File No.: LTD/2146
LTD/2147

September 2020

AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

LTD/2146: 1-Penten-3-one, 1-(4,6-dimethyl-3-cyclohexen-1-yl)-2-methyl-, (1*E*)-
LTD/2147: 1-Penten-3-one, 1-(3,6-dimethyl-3-cyclohexen-1-yl)-2-methyl-, (1*E*)-

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals Act 2019 (the IC Act) and Industrial Chemicals (General) Rules 2019 (the IC Rules) by following the Industrial Chemicals (Consequential Amendments and Transitional Provisions) Act 2019 (the Transitional Act) and Industrial Chemicals (Consequential Amendments and Transitional Provisions) Rules 2019 (the Transitional Rules)*. The legislations are Acts of the Commonwealth of Australia. The Australian Industrial Chemicals Introduction Scheme (AICIS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Agriculture, Water and the Environment.

This Public Report is available for viewing and downloading from the AICIS website. For enquiries please contact AICIS at:

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

Executive Director
AICIS

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SUMMARY

The following details will be published on the AICIS website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2146	International Flavours and Fragrances (Australia) Pty Ltd.	1-Penten-3-one, 1-(4,6-dimethyl-3-cyclohexen-1-yl)-2-methyl-, (1*E*)-	Yes	≤ 0.6 tonne per annum	Fragrance ingredient
LTD/2147	International Flavours and Fragrances (Australia) Pty Ltd.	1-Penten-3-one, 1-(3,6-dimethyl-3-cyclohexen-1-yl)-2-methyl-, (1*E*)-	Yes	≤ 0.6 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard Classification
Based on the available information, the assessed chemicals are hazardous chemicals 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 assessed chemicals is presented in the following table.

Hazard Classification	*Hazard Statement*
Acute toxicity (Category 4)	H302 – Harmful if swallowed
Skin corrosion/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*
Aquatic chronic (Category 1)	H410 – Very 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 assessed chemicals are not considered to pose an unreasonable risk to the health of workers.

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

Environmental Risk Assessment
On the basis of the PEC/PNEC ratio, the assessed chemicals are not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The assessed chemicals should be classified as follows:
  - Acute toxicity (Category 4): H302 – Harmful if swallowed
  - Skin irritation (Category 2): H315 – Causes skin irritation
  - Skin sensitisation (Category 1B): H317 – May cause an allergic skin reaction

  In the absence of hazard data for end-use products, concentrations at ≥ 1% warrant classification as causing skin sensitisation (Category 1B) according to the GHS criteria.

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

Health Surveillance

- As the assessed 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 assessed chemicals during reformulation:
  - Enclosed/automated processes, where possible
  - Local exhaust ventilation

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

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

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

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

- If products and mixtures containing the assessed 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

- As the assessed chemical is a weak skin sensitiser, product labels and/or associated information leaflets should include warning statements regarding the risk of allergic reactions to the assessed chemical when

used it at ≥ 1% concentration in cosmetic products for consumer use. This is not required if skin sensitisation data are available for the consumer products to indicate that there is no skin sensitisation risk.

Emergency procedures

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

Disposal

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

Regulatory Obligations

Specific Requirements to Provide Information
This risk assessment is based on the information available at the time of the application. The Executive Director may initiate an evaluation of the chemicals based on changes in certain circumstances. Under Section 101 of the IC Act the applicant of the assessed chemicals has post-assessment regulatory obligations to provide information to AICIS when any of these circumstances change. These obligations apply even when the assessed chemicals are listed on the Australian Inventory of Industrial Chemicals (the Inventory).

Therefore, the Executive Director of AICIS must be notified in writing within 20 working days by the applicant or other introducers if:

- the importation volume exceeds one tonne per annum for each assessed chemical;
- the final use concentration of the isomer mixture containing the assessed chemicals exceeds:
  - 0.61%, 0.66%, 0.45%, 0.22% and 0.23% for face cream, hand cream, fine fragrances, deodorant (non-spray) and deodorant (spray), respectively
  - 1% in leave-on cosmetic products, except for the product types mentioned above
  - 3% in rinse-off cosmetic products
  - 3% in hair care products, except for 1% in hair spray and hair styling products
  - 3% in all other household or domestic cleaning products;
- the function or use of the chemicals has changed from a fragrance ingredient, or is likely to change 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 chemical on human health, or the environment.

The Executive Director will then decide whether an evaluation of the introduction is required.

Safety Data Sheet
The SDS of the assessed chemicals provided by the applicant was reviewed by AICIS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND APPLICATION DETAILS

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

APPLICATION CATEGORY
LTD/2146: Limited-small volume: Chemical other than polymer (1 tonne or less per year)
LTD/2147: Limited (reduced fee application): Chemical other than polymer (1 tonne or less per year)
Chemical Group Assessment

PROTECTED INFORMATION (SECTION 38 OF THE TRANSITIONAL ACT)
No details are taken to be protected information.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are varied for Hydrolysis as a function of pH, dissociation constant, and reactivity.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT(S)
None

APPLICATION IN OTHER COUNTRIES
EU REACH (2019) and China MEE (2019)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Satinone (containing the assessed chemical in LTD/2146 at 50-60% and the assessed chemical in LTD/2147 at 35-45%)
X00002596217 (containing the assessed chemical in LTD/2146 and LTD/2147 at 10-30%)
PG-RAW-90-032 (containing the assessed chemical in LTD/2146 and LTD/2147 at 99%)

CAS NUMBER
LTD/2146: 2248118-60-5
LTD/2147: 2248118-61-6

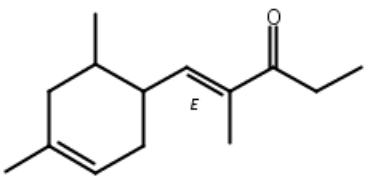
CHEMICAL NAME
LTD/2146: 1-Penten-3-one, 1-(4,6-dimethyl-3-cyclohexen-1-yl)-2-methyl-, (1*E*)-
LTD/2147: 1-Penten-3-one, 1-(3,6-dimethyl-3-cyclohexen-1-yl)-2-methyl-, (1*E*)-

OTHER NAME(S)
LTD/2146: (*E*)-1-(4,6-dimethylcyclohex-3-en-1-yl)-2-methylpent-1-en-3-one
LTD/2147: (*E*)-1-(3,6-dimethylcyclohex-3-en-1-yl)-2-methylpent-1-en-3-one

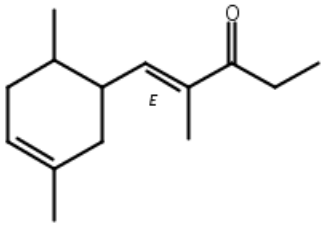
MOLECULAR FORMULA
$C_{14}H_{22}O$

STRUCTURAL FORMULA

LTD/2146:



LTD/2147:



MOLECULAR WEIGHT
206.32 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
LTD/2146: 50-60%
LTD/2147: 35-45%
The assessed chemicals are manufactured overseas as an inseparable isomer mixture.

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: clear colourless liquid*

Property	*Value*	*Data Source/Justification*
Freezing Temperature	< -20 °C	Measured
Boiling Point	274.8 °C at 102 kPa	Measured
Density	928 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.15 × 10^{-3} kPa at 25 °C	Measured
Water Solubility	0.0105 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains no hydrolysable functional groups
Partition Coefficient (n-octanol/water)	log Pow = 4.61 at 25 °C	Measured
Surface Tension	58.5 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.67 – 3.83	Measured
Dissociation Constant	Not determined	Contains no dissociable functional groups
Flash Point	127 °C at 99.8 kPa	Measured
Autoignition Temperature	244 °C	Measured
Explosive Properties	Predicted negative	Based on the chemical structure.
Oxidising Properties	Predicted negative	Based on the chemical structure.

*Isomer mixture containing the assessed chemicals up to 100%

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

Reactivity
The assessed chemicals are expected to be stable under normal conditions of use. Direct sources of heat and contact with strong acids, alkali or oxidising agents should be avoided.

Physical Hazard Classification
Based on the submitted physico-chemical data depicted in the above table, the assessed 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.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
The assessed chemicals will not be manufactured in Australia. The assessed chemicals will be imported into Australia as inseparable isomer mixture in fragrance oils at ≤ 10% concentrations.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes for each chemical	≤ 0.6	≤ 0.6	≤ 0.6	≤ 0.6	≤ 0.6

PORT OF ENTRY
Melbourne

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

TRANSPORTATION AND PACKAGING
The imported fragrance oils containing the inseparable isomer mixture (at ≤ 10% concentration) in polypropylene-lined steel drums (usually 208 L) will be transported mainly by road to the applicant's facility, then distributed to reformulation sites. The end-use products will be packaged in containers suitable for retail sale and transported primarily by road to retail stores.

USE
The assessed chemicals will be used as a fragrance ingredient in cosmetic products, detergents, cleaning and other household products. The proposed maximum use concentrations of the isomer mixture in different products will be:

- 1% in leave-on cosmetic products except for the following products:
  - face cream at 0.61%
  - hand cream at 0.66%
  - fine fragrances at 0.45%
  - deodorant (non-spray) at 0.22% and
  - deodorant (spray) at 0.23%
- 3% in rinse-off cosmetic products
- 3% in hair care products, except for 1% in hair spray and hair styling products
- 3% in all other consumer products

OPERATION DESCRIPTION
At the applicant's customer reformulation sites, reformulation of fragrance oil formulations containing the assessed chemicals (at ≤ 10% concentration of the isomer mixture) into finished consumer products may vary depending on the type of products and may involve both automated and manual transfer steps. Typically, reformulation processes may include blending operations which are largely automated and occur in an enclosed/contained environment, followed by automated filling of the reformulated end-use products into containers of various sizes.

End-use products containing the assessed chemicals (at ≤ 3% concentration for the isomer mixture) will be used by consumers and professionals such as hairdressers, beauticians or cleaners. Depending on the nature of the product, these could be applied in a number of ways, such as by hand, using an applicator or sprayed.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

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

EXPOSURE DETAILS

Transport and storage
Transport and storage workers may come into contact with the assessed chemicals (at ≤ 10% concentration) as components of fragrance oils or as components of end-use products only in the unlikely event of accidental rupture of containers.

Reformulation
During reformulation, dermal and ocular exposure of workers to the assessed chemicals (at ≤ 10% concentration of the isomer mixture) may occur during weighing and transfer stages, blending, quality control analysis, and cleaning and maintenance of equipment. Due to the low vapour pressure of the assessed chemicals (1.15×10^{-3} kPa at 25 °C for the isomer mixture), inhalation exposure is not expected, unless aerosols or mists are formed.

The applicant stated that exposure is expected to be minimised through the use of local exhaust ventilation and/or enclosed systems, and through workers using personal protective equipment (PPE) such as protective overalls, hard hats, chemical resistant gloves and safety glasses.

End-use
Exposure to the assessed chemicals in end-use products (at ≤ 3% concentration for the isomer mixture) may occur in professions where the services provided involve the application of cosmetics to clients (e.g. hair dressers and workers in beauty salons), or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposures are also possible. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using the products containing the assessed chemicals.

6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the assessed chemicals (at ≤ 3% concentration for the isomer mixture) through the use of a wide range of cosmetic and household products. The principal route of exposure will be dermal, while ocular and inhalation exposure are also possible, particularly if the products are applied by spray.

Data on typical use patterns of cosmetic and household cleaning product categories (SCCS, 2012; Cadby *et al*., 2002; ACI, 2010; Loretz *et al*., 2006), in which the isomer mixture of the assessed chemicals may be used are shown in the following tables. For the purposes of exposure assessment via the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, a dermal absorption (DA) of 100% was assumed for the assessed chemicals (ECHA, 2017). For inhalation exposure assessment, taking hairspray as a typical example, a 2-zone approach was used (Steiling *et al*., 2014; Rothe *et al*., 2011; Earnest, Jr., 2009). An adult inhalation rate of 20 m^3/day (enHealth, 2012) was applied and it was conservatively assumed that the fraction of the assessed chemicals inhaled is 50% of the amount sprayed, with remaining fraction ending up on the hair as intended for hair sprays. A lifetime average female body weight (BW) of 70 kg (enHealth, 2012) was used for calculation purposes.

Cosmetic products (dermal exposure)

Product type	Amount (mg/day)	C (%)	RF	Daily Systemic Exposure (mg/kg bw/day)
Body lotion	7,820	1	1	1.1171
Face cream	1,540	0.61	1	0.1342
Hand cream	2,160	0.66	1	0.2037
Fine fragrances	750	0.45	1	0.0482
Deodorant spray	1,430	0.23	1	0.0470
Deodorant non-spray	1,500	0.22	1	0.0471
Shampoo	10,460	3	0.01	0.0448
Hair conditioner	3,920	3	0.01	0.0168
Shower gel	18,670	3	0.01	0.0800
Hand wash soap	20,000	3	0.01	0.0857
Hair styling products	4,000	1	0.1	0.0571
Facial cleanser	800	3	0.01	0.0034
Total				**1.8853**

C = Concentration (%); RF = Retention Factor.
Daily Systemic Exposure = Amount × C × RF × Dermal Absorption / Body Weight

Household products (Indirect dermal exposure - from wearing clothes):

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily Systemic Exposure (mg/kg bw/day)
Laundry liquid	230	3	0.95	10	0.0936
Fabric softener	90	3	0.95	10	0.0366
Total					**0.1303**

Daily Systemic Exposure = Amount × C × PR × PT × Dermal Absorption /Body Weight

Household products (Direct dermal exposure):

Product type	Frequency (use/day)	C (%)	Contact Area (cm^2)	Product Use C (g/cm^3)	Film Thickness (cm)	Time Scale Factor	Daily Systemic Exposure (mg/kg bw/day)
Laundry liquid	1.43	3	1980	0.01	0.01	0.007	0.0008
Dishwashing liquid	3	3	1980	0.009	0.01	0.03	0.0069
All-purpose cleaner	1	3	1980	1	0.01	0.007	0.0594
Total							**0.0671**

Daily Systemic Exposure = Frequency × C × Contact Area × Product Use Concentration × Film Thickness on Skin × Time Scale Factor × Dermal Absorption/Body Weight

Aerosol products (Inhalation exposure):

Product type	Amount	C	Inhalation Rate	Exposure Duration (Zone 1)	Exposure Duration (Zone2)	Fraction Inhaled	Volume (Zone 1)	Volume (Zone 2)	Daily Systemic Exposure
	(g/day)	(%)	(m^3/day)	(min)	(min)	(%)	(m^3)	(m^3)	(mg/kg bw/day)
Hairspray	9.89	1	20	1	20	50	1	10	0.0294

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 assessed chemicals. This would result in a combined internal dose of 2.112 mg/kg bw/day.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the assessed chemicals together (isomeric mixture) 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 > 300 and < 2,000 mg/kg bw; harmful
Skin irritation - *in vitro* Skin Corrosion: Reconstructed Human EpiDermis (RHE) Test	non-corrosive
Skin irritation – *in vitro* EpiDerm Reconstructed Human Epidermis Model	not classified as a skin irritant
Skin irritation – rabbit	irritating
Eye irritation – *in vitro* Bovine Corneal Opacity and Permeability (BCOP) Test	not classified for eye irritation or serious eye damage
Eye irritation – *in vitro* Reconstructed Human Cornea-like Epithelium (RhCE) Test	not classified for eye irritation or serious eye damage
Eye irritation – rabbit	slightly irritating
Skin sensitisation – *in chemico DPRA test*	negative
Skin sensitisation – *in vitro* KeratinoSens test	negative
Skin sensitisation – *in vitro* h-CLAT test	positive
Repeat dose oral (dietary) toxicity – rat, 28 days	NOAEL of approximately 1,000 mg/kg bw/day (12,000 ppm)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* Chromosome Aberration Test	non genotoxic

Toxicokinetics, Metabolism and Distribution
No toxicokinetic data were provided for the assessed chemicals. Given the low molecular weight of the assessed chemicals (206.32 g/mol) and partition coefficient (log Pow = 4.61 at 25 °C), absorption across biological membranes may occur.

Acute Toxicity
The isomer mixture containing the assessed chemicals was harmful via oral route based on a study conducted in rats. No dermal or inhalation toxicity data were provided for the assessed chemicals.

Irritation
The isomer mixture containing the assessed chemicals was not considered to be corrosive to the skin in an *in vitro* Skin Corrosion: Reconstructed Human EpiDermis (RHE) Test. The isomer mixture was not classified as a skin irritant in an *in vitro* Skin Irritation: Reconstructed Human Epidermis Test. The isomer mixture was found to be irritating to the skin when tested in rabbits. The skin reactions were not fully recovered at the end of the study period (14 days). Based on the results of this study, the assessed chemicals warrant hazard classification for skin irritation according to the GHS criteria.

The isomer mixture containing the assessed chemicals was not classified for eye irritation or serious eye damage in an *in vitro* Bovine Corneal Opacity and Permeability (BCOP) Test and an *in vitro* Reconstructed Human Cornea-like Epithelium (RhCE) Test. The isomer mixture was found to be slightly irritating to the eyes when tested in rabbits.

Sensitisation
One *in chemico* and two *in vitro* cell based assays were conducted to evaluate the skin sensitisation potential of the assessed chemicals. The tests are part of Integrated Approach to Testing and Assessment (IATA) which address specific events of the Adverse Outcome Pathway (AOP) leading to development of skin sensitisation (OECD, 2016). The tests are thus considered relevant for assessment of the skin sensitisation potential of the assessed chemicals, 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 assessed chemicals with cysteine and lysine, small synthetic peptides representing the nucleophilic centres in skin proteins. The ARE-Nrf2 Luciferase Assay aims to address the second key event (keratinocyte activation) of the AOP by measuring the expression of a reporter luciferase gene under the control of a promoter from the antioxidant response element (ARE), a responding gene known to be upregulated by contact sensitisers. The *in vitro* h-CLAT assay aims to address the third key event (dendritic cell activation) of the AOP by measuring the expression of cell surface markers (such as CD54 and CD86) in human monocyte leukaemia cells (THP-1) upon stimulation with the assessed chemicals.

The assessed chemicals showed positive response in an in vitro h-CLAT test (third key event assay of the AOP) and negative results in both *in chemico DPRA test* and in *in vitro KeratinoSens test*. However, there are limitations

for KE1 & KE2 assays, as pre- and pro-haptens might not be reliably predicted by these two assays due to lack of metabolic capacity. In addition, the chemicals have a structural alert (α-, β-unsaturated ketone or precursor) for skin sensitisation (Barratt *et al.,* 1994). An EC value of 21.8% was derived (Belsito *et al.,* 2007) for an analogue chemical, 3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- (CAS No. 127-51-5), containing the same structural alert as the assessed chemical.

QSAR predictions using the version 4.2 indicated that the assessed chemicals were likely to be skin sensitisers. Using the weight of evidence, the assessed chemicals are considered to have skin sensitising potential and classified as weak skin sensitisers.

Repeated Dose Toxicity
In a 28-day repeated dose oral (dietary) toxicity study in rats with a 14-day recovery period, the isomer mixture containing the assessed chemicals was administered daily via the diet at dose levels of 0, 1,300, 4,000 and 12,000 ppm (approximately 0, 110, 330, and 1,000 mg/kg bw/day).

There was no mortality, morbidity or abnormalities in treated rats throughout the study period. The decrease in mean body weight and body weight gain (< 10%) was considered to be related to the treatment. The effect tended to recover during recovery period and there was marginal reduction of body weight (reduced to 2- 5% from the control). The mean liver weight increases observed in the test groups (over 10% increase compared to the control mean in all treated male groups and high dose females) appeared to recover during the recovery period (< 10% increase at the end of recovery).

Apart from the marginally reduced body weight and weight gain reductions in the high dose animals and increased liver weights in all treated animals, the test substance did not produce any severe toxicity or adverse effects at up to the highest dose level tested in rats. The No Observed Adverse Effect Level (NOAEL) of both male and female rats was established by the study author as 12,000 ppm (corresponding to 1,086.31 mg/kg bw/day for male rats and 1,121.33 mg/kg bw/day for female rats). However, the increased mean liver weights in rats treated at the highest dose (approximately 1,000 mg/kg bw/day) were not completely recovered during the 2-week recovery period (7% increase compared to the control group). The body weight recovered but there was a slight decrease of 2-5% at the end of recovery period. Therefore, the NOAEL could be below 1,000 mg/kg bw/day.

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

Health Hazard Classification
Based on the available information, the assessed chemicals are hazardous chemicals 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 assessed chemicals is presented in the following table.

Hazard Classification	***Hazard Statement***
Acute toxicity (Category 4)	H302 – Harmful if swallowed
Skin corrosion/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 available toxicological information, the isomer mixture containing the assessed chemicals is harmful if swallowed, a skin irritant and a skin sensitiser. The isomer mixture is slightly irritating to eyes. No inhalation toxicity data were available.

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

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

End-use
Cleaners and beauty care professionals will be exposed to the assessed chemicals (at ≤ 3 % concentration for the isomer mixture) in end use products, similar to the public. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. Therefore the risk to workers who use products containing the assessed chemicals is expected to be of a similar or lesser extent than consumers who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health

Members of the public may experience repeated exposure to the assessed chemicals (at ≤ 3% concentration for the isomer mixture) in individual products through the use of cosmetic and household products. The main route of exposure is expected to be dermal, while ocular and inhalation exposure are also expected where products are applied by spray.

Acute toxicity and irritation
The assessed chemicals are harmful by the oral route, irritating to the skin and slightly irritating to eyes. However, these effects are not expected from the use of products containing assessed chemicals at the proposed low use concentrations in cosmetic and household products.

Sensitisation
Based on the available data and results of an LLNA for an analogue chemical, the assessed chemicals are considered as weak skin sensitisers. As potency data were not available for the assessed chemicals, for the quantitative skin sensitisation risk assessment the EC3 value of 21.8% reported for the analogue chemical was used.

Using deodorant (non-spray) as an example of cosmetic products that may contain assessed chemicals (at ≤ 0.22% concentration), the Consumer Exposure Level (CEL) is estimated to be 16.50 μg/cm^2/day. Consideration of available information and application of appropriate safety factors, an Acceptable Exposure Level (AEL) of 16.70 μg/cm^2/day is estimated for assessed chemicals. 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 AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of deodorant (non-spray) at ≤ 0.22% concentration is not considered to be unreasonable. However, it is acknowledged that consumers may be exposed to multiple products containing assessed chemicals, and a quantitative risk assessment based on aggregate exposure has not been conducted.

Therefore, based on the information available, the risk to the public associated with use of assessed chemicals at concentrations of 0.61, 0.66, 0.45, 0.22% and 0.23% for face cream, hand cream, fine fragrances, deodorant (non-spray) and deodorant (spray) respectively and proposed concentrations for other cosmetic and household products (≤ 3%), is not considered to be unreasonable.

Repeated dose toxicity
The repeat dose toxicity risk was estimated based on the margin of exposure (MoE).

The potential systemic exposure to the public from use of the assessed chemicals as an isomer mixture in cosmetic and household products was estimated to be 2.112 mg/kg bw/day. Using a NOAEL of approximately 1,000 mg/kg bw/day, which was derived from a 28-day repeated dose toxicity study on the assessed chemicals as an isomer mixture, the margin of exposure (MoE) value was calculated as 473. An MoE of greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences, and to account for long-term exposure. Therefore the risk from repeated exposure is considered to be acceptable.

The MoE would be still acceptable (>100) even if the NOAEL was used as 400 ppm (~ 362 mg/kg bw/day), indicating that the risk from repeated exposure to the assessed chemicals is acceptable.

When used as a fragrance ingredient at a maximum concentration of 3% in cosmetic and household products, assessed chemicals are not considered to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The assessed chemicals will be imported as a component of fragrance oils and reformulated into personal care and household products by blending in an enclosed environment. Release of the assessed chemicals is only expected to be from spills during the transport, storage and product reformulation of the assessed chemicals. Accidental spills and equipment washings are to be collected for disposal, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The majority of the assessed chemicals will be released to sewer across Australia as a result of consumer use in personal care and household products.

RELEASE OF CHEMICAL FROM DISPOSAL

A small proportion of the assessed chemicals may remain as residues within empty import or empty end use containers. These containers are expected to be recycled or disposed of to landfill, with the assessed chemicals being disposed of to sewer via trade waste or to landfill.

7.1.2. Environmental Fate

The majority of the assessed chemicals is expected to enter the sewer system before potential release to surface waters on a nationwide basis. A proportion of the assessed chemicals may be partition to air. The half-life of the assessed chemicals in air is calculated to be < 1 h, based on reactions with hydroxyl radicals (US EPA, 2012; calculated using AOPWIN v1.92). Therefore, the assessed chemicals are not expected to persist in the air compartment. Ready biodegradation tests determined that the assessed chemicals are not readily biodegradable. For further details on the biodegradability studies, refer to Appendix C.

The assessed chemicals are expected to be effectively removed at sewage treatment plants (STPs) through adsorption to sludge based on their low water solubility, log Pow and log Koc. A proportion of the assessed chemicals may be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. The residues of assessed chemicals in landfill and soils are expected to have medium mobility based on their soil adsorption coefficient. The assessed chemicals may have a potential for bioaccumulation based on their measured partition coefficient (log Pow = 4.61), however, this is likely to be limited by their low water solubility and metabolism of the functional groups present in the assessed chemicals (Boethling & Mackay 2000). In the aquatic and soil compartments, the assessed chemicals are expected to degrade through biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The use pattern will result in most of the inseparable mixture of the assessed chemicals being washed into the sewer. The predicted environmental concentration (PEC) has been calculated assuming the realistic worst-case scenario with 100% release of the assessed chemicals into sewer systems nationwide over 365 days per annum. The extent to which the assessed chemicals are removed from the effluent in STP processes based on the properties of the assessed chemicals has not been considered for this scenario, and therefore no removal of the assessed chemicals during sewage treatment processes, is assumed. The PEC in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import Volume of the assessed chemicals	1,200	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1,200	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	3.29	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:	0.67	µg/L
PEC - Ocean:	0.07	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m^2/year (10 ML/ha/year). The assessed chemicals in this volume are assumed to infiltrate and accumulate in the top 10 cm of soil (density 1,500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.674 µg/L may potentially result in a soil concentration of approximately 4.494 µg/kg. Assuming accumulation of the assessed chemicals in soil for 5 and 10 years under repeated irrigation, the concentration of assessed chemicals in the applied soil in 5 and 10 years may be approximately 22.47 µg/kg and 44.94 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 0.37 mg/L	Very toxic to fish
Daphnia Toxicity	48 h EC50 = 0.92 mg/L	Very toxic to aquatic invertebrate
Algal Toxicity	72 h ErC50 = 2.9 mg/L NOEC = 0.83 mg/L	Toxic to algae
Inhibition of Bacterial Respiration	3 h EC50 > 1,000 mg/L	No inhibition to microorganisms in sewage treatment plants
Earthworms Toxicity	14 d EC50 = 90 mg/kg dried soil	Moderately toxic to earthworms

Based on the above ecotoxicological endpoints for the inseparable mixture of the assessed chemicals, it is expected to be acutely very toxic to fish and daphnia and toxic to algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the assessed chemicals warrant classification for "Aquatic chronic (Category 1); Very toxic to aquatic life with long lasting effects". Based on the acute toxicity and not being rapidly degradable, the assessed chemicals are formally classified as "Chronic Category 1; Very toxic to aquatic life with long lasting effects".

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) was calculated using the most sensitive endpoint for ecotoxicity (Fish, 96 h LC50 = 0.37 mg/L) with an assessment factor of 100 as all three measured acute endpoints were available for the inseparable mixture of the assessed chemicals.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish)	0.37	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC	3.7	µg/L

For the terrestrial environment one acute endpoint is available and an assessment factor of 1,000 was used.

Predicted No-Effect Concentration (PNEC) for the Terrestrial Compartment		
EC50 (Earthworms)	90	mg/kg
Assessment Factor	1,000	
Mitigation Factor	1.00	
PNEC	90	µg/kg

7.3. Environmental Risk Assessment

The Risk Quotient for the aquatic environment (Q = PEC/PNEC) was calculated based on the PEC of the assessed chemicals as part of an inseparable mixture and PNEC for the inseparable mixture of the assessed chemicals.

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q - River:	0.67	3.7	0.18
Q - Ocean:	0.07	3.7	0.02

The risk quotient for discharge of treated effluents containing the assessed chemicals to the aquatic environment indicates that the assessed chemicals are unlikely to reach ecotoxicologically significant concentrations in surface waters, based on its maximum annual importation quantity.

The Risk Quotient (Q = PEC/PNEC) for the terrestrial environment was also calculated in a similar manner to that for the aquatic environment as follows.

Risk Assessment	PEC (µg/kg)	PNEC (µg/kg)	Q
Q – soil	44.94	90	0.49

Therefore on the basis of the aquatic and the terrestrial PEC/PNEC ratios, the assessed chemicals as part of an inseparable mixture are not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES OF THE ISOMER MIXTURE

Freezing Temperature < -20 °C

Method	OECD TG 102 Melting Point/Melting Range (1995) EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	Crystallizing point method was used.
Test Facility	Covance (2019a)

Boiling Point 274.8 ± 0.5 °C at 102 kPa

Method	OECD TG 103 Boiling Point (1995) EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Differential scanning calorimetry method was used.
Test Facility	Covance (2019b)

Density 928 kg/m^3 at 20 ± 0.5 °C

Method	OECD TG 109 Density of Liquids and Solids (2012) EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Pycnometer method was used.
Test Facility	Covance (2019b)

Vapour Pressure 1.15×10^{-3} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure (2006) EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Vapour pressure balance method was used.
Test Facility	Envigo (2019a)

Water Solubility 0.0105 g/L at 20 °C

Method	OECD TG 105 Water Solubility
Remarks	Flask Method
Test Facility	Covance (2019a)

Partition Coefficient (n-octanol/water) log Pow = 4.61 at 25 °C

Method	OECD TG 123 Partition Coefficient (n-octanol/water).
Remarks	The proposed slow-stirring method is optimal for highly hydrophobic substances and is preferred to the shake-flask with a systematic underestimation of the partition coefficient value. The test substance was analysed by GC Method.
Test Facility	Covance (2019a)

Surface Tension 58.5 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions EC Council Regulation No 440/2008 A.5 Surface Tension
Remarks	Test concentration: 90% saturated solution. The test substance is surface active.
Test Facility	Covance (2019b)

Adsorption/Desorption log K_{oc} = 3.67 – 3.83

Method	OECD TG 121Adsorption Coefficient
Remarks	HPLC method with the column temperature at 30 °C.
Test Facility	Covance (2019c)

Flash Point 127 ± 2 °C at 99.8 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Based on, Anon Determination of Flash no Flash and Flash Point – Rapid Equilibrium Closed Cup Method, *International Standard ISO 3679:2015* Second Edition, P1-22 and Anon (2011) Flash Point of Liquids by Small Scale Closed Cup Apparatus, ASTM D-3278-89, P1-8.
Test Facility	Covance (2019d)

Autoignition Temperature 244 °C at 99.8-103.4 kPa

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks	Based on, Anon (2010), Explosive atmospheres – Part 20-1: Material characteristics for gas and vapour classification – test methods and data, Method of test for auto-ignition temperature. *IEC 60079-20-1:2010* P1-77.
Test Facility	Covance (2019d)

Explosive Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	There were no structural alerts within the chemical structure of the test substance to imply explosive properties.
Test Facility	Covance (2019d)

Oxidizing Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks	The structure contains oxygen but it is not bonded to nitrogen or oxygen.
Test Facility	Covance (2019d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

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

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method (2001)
Species/Strain	Rat/Wistar Han™
Vehicle	Corn oil
Remarks – Method	Minor protocol deviations, such as relative humidity, age of animals and observation time points, did not affect the validity of the study.

Main Study

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

Discriminating Dose	LD50 > 300 and < 2000 mg/kg bw
Signs of Toxicity	At 2,000 mg/kg bw dose, the animal showed partially closed eyes, decreased activity, sunken flanks, piloerection, prostration, lethargy, ataxia, hunched back, and increased respiration rate. The animal was euthanised 4 hours after treatment due to deterioration. At necropsy, the stomach was filled with the diet and the test substance with an aromatic, sweet smell, noting hardened faeces at proximal to the caecum. At 300 mg/kg bw, the animals showed no signs of systemic toxicity.
Effects in Organs	All animals showed expected gains in body weight. No abnormalities were noted at necropsy except for one animal found with clear liquid in the uterus.
CONCLUSION	The test substance is harmful via the oral route.
TEST FACILITY	Envigo (2019b)

B.2. Skin Irritation – *In Vitro* Epidermi Skin Corrosion Test

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 431 *In vitro* Skin Corrosion: Reconstructed Human EpiDermis (RHE) Test Method (2016)
Vehicle	None
Remarks – Method	No protocol deviations. In pre-tests, the test substance did not have any interference with MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazoliumbromide) reduction or with the test colour change. Negative control: sterile distilled water Positive Control: 8.0 N potassium hydroxide

RESULTS

Test Material	*Mean OD_{570} of duplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	1.577/1.622	100/100	0.061/0.045
Test substance	1.869/2.009	118.5/123.9	0.095/0.130
Positive control	0.073/0.072	4.6/4.4	0.017/0.006

OD = optical density; SD = standard deviation
Values are for exposure periods (3 minutes/60minutes)

Remarks – Results	Based on the mean tissue viability of > 50% (118.5% for 3 minutes exposure period and 123.9% for 60 minutes exposure period), the test substance is not classified as a skin corrosive according to the test guidelines, using GHS criteria. The positive and negative controls performed as expected and the standard deviation of the relative mean viability of the test substance-treated tissues was within acceptable range, confirming the validity of the test system.
CONCLUSION	The test substance was considered non-corrosive to the skin according to the GHS criteria.
TEST FACILITY	Covance (2019i)

B.3. Skin Irritation – *In Vitro* EpiDermTM Reconstructed Human Epidermis Model

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2015) using EpiDerm™ Reconstructed Human Epidermis Model
Vehicle	None
Remarks – Method	No protocol deviations. In pre-test, the test substance did not have any interference with MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazoliumbromide) reduction or with the colour change. Positive and negative controls were run in parallel with the test substance: - Negative control: Dulbecco's Phosphate Buffered Saline (DPBS) with Ca^{++} and Mg^{++} - Positive control: sodium dodecyl sulphate (99.5%)

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.857 ± 0.008	100	1.0
Test substance	0.501 ± 0.007	58.5	0.8
Positive control	0.038 ± 0.006	4.4	0.7

OD = optical density; SD = standard deviation

Remarks – Results	The positive and negative controls performed as expected and the standard deviation of the relative mean viability of the test substance-treated tissues was within acceptable range, confirming the validity of the test system.
CONCLUSION	Based on the mean tissue viability of > 50%, the test substance is not classified as a skin irritant according to the GHS criteria.
TEST FACILITY	Covance (2019e)

B.4. Skin Irritation – Rabbit

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion (2015) EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 F
Vehicle	None

Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks – Method	No protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	2.0	1.0	1.3	3	14 days	1
Oedema	0.3	0.0	0.0	3	< 10 days	0

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

Remarks – Results	There were very-slight or well-defined erythema, very slight to slight oedema, thickened skin, loss of elasticity, loss of flexibility and exfoliation noted in the test animals during the study with some effects persistent until 14 days.
CONCLUSION	The test substance is irritating to the skin.
TEST FACILITY	Covance (2020a)

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

TEST SUBSTANCE	PG-RAW-90-032
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 (2017)
Vehicle	None
Remarks – Method	A minor protocol deviation was not considered to affect the validity of the study: the permeability mean score for the negative control is marginally outside of the historical control range at 0.036 (≤ 0.035). Negative control: sodium chloride 0.9% w/v Positive control: ethanol

RESULTS

Test Material	*Mean Opacities of Triplicate Tissues (SD)*	*Mean Permeabilities of Triplicate Tissues (SD)*	*IVIS (SD)*
Negative control	0.0	0.036	0.5
*Test substance**	0.0	0.000	0.0
*Positive control**	32.7	1.410	53.8

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

Remarks – Results	The corneas treated with the test substance and the negative control were clear post treatment and post incubation. The corneas treated with the positive control were cloudy post treatment and post incubation. The IVIS for the test substance was ≤ 3 ('no category'). The positive and negative control acceptance criteria were satisfied.
CONCLUSION	The test substance does not require classification for eye irritation or serious eye damage.
TEST FACILITY	Covance (2019h)

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

TEST SUBSTANCE	PG-RAW-90-032
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 (2018)
Vehicle	None
Remarks – Method	A minor protocol deviation was not considered to affect the validity of the study: vitality calculation was done as percent values related to the main negative control instead of the relating negative control. Positive and negative controls were run in parallel with the test substance: - negative control: deionised water - positive control: methyl acetate Additional tests with freeze-killed tissues were conducted, since the test substance proved to be a MTT reducer.

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*	*Relative Mean Viability (%)*
Negative Control	1.940	100.0
Test Substance	2.064	106.39
Positive Control	0.422	21.74

OD = optical density; SD = standard deviation

Remarks – Results	The optical pre-test to investigate the test substance's colour change potential in water or isopropanol did not show a change in colour. The relative mean tissue viability for the test substance as compared to the negative control was 106.39%. Given that the relative mean tissue viability for the test substance was > 50%, it is not considered to cause eye irritation. The positive and negative controls gave satisfactory results, confirming the validity of the test.
CONCLUSION	The test substance does not require classification for eye irritation or serious eye damage.
TEST FACILITY	Envigo (2019f)

B.7. Eye Irritation – Rabbit

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion (2012)
Species/Strain	Rabbit/New Zealand White
Number of Animals	2 F
Observation Period	72 hours
Remarks – Method	A minor protocol deviation related to the supplier of animals was not considered to affect the validity of the study.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva – Redness	0.3	0.3	3	< 48 hours	0
Conjunctiva – Chemosis	0.3	0.3	2	< 48 hours	0
Conjunctiva – Discharge	0	0	3	< 24 hours	0
Corneal Opacity	0	0	0	-	0
Iridial Inflammation	0	0	0	-	0

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

Remarks – Results: After one hour the application of the test substance to one eye of the rabbit resulted in swelling of the conjunctiva with slight discharge and diffuse crimson colour. The second animal showed diffuse beefy redness and swelling of the conjunctiva with slight discharge. Injection of the conjunctival blood vessels with some swelling was observed in both animals 24 hours after treatment. The adverse effects were fully reversible by the end of 48 hours observation period.

No initial pain response or sign of toxicity or ill health was noted in the animals during the study.

CONCLUSION: The test substance is slightly irritating to the eye.

TEST FACILITY: Covance (2020b)

B.8. Skin Sensitisation – *In Chemico* DPRA Test

TEST SUBSTANCE: PG-RAW-90-032

METHOD: OECD TG 442c *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)

Vehicle: Acetonitrile

Remarks – Method: No protocol deviations.

Negative control: synthetic peptide containing cysteine or lysine
Positive control: cinnamic aldehyde

RESULTS

Sample	*Cysteine Peptide Depletion (% ± SD)*	*Lysine Peptide Depletion (% ± SD)*
Negative Control	0.48 ± 0.12	0.66 ± 0.67
Test Substance	1.24 ± 0.12	-1.32 ± 0.67
Positive Control	71.6 ± 0.16	57.1 ± 0.68

SD = Standard Deviation

Remarks – Results: The overall mean depletion peptides was 0.622%, indicating minimal reactivity.

Positive and negative controls performed as expected. All quality criteria were met. The reactivity of the test substance was classed as no or minimal indicating a negative DPRA prediction.

CONCLUSION: The test substance was considered to have low 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 Envigo (2019c)

B.9. Skin Sensitisation – *In Vitro* ARE-Nrf2 Luciferase Test

TEST SUBSTANCE PG-RAW-90-032

METHOD OECD TG 442d *In Vitro* Skin Sensitisation Assays Addressing the AOP Key Event on Keratinocyte Activation (2018)
- The ARE-Nrf2 luciferase KeratinoSens™ test method (Appendix IA).

Vehicle Dimethyl sulphoxide (DMSO)

Remarks – Method No protocol deviations.
Positive control: cinnamic aldehyde

RESULTS

Sample	*Concentration (µM)*	*Mean Cell viability (%)*	*Mean Luciferase Induction (%)*
Vehicle Control	-		
Negative Control	-		
Test substance		Test1/Test2	Test1/Test2
Dose Level 1	0.98	125.15/101.25	1.01/0.96
Dose Level 2	1.95	117.33/99.66	1.07/0.92
Dose Level 3	3.91	105.72/95.33	1.07/0.97
Dose Level 4	7.81	106.80/108.64	1.08/0.96
Dose Level 5	15.63	110.24/110.51	1.12/1.01
Dose Level 6	31.25	110.72/118.10	1.25/1.05
Dose Level 7	62.5	3.17/3.06	0.01/0.14
Dose Level 8	125	3.31/3.26	0.00/0.00
Dose Level 9	250	3.37/3.59	0.00/0.00
Dose Level 10	500	2.90/3.13	0.00/0.00
Dose Level 11	1,000	3.10/3.26	0.00/0.00
Dose Level 12	2,000	2.63/3.00	0.05/0.00
Positive Control			
	4	103.50/116.70	1.38/1.23
	8	103.56/108.04	1.22/1.48
	16	103.56/107.51	1.64/1.99
	32	107.07/108.04	2.00/2.87
	64	102.08/101.85	4.64/6.36

Remarks – Results As the Imax for both tests was < 1.5 fold compared with the negative control, the EC1.5 could not be established. The cellular viability was < 70% in both tests. The IC30 value was 43.08 µM in test 1 and 44.32 µM in test 2 and the IC50 values were 48.89 µM and 49.75 µM in tests 1 and 2. There was no overall dose-response for luciferase induction for the test substance.

Luciferase induction activity obtained with the positive control was statistically significant above the threshold of 1.5 in at least one of the test concentrations in both tests.

The EC1.5 values of the positive control were 13.26 µM and 8.36 µM for tests 1 and 2, which were within the historical control range. The average induction of the positive control at 64 µM were 4.64 and 6.36 for tests 1 and 2, within the acceptance criterion of between 2 and 8.

The average coefficient of variation of the luminescence reading for the negative control was 8.6% and 11.8% for tests 1 and 2, meeting the acceptance criterion of below 20%.

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

TEST FACILITY Envigo (2019d)

B.10. Skin Sensitisation – *In Vitro* Skin Sensitisation Test - Human Cell Line Activation Test (h-CLAT)

TEST SUBSTANCE PG-RAW-90-032

METHOD OECD TG 442e *In Vitro* Skin Sensitisation Assays Addressing the Key Event on Activation of Dendritic Cells on the Adverse Outcome Pathway for Skin Sensitisation *In Vitro* Skin Sensitisation (2018)
- Human Cell Line Activation Test (h-CLAT)

Vehicle DMSO

Remarks – Method Minor protocol deviations, such as staining of cells and sample preparation for measurement, did not affect the validity of the study.

The concentration selection for main experiments was based on the cytotoxic effects in a pre-test performed at up to 5,000 µg/mL. There were 6 independent runs in the study.

Negative control: culture medium
Vehicle control: DMSO in culture medium
Positive control: 2,4-dinitrochlorobenzene

RESULTS

Sample	*Concentration (µg/mL)*	*Mean RFI* CD86 (%)*	*Mean RFI* CD54 (%)*	*Relative Viability (%)*
Vehicle Control	-	100.0	100.0	95.04
Negative Control	-	100.0	100.0	96.04
Test substance				
Dose Level 1	24	154.1	72.8	94.46
Dose Level 2	29	142.3	91.5	93.99
Dose Level 3	35	136.7	104.5	94.29
Dose Level 4	42	114.3	103.2	93.14
Dose Level 5	50	157.1	107.6	94.89
Dose Level 6	60	147.0	110.9	94.43
Dose Level 7	72	128.4	140.7	94.79
Dose Level 8	73	139.9	75.1	93.50
Dose Level 9	87	137.9	127.4	92.83
Dose Level 10	104	175.3	184.0	92.47
Dose Level 11	125	185.8	202.1	92.27
Dose Level 12	150	311.2	143.3	90.36
Dose Level 13	180	269.0	140.0	92.49
Positive Control	3	513.9	333.0	84.09
	4	561.6	463.8	80.13

*RFI = relative fluorescence intensity
All results were averaged from up to six h-CLAT runs for the test substance PG-RAW-90-032, vehicle control, negative control and positive control

Remarks – Results As the RFI of CD86 and CD54 was greater than 150% and 200% respectively in at least one of the concentrations in more than one independent run (6 runs in total), the test substance was predicted to be positive under the conditions of this test.

The study authors considered one run was invalid, as the cell viability was not below 90% at any tested concentration and the result was negative.

	Positive and negative controls performed as expected.
CONCLUSION	The test substance was positive in the third key event (dendritic cell activation) of the adverse outcome pathway (AOP) for skin sensitisation as defined in the test guideline.
TEST FACILITY	Envigo (2019e)

B.11. Repeat Dose Oral – Dietary Toxicity – Rats

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (2008)
Species/Strain	Rats/Wistar (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	None
Remarks – Method	No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (ppm)*	*Mortality*
Control	5 per sex	0	0/10
Low Dose	5 per sex	1,300	0/10
Mid Dose	5 per sex	4,000	0/10
High Dose	5 per sex	12,000	0/10
Control Recovery	5 per sex	0	0/10
High Dose Recovery	5 per sex	12,000	0/10

Mortality and Time to Death

No mortality or morbidity was observed in animals, throughout the study period.

Clinical Observations

No treatment related clinical observations, ophthalmological effects, neurobehavioural observations, and functional battery changes were observed in any dose group.

The mean body weights of male and female rats from all dose groups were comparable with those of the control group throughout the study. There was a statistically significant decrease in the mean body weight in male rats at week 1 and female rats at weeks 1 to 4 in high dose recovery group. There were no changes in food consumption of treated animals, compared to the control animals.

The changes in mean body weight of male and female rats from low, mid and high dose groups were comparable with those of the control group except that a statistically significant decrease at week 1 in male rats of high dose group and in female rats of mid and high dose groups. There was a statistically significant decrease in the mean body weight change in male rats at week 1 and female rats at weeks 1 to 6 in the high dose recovery group.

The decrease in the mean body weight (2-8%) and mean body weight gain (either statistically significant or not) of both sexes of high dose groups (treatment and recovery) was considered as a possible treatment related effect. However, the effect disappeared during the recovery period and there was marginal reduction of mean body weight (2- 5% decrease from the control mean). Therefore the effect was not considered as adverse by study authors.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no significant changes in haematological and urinalysis parameters in all treated animals compared to controls. However, there was statistically significant variation in clinical chemistry parameters, including

increase in total cholesterol, triglyceride and calcium in high dose of both sexes, total protein (albumin and globulin) in mid and high dose males, globulin in high dose females,, decrease in total bile acids and chloride in high dose males, alanine aminotransferase (ALT) in mid and high dose males and aminotransferase (AST) in high dose males. These levels were comparable to the control levels after the recovery period.

Effects in Organs

There was a statistically significant increase in relative liver weight in all treated groups, possibly related to hepatocyte hypertrophy noted in histopathological examination, except in the low dose female group. The mean absolute liver weights were statistically significantly increased in all treated males and high dose females (14.4%, 20.5% and 44.2% in males at low, mid and high dose groups respectively, compared to the control group and 28.5% in high dose females, compared to the control group). Although the mean liver weight in the high dose recovery group was not completely reversed back to the level of the control group during the recovery period, the weight increase was < 10% (7.3%) compared to the control group, indicating recovery.

A statistically significant relative kidney weights were observed in high dose male group. The study author considered this may be associated with basophilic tubules observed in histopathological examination.

Macroscopic finding did not reveal any abnormalities in treated animals, except that one female animal in the high dose group showed uterus distended with purulent exudate.

Microscopic examination noted lesions in the liver, kidneys and pancreas of animals mainly at mid and high doses: in liver, there was hepatocyte hypertrophy in all rats of high dose group and some rats in the mid dose group. The effect was considered by study authors as adaptive due to the absence of changes in clinical chemistry parameters related to liver function (ALT, AST, alkaline phosphatase, gamma glutamyl transferase, total bilirubin and bile acid) and marked recovery in the recovery group. The increased zymogen granules in pancreas was considered non-adverse since this lesion was found in one male from the control recovery group.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established by the study author as 12,000 ppm in diet (corresponding to 1,086.31 ± 81.85 mg/kg bw/day for male rats and 1,121.33 ± 131.10 mg/kg bw/day for female rats) in this study.

TEST FACILITY	Jay Research Foundation (2019)

B.12. Genotoxicity – Bacteria

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 471 Bacterial Reverse Mutation Test (1997) Test 1: Plate incorporation procedure/Test 2: Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix prepared from phenobarbital and β-naphtha flavone induced rat liver homogenateS9
Concentration Range in Main Test	a) With and without metabolic activation in Test 1: 0, 1.5, 5, 15, 50, 150, 500, 1,500, 5,000 µg/plate b) With and without metabolic activation in Test 2: 0, 15, 50, 150, 500, 1,500, 5,000 µg/plate)
Vehicle	DMSO
Remarks – Method	No protocol deviations. There was no preliminary test. Positive controls: With metabolic activation: 2-Aminoanthracene (TA100, TA1535, TA1537, Wp2uvrA), Benzo(a)pyrene (TA98) Without metabolic activation: N-ethyl-N'-nitro-N-nitrosoguanidine (Wp2uvrA, TA100, TA1535), 9-Aminoacridine (TA1537), 4-Nitroquinoline-1-oxide (TA98)

RESULTS

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

Remarks – Results

There was no visible reduction in the growth of the bacterial background lawn at any dose level, either in the presence or absence of metabolic activation in both tests.

No biologically relevant increases in the frequency of revertant colonies and no test substance precipitate were observed for any of the bacterial strains either with or without metabolic activation in both tests.

Positive and negative controls performed as expected in both tests.

CONCLUSION

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

TEST FACILITY

Envigo (2019g)

B.13. Genotoxicity – *In Vitro* Chromosome Aberration Test in Human Lymphocytes

TEST SUBSTANCE

PG-RAW-90-032

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (2016)

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

Metabolic Activation System: S9 mix prepared from phenobarbital and β-naphtha flavone induced rat liver homogenate

Vehicle: DMSO

Remarks – Method: No protocol deviations.

Negative control: DMSO Positive controls: with metabolic activation: cyclophosphamide; without metabolic activation: Mitomycin C.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 4, 8, 16, 32*, 40*, 48*, 64	4	20
Test 2	0*, 4, 8, 16, 32*, 40*, 48*, 64*	24	-
Present			
Test 1	0*, 16, 32, 48, 64, 72*, 80*, 88*,96	4	20

*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	> 31.25			
Test 1		> 40	≥ 32*	negative
Test 2		> 48	≥ 32*	negative
Present	> 62.5			
Test 1		> 96	≥ 48*	negative

*Haemolysis

Remarks – Results	The test substance did not induce any statistically significant increases in the frequency of cells with chromosomal aberrations in the absence of metabolic activation. Statistically significant small increase was noted in the frequency of cells with chromosomal aberrations at 80 µg/mL in the presence of metabolic activation. The concentrations above and below that did not show any increases and therefore this result was considered of no toxicological significance. The test substance did not induce a statistically significant increase in the numbers of polyploid cells at any concentration tested. Positive and negative controls performed as expected in both tests.
CONCLUSION	The test substance was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Envigo (2019h)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability Study 1

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated sludge from a domestic sewage treatment plant (STP)
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical oxygen demand (BOD)
Remarks – Method	The test was conducted according to good laboratory practice (GLP) principles with no significant deviations.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0.789	14	84.3
14	-0.789		
28	-0.789		

Remarks – Results	All validity criteria for the test were satisfied. The biodegradation of sodium benzoate was > 60% in 14 days, indicating the suitability of the inoculum. Oxygen consumption in the inoculum blank was > 60% in 28 days. The pH was maintained between 7.21 – 7.69. No degradation of the test substance was observed after 28 days.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	Yaxin (2019a)

C.1.2. Ready Biodegradability Study 2

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 310 Ready Biodegradability: CO_2 in Sealed Vessels
Inoculum	Sewage sludge microorganisms from a domestic STP
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved organic carbon (DOC)
Remarks – Method	No significant protocol deviation. A toxicity control was run.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
14	0	14	77
28	4		

Remarks – Results	Validity criteria were met. The degradation in the toxicity control was 32% and therefore the test substance was not toxic to the sewage treatment microorganisms. The percentage degradation of the test substance at the end of the test was 4%.

CONCLUSION	The test substance is not readily biodegradable
TEST FACILITY	Envigo (2019b)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 203 Fish, Acute Toxicity Test -static conditions
Species	Rare minnow (*Gobiocypris rarus*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	146 mg $CaCO_3$/L
Analytical Monitoring	High Performance Liquid Chromatography with Diode-Array Detector (HPLC – DAD)
Remarks – Method	The test was conducted according to good laboratory practice (GLP) principles. Following a range-finding test, a limit test was conducted with no major deviations from the test guidelines. Test solutions were prepared by diluting the stock solution. Potassium dichromate was used as a reference substance.

RESULTS

Concentration		*Number of Fish*	*Mortality*				
Nominal (%)	*Actual (mg/L)*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	10	0	0	0	0	0
8.80	0.255	10	0	0	0	0	2
13.3	0.375	10	0	0	1	2	5
19.8	0.601	10	5	10	10	10	10
29.7	0.792	10	7	10	10	10	10
46.5	1.36	10	8	10	10	10	10
66.7	2.03	10	10	10	10	10	10
100	3.31	10	10	10	10	10	10

LC50	0.37 mg/L at 96 hours (95% CL: 0.49 - 0.46 mg/L)
Remarks – Results	All validity criteria were fulfilled. Dissolved oxygen concentration at the end of the test was ≥ 60% of the air-saturation value in controls and test vessels. Based on measured concentration, the 96 h LC50 was 0.37 mg/L with the 95% confidence limit of 0.29 to 0.46 mg/L. The 24 h LC50 = 346 mg/L for fish exposed to potassium dichromate was within the range of expected responses.
CONCLUSION	The test substance is very toxic to fish.
TEST FACILITY	Yaxin (2019b)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None

Water Hardness	Not reported
Analytical Monitoring	Gas Chromatography (GC)
Remarks – Method	The test was conducted according to good laboratory practice (GLP) principles. No major deviations from the test guidelines were reported. Following a range-finding test, a definitive test was conducted with no major deviations from the test guidelines. Test concentrations in a table below were prepared by diluting the stock solution. Potassium dichromate was used as a reference substance.

RESULTS

Concentration		*Number of D. magna*	*Number Immobilised*	
Nominal (% saturated solution)	*Actual (mg/L)*		*24 h*	*48 h*
Control	0	20	0	0
1.9	0.19	20	0	2
4.1	0.43	20	0	0
9.1	0.98	20	7	11
20	2.3	20	13	18
45	4.9	20	20	20
100	9.6	20	20	20

LC50	0.92 mg/L at 48 hours (95% CL: 0.37 – 2.1 mg/L)
Remarks – Results	All validity criteria were fulfilled. In the control, no daphnids were trapped at the surface of the water or showed signs of stress. Concentrations of dissolved oxygen were in the range 8.7 – 9.5 mg O_2/L equivalent to > 83% oxygen saturation in fresh water (U.S. Geological Survey, 2011). The pH of test water was in the range of 7.9 to 8.2. The 48 h EC50 = 0.80 mg/L for daphnids exposed to potassium dichromate was within the range of expected responses.
CONCLUSION	The test substance is very toxic to daphnids.
TEST FACILITY	Covance (2019f)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	PG-RAW-90-032
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	Freshwater Green Alga, *Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 3.2, 10, 32 and 100% v/v saturated solution (Equivalent to 0.11 to 8.0 mg/L) Actual: 0.09, 0.28, 0.83, 2.7 and 6.70 mg/L (Average geometric mean measured test concentrations for 0, 24, 48 and 72 h)
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	Gas chromatography (GC)
Remarks – Method	The test was conducted according to good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. Mean measured concentrations were calculated as the arithmetic mean of the measured concentrations. Potassium dichromate was used as a reference substance.

RESULTS

Biomass		*Growth*	
EbC50 (mg/L at 72 h)	*NOEbC (mg/L)*	*ErC50 (mg/L at 72 h)*	*NOErC (mg/L)*
1.6	0.83	2.9	0.83

Remarks – Results: All the validity criteria for the study were satisfied. The cell density in the control increased by a factor of 128 within 72 hours. The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 19%. The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 3%. The ErC50 = 1.5 mg/L was within the acceptable range for the reference substance.

CONCLUSION: The test substance is toxic to algae.

TEST FACILITY: Covance (2019g)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE: PG-RAW-90-032

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum: Activated sludge
Exposure Period: 3 hours
Concentration Range: Nominal: 10, 32 and 100 mg/L

Remarks – Method: The test was conducted according to the good laboratory practice (GLP) principles with no significant protocol deviations. Following the results of range finding test at 100, 500 and 1000 mg/L, a definitive test was conducted at the nominal concentration of 10, 32 and 100 mg/L. The 3,5-dichlorophenol was used as reference item.

RESULTS
EC50: > 1,000 mg/L
Remarks – Results: All validity criteria were satisfied. The 3 h EC50 = 7.7 for the 3,5 dichlorophenol was within the acceptable range.

CONCLUSION: The test substance is not harmful to microbial activity.

TEST FACILITY: Envigo (2019c)

C.2.5. Earthworms Toxicity Study

TEST SUBSTANCE: PG-RAW-90-032

METHOD: OECD TG 207 Earthworm Acute Toxicity Test
Species: *Eisenia foetida*

Exposure: 14 days
Concentration range: Nominal: 20, 40, 80, 160 and 320 mg/kg soil
Auxiliary solvent: None
Remarks – Method: Following a range finding test, a definitive test was conducted at 20, 40, 80, 160 and 320 mg/kg dw soil. 2-Chloracetamide was used as a reference substance.

RESULTS

Nominal Concentration (mg/kg dry weight)	*Total number of test earthworms*	*Exposure duration*	
		7 d *Cumulative mortality (%)*	*14 d* *Cumulative mortality (%)*
Control	40	0	0
20	40	0	0
40	40	0	0
80	40	5	13
160	40	29	40
320	40	40	40

14 d LC50	90.3 mg/kg dry weight soil (95% CL: 81.5 – 100 mg/kg dry weight soil).
Remarks – Results	The LC50 and 95% confidence intervals were calculated using Trimmed Spearman-Karber method. All the validity criteria were met. During the test, pH values ranged from 6.0 to 6.1. The 7 d LC50 and 14 d LC50 for the test substance were 126 mg/kg dry weight (dw) soil (95% CI: 111 to 142 mg/kg dw soil) and 90.3 mg/kg dw soil (95% CI: 81.5 to 100 mg/kg dw soil), respectively. The 7 d LC50 and 14 d LC50 for the reference substance were 48.6 and 34.2 mg/kg dw soil, respectively.
CONCLUSION	The test substance is moderately toxic to earthworms.
TEST FACILITY	Yaxin (2019c)

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