD

Repeated insult patch test with challenge

Study Design

Induction procedure: patches containing 0.15 mL of the test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications during the induction period. Patches were removed by the subjects after 24 hours and graded by technicians after an additional 24 hours (or 48 hours for patches applied on Friday).

Rest Period: ~10-21 days

Challenge Procedure: Patches were applied to a naïve site. The sites were scored 24, 48 and 72 hours after application. If reactions were observed at the 72 hour observation, these were re-evaluated at 96 hours.

Study Group	92F/20M; age range 18-68 years
Vehicle	Alcohol SD40B:diethyl phthalate (1:3)
Remarks – Method	Occluded. The test substance was spread on a 3.63 cm^2 patch. Negative control [0.8% distilled water in alcohol:diethyl phthalate (1:3)] was conducted in parallel with the test substance.
RESULTS	
Remarks – Results	107/112 subjects completed the study. Five subjects discontinued with the study for reasons unrelated to the test substance. A female exposed to the negative control showed barely perceptible erythema at the 72 hour evaluation which resolved at the 96 hour evaluation. No adverse responses were noted at induction and challenge.
CONCLUSION	The test substance was non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2017)

B.12. Skin Sensitisation – Human Volunteers

TEST SUBSTANCE	Notified chemical (5%)
METHOD	Repeated insult patch test with challenge
Study Design	Induction procedure: patches containing 0.15 mL of the test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications during the induction period. Patches were removed by the subjects after 24 hours and graded by technicians after an additional 24 hours (or 48 hours for patches applied on Friday). Rest Period: ~10-21 days Challenge Procedure: Patches were applied to a naïve site. The sites were scored 24, 48 and 72 hours after application.
Study Group	76F/37M; age range 18-67 years
Vehicle	Alcohol SD40B:diethyl phthalate (1:3)
Remarks – Method	Occluded. The test substance was spread on a 3.63 cm^2 patch. Negative control [5% distilled water in alcohol:diethyl phthalate (1:3)] was conducted in parallel with the test substance.
RESULTS	
Remarks – Results	102/113 subjects completed the study. Eleven subjects discontinued with the study for reasons unrelated to the test substance. No adverse responses were noted at induction and challenge.
CONCLUSION	The test substance was non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2018)

B.13. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Rat/Wistar Han™:RccHan™:Wist
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week

	Post-exposure observation period: 14 days
Vehicle	Nil
Remarks – Method	No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*		*Mortality*
		Male	*Female*	
Control	5M/5F	0	0	0/10
Low Dose	5M/5F	114.5	114.1	0/10
Mid Dose	5M/5F	340.5	311.9	0/10
High Dose	5M/5F	676.1	615.4	0/10
Control Recovery	5M/5F	0	0	0/10
High Dose Recovery	5M/5F	676.1	615.4	0/10

Mortality and Time to Death

No treatment related mortalities occurred during the study.

Clinical Observations

High dosed females showed statistically significant reduction (30.7% reduction than the control group) in forelimb grip strength. The study authors stated as the intergroup difference was confined to 1/3 tests and in the absence of relevant clinical observations, this symptom is not considered to be toxicological significant.

Following statistically significant mean body weight gain changes were noted:

- 61% and 128% reductions in mid and high dose group males, respectively, in week 1-2;
- 34% increase in high dose males in week 2-3 and 78.6% in recovery males in week 5-6;
- 72.6% and 106% decrease in mid and high dose females respectively in week 1-2 and, 61% reduction in mid dose group females in week 2-3 and, 30% reduction in high dose group females in week 3-4; and
- 136% increase in recovery females in week 5-6.

During the first week of treatment, food consumption was reduced in all female dose groups and in mid and high dose males. Mid and high dose females also showed reduced food consumption in weeks 3 and 4.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

Following statistically significant changes were observed in treated animals:

- increased red blood cell counts in high dose females (11.4% increase compared to the control group);
- increased haematocrit values in high dose females (8.1% increase compared to the control group);
- decreased mean corpuscular haemoglobin concentration in high dose (5.6% reduction compared to the control group) males, and mid and high (5.2% and 4.4% reduction than control group respectively) dose females;
- decreased mean corpuscular volume in mid (4% reduction compare to control group) and high (3% decrease compare to control group) dose females;
- decreased mean corpuscular haemoglobin volume in mid (1.2% decrease than control group) and high (1.5% decrease than control group) dose females;
- decreased activated partial thromboplastin time in low (12.8% decrease than control group), mid (5.8% decrease than control group) and high (4.3% decrease than control group) dose males; and
- increased reticulocyte count in low (24% and 26% increase than control groups in males and females respectively), mid (19.4% and 25.4% increase than control groups in males and females respectively) and high (36.6% and 33% increase than control group in males and females respectively) dose males and females.

Recovery groups

- decreased haemoglobin in high dose recovery males (4.9% decrease than control recovery group) and females (3.8% decrease than control recovery group);
- decreased (6.5% decrease than control recovery group) red blood cell count in high dose recovery females;
- decreased (3.7% decrease than control recovery group) haematocrit in high dose recovery females;

- decreased mean corpuscular haemoglobin concentration (1.9% reduction than control recovery group) in high dose recovery males;
- increased (33% increase than control recovery group) reticulocyte count in high dose recovery males; and
- increased (150% increase than control recovery group) eosinophils and prothrombin time (4.6% increase than control recovery group) in high dose recovery females.

Clinical chemistry
Following statistically significant changes were observed:

Treatment groups
- increased (16.8% increase than control group) urea in high dose males;
- decreased triglycerides in mid (48% decrease than control group) and high (52% decrease than control group) dose males;
- increased cholesterol (59% increase than control group) in high dose males; and
- increased bilirubin in high dose males (4.2% increase than control group) and females (51.2% increase than control group).

Recovery groups
- decreased urea (10.7% decrease than control recovery group) and calcium (7.4% decrease than control recovery group) in high dose recovery males;
- increased (28.7% increase than control recovery group) alanine aminotransferase in high dose recovery males;
- decreased (37.5% decrease than control recovery group) triglycerides in high dose recovery males; and
- increased (116% increase than control recovery group) bile acids in high dose recovery females.

Effects in Organs
Following statistically significant changes were observed:
- decreased thyroid weight in low (9.1% decrease than control group) and mid (7.3% decrease than control group) dose males;
- increased liver weights in low (7.6% increase than control group), mid (1.9% increase than control group) and high (8.1% increase than control group) dose males and decreased liver weights in high (3.3% decrease compare to control group) dose females; and
- decreased prostrate and seminal vesicle weights in mid (15.9% decrease than control group) and high (25.1% decrease than control group) dose males.

Following observations were made during histopathology:
- minimal hyperplasia in caecum in low (1 male) and high (3 males) dose males;
- minimal mixed cellular inflammation in caecum in low (1 male) and high (5 males) dose males and mild mixed cellular inflammation in 1 high dose female;
- minimal hyperplasia in colon in 1 high dose male;
- minimal mineralisation (1 male) and basophilic tubules (1 male) in kidney in high dose males and minimal mineralisation in a control group female;
- moderate unilateral pelvis dilation in a high dose male and minimal dilation in a low dose female;
- minimal bilateral pelvis dilation in a low dose female;
- minimal centrilobular hepatocyte hypertrophy in liver in 3 high dose males and 4 high dose females;
- minimal mixed cellular inflammation in liver in low (2 males and 1 female), mid (2 females) and high (1 male) dose groups;
- mimimal increased alveolar macrophages in lungs in a control and a high dose female; and
- mimimal follicular hypertrophy in thyroid glands in low (1 male), mid (1 male) and high (2 males) dose males.

Remarks – Results
The study authors stated the changes observed in the caecum (mixed cellular inflammation and reactive hyperplasia of the caecal mucosa) were unusual and may be related to secondary changes in the gut flora, pH or an irritant effect of the test substance at high concentrations. Nevertheless they are considered as an adverse effect by the study authors.

The changes observed in the liver (centrilobular hepatocyte hypertrophy) and thyroid (follicular epithelial hypertrophy) were considered adaptive in nature in response to a xenobiotic.

CONCLUSION
The NOAEL was established by the study authors as 340.5 mg/kg bw/day for males and 311.9 mg/kg bw/day for females in this study, based on adverse microscopic changes observed in the caecum in both sexes.

TEST FACILITY ERL (2017b)

B.14. Genotoxicity – Bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test
Plate incorporation method

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

Metabolic Activation System S9 mix from Aroclor 1254-induced rat liver

Concentration Range in Main Test Two tests were conducted.

Test 1 had 4 experiments:

a) Experiment 1: 21-5,000 µg/plate in all strains, with and without S9-mix;
b) Experiment 2: 1.4-1,000 µg/plate, with or without S9-mix in *S. typhimurium* strains and in *E. coli* 62-5,000 µg/plate with S9-mix; and
c) Experiments 3 and 4: 4.1-1,000 µg/plate in TA1535 only without S9-mix.

Test 2 had 2 experiments:

d) Experiment 1: 2.5-313 (with S9-mix) and 4.9-1,250 µg/plate (without S9-mix) for *S. typhimurium* strains;
a) Experiment 2: 313-5,000 µg/plate in *E. coli* strain with and without S9-mix.

Vehicle Dimethyl sulfoxide (DMSO)

Remarks – Method Negative control: DMSO
Positive control:
with S9-mix: 2-aminoanthracene (TA100, TA98, TA1535 and WP2uvrA) and benzo(a)pyrene (TA1537)
without S9-mix: sodium azide (TA1535 and TA100), 2-nitrofluorene (TA98); *N*-ethyl-*N*-nitrosourea (WP2uvrA) and 9-aminoacridine (TA1537).

Preliminary toxicity study was not conducted.

Actual concentration of the test substance was not determined and therefore the concentrations stated are nominal concentrations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1				
Experiment 1	Not tested	≥ 185	≥ 1,667	Negative
Experiment 2	Not tested	≥ 111	> 1,000	Negative
Experiment 3	Not tested	≥ 333	> 1,000	Negative
Experiment 4	Not tested	≥ 333	> 1,000	Negative
Test 2				
Experiment 1	Not stated	≥ 39	> 1,250	Negative

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Experiment 2	Not tested	> 5,000	≥ 5,000	Negative
Present				
Test 1				
Experiment 1	Not tested	≥ 62	≥ 1,667	Negative
Experiment 2	Not tested	≥ 37	≥ 1,667	Negative
Test 2				
Experiment 1	Not stated	≥ 39	> 1,250	Negative
Experiment 2	Not tested	> 5,000	≥ 5,000	Negative

Remarks – Results: No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level, with or without S9-mix.

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

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

TEST FACILITY: TNOT (2016)

B.15. Genotoxicity – *In Vitro* Chromosomal Aberration Test (Cultured Human Lymphocytes)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
Species/Strain: Human
Cell Type/Cell Line: Lymphocytes
Metabolic Activation System: S9 mix from Aroclor 1254-induced rat liver
Vehicle: DMSO
Remarks – Method: vehicle control: DMSO (1%)
positive control: cyclophosphamide and mitomycin C

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	2.0, 3.9, 7.8, 15.6*, 31.3*, 62.5*, 125, 250, 500 and 1,000	4 h	24 h
Test 2	6.25*, 12.5, 25*, 50*, 75, 100, 125, 150, 175 and 200	24 h	24 h
Present			
Test 1	2, 3.9, 7.8*, 15.6, 31.3, 62.5*, 125*, 250, 500 and 1,000	4 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	Not conducted	≥ 62.5	> 1,000	Negative
Test 2	Not conducted	≥ 50	> 200	Negative
Present				
Test 1	Not conducted	≥ 125	> 1,000	Negative

Remarks – Results: In Test 1, the test substance was severely toxic to cells at 250, 500 and 1,000 µg/mL with S9-mix and 125, 250, 500 and 1,000 µg/mL without S9-mix.

	In Test 2, the test substance was severely toxic to cells at 125, 150, 175 and 200 µg/mL. No statistically significant increases were noted in chromosome aberrations, either with or without metabolic activation. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic to human lymphocyes treated *in vitro* under the conditions of the test.
TEST FACILITY	TNOT (2015)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 310: Ready Biodegradability: CO_2 in sealed vessels (Headspace Test)
Inoculum	Secondary effluent from a treatment plant which receives primarily domestic sewage
Exposure Period	28 days
Auxiliary Solvent	Acetone
Analytical Monitoring	IC analysis of headspace CO_2 (TOC analyser)
Remarks – Method	The test substance was dissolved in acetone to give a solvent stock solution (1070 mg/10 mL). An aliquot of this solvent stock solution was dispensed onto a filter paper and the solvent allowed to evaporate to dryness. The filter paper was added to inoculated mineral medium to give a final concentration of 24.0 mg/L, equivalent to 20 mg carbon/L. Controls were prepared with acetone in the same manner but no test item. A positive control and a toxicity control were prepared by adding a stock solution of the reference compound (sodium benzoate) directly to the test medium.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	-1	0	-1
14	-1	14	61
28	0	28	70

Remarks – Results	All validity criteria for the test were satisfied. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor in inhibiting the biodegradability of the test substance. No biodegradation of the test substance was observed after 28 days. One possible explanation for this result is that the test substance was retained by the filter paper and was not bioavailable to the microorganisms. This possibility was not considered in the study; however, the toxicity control experiment showed that the test substance had some effect on the microorganisms (*i.e.* it inhibited degradation of sodium benzoate compared to the control-only experiment). This result confirms that the test substance was at least partially bioavailable. Thus the results of the study are considered to be relevant.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	ERL (2018e)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203: Fish, Acute Toxicity Test – Semi-static
Species	*Gobiocypris rarus* (rare minnow)
Exposure Period	96 hours
Auxiliary Solvent	None

Water Hardness	75 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	A stock solution containing the test substance was prepared by dissolving a measured quantity of the solid test substance in water and stirring for 3 h. Test media were prepared by diluting the stock solution to the desired concentration. Based on the results of a range finding test, solutions for the definitive test were prepared by diluting the stock solution to nominal concentrations of between 0.20 and 3.20 mg/L. A closed, semi-static system was used with media replaced daily. Concentrations of the test item were measured in fresh and aged media (immediately before replacement) at 24, 48, 72 and 96 hours. The geometric mean of the measured concentrations was calculated at 0-24(aged), 24(fresh)-48(aged), 48(fresh)-72(aged) and 72(fresh)-96 hour intervals. The arithmetic mean of these values was used to determine LC50 and NOEC. The limit of quantitation (LOQ) and limit of detection (LOD) were 0.050 mg/L and 0.015 mg/L respectively. A negative control was also run as a separate test using potassium dichromate.

RESULTS

Nominal Concentrations (mg/L)	*Measured concentration* (mg/L)*	*Number of Fish*	*Mortality*				
			3 h	*24 h*	*48 h*	*72 h*	*96 h*
Control	Not Measured	7	0	0	0	0	0
0.20	0.189	7	0	0	0	0	0
0.40	0.399	7	0	0	0	0	0
0.80	0.742	7	0	0	1	1	2
1.60	1.373	7	0	0	3	4	5
3.20	2.594	7	0	1	5	7	7

* *Arithmetic mean of geometric mean*

LC50	1.010 mg/L at 96 hours
NOEC	0.399 mg/L at 96 hours
Remarks – Results	All validity criteria were satisfied. The dissolved oxygen concentration in the test solutions during the test were ≥ 65 %. No abnormalities or mortality was observed in any of the control fish up to 96 hours. Test fish displayed signs of unbalance, diminished swimming capacity and abnormal movement in solutions containing ≥ 0.742 mg/L of test substance. The 96 h LC50 of potassium dichromate was 281.14 mg/L.

CONCLUSION	The test substance is toxic to fish.
TEST FACILITY	SXZDR&D (2017)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202: Daphnia sp. Acute Immobilisation Test and Reproduction Test – Semi static conditions
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	GC
Remarks – Method	A saturated stock solution of the test substance was prepared by stirring an excess of the test substance (100 mg/L) in water for ~ 24 hours. After the stirring period any undissolved test item was removed by filtration (0.2 µm filter) to produce a 100% v/v saturated solution of the test substance. Based on the results of a range finding test, solutions for definitive test were prepared by diluting the 100% v/v saturated stock

solution to 10, 18, 32, 56 and 100% v/v concentrations. Test solutions were renewed daily. Concentrations are reported as the measured average in freshly prepared media (at 0 h). A positive control was also run as a separate test using potassium dichromate at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L.

RESULTS

Concentration		*Number of D. magna*	*Number Immobilised*	
Nominal (% v/v Saturated Stock Solution)	*Actual (mg/L)*		*24 h*	*48 h*
Control	Control	20	0	0
10	1.7	20	0	0
18	3.0	20	1	5
32	4.8	20	11	20
56	6.8	20	20	20
100	13	20	20	20

EC50	3.1 mg/L at 48 hours
NOEC	1.7 mg/L at 48 hours
LOEC	3.0 mg/L at 48 hours
Remarks – Results	All validity criteria were satisfied. The 48-h EC50 of the positive control experiment was 1.2 mg/L which is within the range for the test to be considered valid (0.6 – 2.1 mg/L). The dissolved oxygen concentration in the test and control solutions was ≥ 7.5 mg/L at 22 °C. The pH values of the test solutions ranged from 7.8 – 8.0. No vitamin stock was added to the medium prior to use contrary to the guidelines; however, given that no adverse effects were observed in the control groups, this was considered not to have had significant impact on the outcome of the test.
CONCLUSION	The test substance is toxic to aquatic invertebrates.
TEST FACILITY	ERL (2017c)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201: Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Actual: 0.43 – 4.9 mg/L
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	GC
Remarks – Method	A saturated stock solution of the test chemical was prepared by stirring an excess of test substance (100 mg/L) in water for ~ 24 hours. After the stirring period any undissolved test substance was removed by filtration (through a 0.2 µm filter) to produce a 100% v/v saturated solution of the test substance. Based on the results of the range finding test, solutions for the definitive test were prepared by diluting the 100% v/v saturated stock solution to 0.32, 1.0, 3.2, 10 and 32% v/v. Concentrations are reported as the measured average in freshly prepared media (at 0 h). Temperature maintained at 24 ± 1°C. A positive control experiment was performed separately using potassium dichromate.

RESULTS

Biomass		*Growth*	
E_yC_{50}	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
1.7	0.43	3.5	0.43

Remarks – Results: The pH of some test solutions drifted by more than 1.5 units during the 72 hours (8.0 initial – 9.7 final) exposure time. However, this was not considered to have a significant impact on the outcomes of the study because the pH of the control drifted from 8.1 (initial) to 9.5 (end of test) without any significant effect. The cell growth was 108-fold in the controls and all other validity criteria were satisfied. The 72-h ErC50 for the positive control was 1.4 mg/L.

CONCLUSION: The test substance is toxic to algae

TEST FACILITY: ERL (2018f)

C.2.4. Acute Toxicity to Earthworms

TEST SUBSTANCE: Notified chemical

METHOD: Equivalent to OECD TG 207 Acute Earthworm Toxicity Test
Species: *Eisenia foetida*
Exposure Period: 14 days
Remarks – Method: No deviations to the test guideline were noted. Based on the results of a preliminary range finding test, a series of six nominal concentrations between 14.9 – 480.2 mg/kg (dry/weight) were used in the definitive study.

RESULTS

Concentration (mg/kg dry weight)	*Total number of test earthworms*	*Exposure duration*	
		7 d	*14 d*
		Cumulative mortality (%)	*Cumulative mortality (%)*
Control	40	0	0
14.9	40	2.5	12.5
30.2	40	22.5	40
59.9	40	52.5	67.5
119.8	40	70.0	87.5
240.1	40	87.5	100
480.2	40	100	100

14 day LC50: 39.4 mg/kg (dry weight)
NOEC: < 14.9 mg/kg (dry weight)
Remarks – Results: The validity criterion was satisfied. Pathological symptoms such as weak peristalsis ability, yellow body fluid exudation and body fracture were observed in some surviving animals in the treated group.

CONCLUSION: The test substance is toxic to earthworms (UN, 2006).

TEST FACILITY: SXZDR&D (2016)

C.2.5. Inhibition of Microbial Activity

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum: Activated sludge
Exposure Period: 3 hours

Concentration Range	Nominal: 180, 320 and 560 mg/L
Remarks – Method	Test media were prepared by combining synthetic sewage, activated sewage sludge and the test substance which had been separately dispersed in water by ultrasonication and stirring. Temperature was held at 21 °C. A positive control test was performed separately with 3,5-dichlorophenol.
RESULTS	
3 h EC50	> 1000 mg/L
NOEC	320 mg/L
Remarks – Results	All validity criteria were satisfied. The coefficient of variation of oxygen uptake in the control vessels was 8.2% and the specific respiration rate of the controls was 22.28 mg oxygen per gram dry weight of sludge per hour. No statistically significant toxic effects were shown at the test concentrations of 180 and 320 mg/L, however statistically significantly toxic effects ($p < 0.05$) were observed at the test concentration of 560 mg/L. The 3-h EC50 for 3,5-dichlorophenol was 6.8 mg/L which is within the guidelines for the test to be considered valid (2 – 25 mg/L).
CONCLUSION	The test substance is not inhibitory to microbial organisms
TEST FACILITY	ERL (2016)

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