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August 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Cyclohexane, 1,1'-[oxybis(methylene)]bis-

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(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2084	International Flavours and Fragrances (Australia) Pty Ltd	Cyclohexane, 1,1'-[oxybis(methylene)]bis-	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*
Irritation, skin (Category 2)	H315 – Causes skin irritation
Sensitisation, skin (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*
Hazardous to the aquatic environment, long-term hazard (category 1)	H410 - Very toxic to aquatic life with long lasting effects

Human Health Risk Assessment
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 as fragrance ingredient at a maximum concentration of ≤ 3% in cosmetic and household products, 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, 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:
  - Irritation, skin (Category 2): H315 – Causes skin irritation
  - Sensitisation, skin (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 allergic skin reactions.

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 if possible

- 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 during reformulation processes:
  - Avoid contact with skin and eyes
  - Avoid inhalation of vapours and 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 notified chemical during reformulation processes:
  - Impervious gloves
  - Protective clothing

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

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 1.87% in body lotion, 0.98% in face cream, 0.98% in hand cream, 1% in fine fragrances, 0.15% in deodorants (including non-spray deodorants), or 3% in other household, cosmetic or personal care products.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from 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 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(S)
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, dissociation constant and flammability.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
European Union (2018)
China (2017)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Solarys

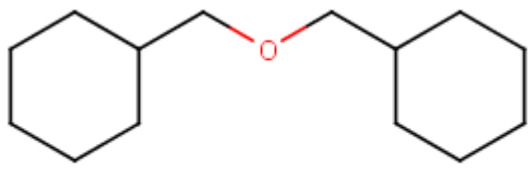
CAS NUMBER
14315-63-0

CHEMICAL NAME
Cyclohexane, 1,1'-[oxybis(methylene)]bis-

OTHER NAME(S)
bis(cyclohexylmethyl) ether
FRET 13-0460

MOLECULAR FORMULA
$C_{14}H_{26}O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
210.36 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
≥ 99%

HAZARDOUS IMPURITIES
None identified

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 liquid

Property	*Value*	*Data Source/Justification*
Freezing Point	< -20 °C	Measured
Boiling Point	171 °C at 5.33 kPa	Measured
Density	907.8 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.0013 kPa at 25 °C	Measured
Water Solubility	0.312 mg/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functional groups but not expected to significantly hydrolyse in the environmentally relevant pH range (4-9)
Partition Coefficient (n-octanol/water)	log Pow = 5.67 at 25 °C (Slow stirring method) log Pow > 6.5 at 20 °C (HPLC method)	Measured
Adsorption/Desorption	log K_{oc} = 4.19	Measured
Dissociation Constant	Not determined	Contains no dissociable functional groups
Flash Point	160 °C at 101.3 kPa	Measured
Flammability	Not determined	Not expected to be highly flammable based on flash point
Autoignition Temperature	190 °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.

The notified chemical has a flash point of 160 ºC which is greater than 93 °C. Based on *Australian Standard AS1940* definitions for combustible liquid, the notified chemical may be considered as a Class C2 combustible liquid if the chemical has a fire point below the boiling point.

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 as a component of a fragrance formula (at a concentration of ≤ 15%).

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 into Australia as a component of fragrance preparations (containing the notified chemical at ≤ 15% concentration) in polypropylene-lined steel drums of 205 L in size. They will be transported by road to the notifier's warehouse for storage and then distributed to reformulation sites. End-use products containing the notified chemical (at concentrations ≤ 3%) will be in packaging suitable for retail sale.

USE
The notified chemical will be used as a fragrance component in cosmetic and household products. The concentration of the notified chemical in final consumer products will vary but the proposed maximum usage concentrations will not exceed 3% in cosmetic products with typical concentrations in fine fragrances at ≤ 1%, body lotions at ≤ 1.87%, face and hand creams at ≤ 0.98%, and antiperspirants and deodorants at ≤ 0.15%.

OPERATION DESCRIPTION

Reformulation
The reformulation procedure will likely vary depending on the nature of the formulated products, and may involve both automated and manual transfer steps. However, in general, it is expected that the reformulation processes will involve blending operations that will be highly automated and use closed systems with adequate ventilation, followed by automated filling (using sealed delivery systems) of the reformulated products into containers of various sizes.

End-use

Household products
Household products containing the notified chemical (at ≤ 3% concentration) may be used by consumers and professional workers (such as cleaners). The products may be used in either closed systems or open manual processes including rolling, brushing, spraying and dipping, using a cloth, sponge, mop or brush followed by wiping. In some cases the household product will be diluted with water prior to application.

Cosmetic products
The finished cosmetic products containing the notified chemical at concentrations of ≤ 1.87% in body lotion, ≤ 0.98% in face cream lotion, ≤ 0.98% in hand cream, ≤ 1% in fine fragrances, ≤ 0.15% in deodorants (including non-spray deodorants), and ≤ 3% in other cosmetic and personal care products will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the product, application of products could be by hand, sprayed or through the use of an applicator.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	Unknown	Unknown
Mixing	4	250
Drum handling	1	250

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Drum cleaning	2	200
Equipment Maintenance	2	250
Quality Control	1	250

EXPOSURE DETAILS

Transport and storage
Transport and storage workers may come into contact with the notified chemical as a component of fragrance preparations or as a component of end-use products at a maximum concentration of 15% only in the event of accidental rupture of containers. The notifier states that such exposures will be minimised through the use of personal protective equipment (PPE) including protective coveralls, chemical resistant gloves and safety glasses.

Formulation of end products
During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical at ≤ 15% concentration may occur during weighing and transfer stages, blending, quality control analysis, packaging of materials, and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of PPE such as coveralls, goggles and impervious gloves, and adequate local exhaust ventilation or self-contained breathing apparatus as required.

Beauty care and cleaning professionals
Exposure to the notified chemical in end-use products at ≤ 3% concentration in cosmetic products may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. hair dressers, workers in beauty salons) or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. Such professionals may use PPE to minimise repeated exposure, but such use is not always expected. However, good hygiene practices are expected to be in place. If appropriate PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using the finished products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical 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 is also possible, particularly if the products are applied by spray (e.g. air fresheners).

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 notified chemical may be used are shown in the following tables. For the purposes of the exposure assessment via the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, a dermal absorption (DA) of 100% was assumed for the notified chemical (ECHA, 2017). 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%, which accounts for a number of other exposure considerations (e.g., the amount ending up on the hair, as intended). A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

Cosmetic products (dermal exposure)

Product type	**Amount** (mg/day)	**C** (%)	**RF** (unitless)	**Daily systemic exposure** (mg/kg bw/day)
Body lotion	7820	1.87	1	2.2849
Face cream	1540	0.98	1	0.2358
Hand cream	2160	0.98	1	0.3308
Fragrances	750	1	1	0.1172
Deodorant (non-spray)	1500	0.15	1	0.0352
Shampoo	10460	3	0.01	0.0490
Hair conditioner	3920	3	0.01	0.0184
Shower gel	18670	3	0.01	0.0875
Hand wash soap	20000	3	0.01	0.0938
Hair styling products	4000	3	0.1	0.1875
Facial cleanser	800	3	0.01	0.0375
Total				**3.4776**

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

Hair spray (inhalation exposure)

Product type	Amount	C	Inhalation Rate	Exposure Duration (Zone 1)	Exposure Duration (Zone 2)	Fraction Inhaled	Volume (Zone 1)	Volume (Zone 2)	Daily systemic exposure
	(g/day)	(%)	(m^3/day)	(min)	(min)	(%)	(m^3)	(m^3)	(mg/kg bw/day)
Hairspray	9.89	3	20	1	20	50	1	10	**0.0966**

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

Household products (Indirect dermal exposure – from wearing clothes)

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	3	0.95	10	0.1024
Fabric softener	90	3	0.95	10	0.0401
Total					**0.1425**

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

Household products (Direct dermal exposure – from wearing clothes)

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	0.0009
Dishwashing liquid	3	3	1980	0.009	0.01	0.03	0.0075
All-purpose cleaner	1	3	1980	1	0.01	0.007	0.0650
Total							**0.0734**

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

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical. This would result in a combined internal dose of 3.7901 mg/kg bw/day. 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 conservative hair spray inhalation exposure assessment parameters, (in particular assuming an airspace volume of 1 m^3 in zone 1), and the aggregate exposure form the use of the dermally applied products (which assumes a conservative 100% absorption rate), is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray cosmetic and household products with lower 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 dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute inhalation toxicity – rat	LC50 > 5.11 mg/L/4 hour; low toxicity
Skin corrosion – *in vitro* EpiDerm™ Reconstructed Human Epidermis Model	non-corrosive
Skin irritation – *in vitro* EpiSkin™ Reconstituted Human Epidermis Model	non-irritating
Skin irritation – rabbit	irritating
Eye irritation – *in vitro* Bovine Corneal Opacity and Permeability Assay	not corrosive or a severe irritant

Endpoint	*Result and Assessment Conclusion*
Eye irritation – *in vitro* EpiOcular™ Human Cornea Model Test	non-irritating
Eye irritation – rabbit	slightly irritating
Skin sensitisation – mouse local lymph node assay	evidence of sensitisation (EC3 = 31%)
Skin sensitisation – HRIPT (5% concentration)	no evidence of sensitisation
Skin sensitisation – HRIPT (10% concentration)	no evidence of sensitisation
Repeat dose oral toxicity – rat, 28 days	NOAEL* – 970.1/942.7 mg/kg bw/day (female/male)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test in human lymphocytes	non genotoxic

** established by the study authors*

Toxicokinetics, Metabolism and Distribution
No toxicokinetic data on the notified chemical were submitted.

For dermal and gastrointestinal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption. Dermal uptake is likely to be low to moderate if the water solubility is between 1-100 mg/L. Dermal uptake through the epidermis may be limited if the partition coefficient (log Pow) values are greater than 4, but uptake into the stratum corneum is expected to be high (ECHA, 2017). Gastrointestinal absorption is also likely to be high if the partition coefficient (log Pow) values are greater than 4. Therefore, absorption of the notified chemical through the skin and gastrointestinal tract is likely to occur based on the moderately low molecular weight (210.36 g/mol). However, dermal uptake through the epidermis may be limited based on the partition coefficient (log Pow > 5) and low water solubility (0.312 mg/L).

Acute Toxicity
The notified chemical is expected to have low acute oral, dermal, and inhalation toxicity based on studies conducted in rats. However, clinical effects including bodyweight loss and respiratory irritation effects were observed in rats in the acute inhalation toxicity study.

Irritation
Two *in vitro* studies were performed using reconstructed human epidermis models. Under the conditions of the EpiDerm™ Model assay, the notified chemical was not considered to be corrosive to the skin. Under the conditions of the EpiSkin™ Model assay, the notified chemical was not considered as a skin irritant.

However, the notified chemical is irritating to the skin based on a study conducted in rabbits. Well defined erythema was observed in both test animals and persisted to the end of the observation period (14 days) at which crust formation in one animal at the exposure site prevented accurate evaluation. Recovery from these effects was not indicated at the end of the observation period.

In vitro tests using the Bovine Corneal Opacity and Permeability Assay and EpiOcular™ Human Cornea Model Test indicated that the notified chemical is not irritating to the eyes, and is not expected to cause serious damage to the eye.

The notified chemical is slightly irritating to the eyes based on a study conducted in rabbits. Slight conjunctival redness and chemosis, and slight to moderate conjunctival discharge was observed in both animals following exposure, but both animals had recovered from all effects within 3 days. No iridial inflammation or corneal opacity were recorded in any of the animals. The irritation effects observed were not sufficient to classify the notified chemical according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

Sensitisation
The notified chemical was positive in a local lymph node assay (LLNA) with a concentration of approximately 31% corresponding to a Stimulation Index of 3 (EC3 = 31%). The notified chemical is expected to be a weak sensitiser.

The notified chemical did not induce skin sensitisation effects in two human repeat insult patch tests (HRIPT) conducted at 5% and 10% concentrations.

Repeated Dose Toxicity
In a 28-day repeated dose oral toxicity study with a 14-day recovery period in rats, the notified chemical was administered daily in the diet at dose levels of 1,500, 4,500 and 12,000 ppm. A reduction in body weight gain was observed in males in all dose groups and females in the mid and high dose groups. No clinical signs of toxicity were observed. No treatment related changes were noted in behavioural parameters, functional performance or sensory reactivity. The changes observed in haematological, blood chemistry, or urinalytical parameters were considered as not toxicologically significant by the study authors as they were either within the historical control range, lacked a clear dose-dependent relationship, were not present in both sexes or had no histopathological findings.

The study authors considered that the observed reduction in body weight gain and food consumption in the high-dose and high-dose recovery groups to be a response to the eating behaviour towards the dietary formula containing the test substance. However, body weight reductions were also observed in rats in the acute inhalation toxicity study.

The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 12,000 ppm (equivalent to 970.1 mg/kg bw/day for females and 942.7 mg/kg bw/day for males) in this study. However, statistically significant higher levels of urea (19% higher than animals in the control group) were observed in high-dose females and statistically significant lower levels of triglycerides were observed in high-dose recovery females. Females in the high-dose and high-dose recovery groups exhibited statistically significantly higher absolute and relative kidney weights compared to control animals (absolute kidney weights were 8% higher in both the high-dose and high-dose recovery groups, and relative kidney weights were 10% higher in both the high-dose and high-dose recovery groups). No supporting histopathological effects were recorded. Males in the low-dose group also exhibited statistically significantly higher absolute and relative kidney weights compared to control animals. The study authors did not consider the observations on the kidneys to be due to exposure to the test substance. When compared to control animals, statistically significantly higher relative and absolute mean ovary weights were observed in females in the high-dose recovery group. There were only 5 animals per sex tested and some effects reported in high-dose animals cannot be ruled out as not treatment related.

Mutagenicity/Genotoxicity
The notified chemical was non-mutagenic in a bacterial reverse mutation assay. The notified chemical was cytotoxic to cultured human lymphocytes, but was non-genotoxic in an *in vitro* mammalian chromosome aberration test in cultured peripheral 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***
Irritation, Skin (Category 2)	H315 – Causes skin irritation
Sensitisation, skin (Category 1B)	H317 – May cause an allergic skin reaction

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Transport, Storage and Reformulation
Transport and storage workers may experience dermal and accidental ocular exposure to the notified chemical (at ≤ 15% concentration) in the event of a discharge via spill or drum leakage. Exposure of reformulation workers to the notified chemical (at ≤ 15% concentration) may occur during blending operations.

The notified chemical is considered to be a weak skin sensitiser, and has the potential to cause skin irritation and slight eye and respiratory irritation. The use of PPE (e.g. impervious gloves, goggles, coveralls, and respiratory protection, if necessary) should minimise the potential for exposure during reformulation.

Under the conditions of the occupational settings described, provided adequate control measures and safe work practices are in place to minimise worker exposure, including the use of PPE, the risk to workers from 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. Therefore the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experience by members of the public who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health

Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic and household products (containing the notified chemical at ≤ 3% concentration in individual products). The main route of exposure is expected to be dermal and inhalation, with some potential for accidental ocular or oral exposure.

The notified chemical is irritating to the skin and slightly irritating to the eyes and respiratory tract. However, given the low proposed use concentrations in cosmetic and household products irritation effects are not expected from the end use.

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 resulting an internal dose of 3.7901 mg/kg bw/day (see Section 6.1.2) and the NOAEL of 942.7 mg/kg bw/day, which was established in a 28-day repeated dose toxicity study on the notified chemical. Therefore a MoE of 249 was calculated. Considering some effects seen in the kidneys of female rats exposed to the highest dose, the NOAEL could be lower than the highest dose tested. However, even using a NOAEL of 358.1 mg/kg bw/day (the lowest dose administered to animals in the mid-dose group), the MoE is close to 100 while still assuming a worst case exposure scenario from use of multiple products and 100% dermal absorption of the chemical used in cosmetic, personal care, and cleaning products.

Skin sensitisation
Methods for the quantitative risk assessment for dermal sensitisation have been proposed and been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). Using fine fragrance as an example product that may contain the notified chemical at 1% concentration, as a worst case scenario, the Consumer Exposure Level (CEL) for the notified chemical is estimated to be 37.5 μg/cm^2/day (Cadby et al., 2002).

When the notified chemical was tested at 5% and 10% concentration in two separate human repeat insult patch test (HRIPT) studies at the level of 0.15 mL applied to 3.63 cm^2 patch, the notified chemical was determined by the study authors to not cause allergic skin reactions at the levels tested. Given the HRIPT studies were each conducted on more than 100 subjects, the HRIPT study examining the skin sensitisation potential of the notified chemical at 10% concentration was chosen for the purposes of quantitative risk assessment.

Consideration of the study details and application of appropriate safety factors (total of 100), the Acceptable Exposure Level (AEL) was above the CEL for fine fragrances. The availability of additional information including the LLNA study and the lower concentration HRIPT study was also taken into account when determining the safety factors.

As the AEL > CEL, the risk to the public of the induction of sensitisation that is associated with the use of fine fragrances (a worst case example of a leave-on cosmetic product) at ≤ 1% concentration is not considered to be unreasonable. Based on the lower estimated CEL for other cosmetic and household products containing the notified chemical, by inference, the risk of induction of skin sensitisation associated with the use of these products is also not considered to be unreasonable. However, it is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on the aggregate exposure has not been conducted.

When used as a fragrance ingredient at a maximum concentration of 1.87% in body lotion, 0.98% in face cream, 0.98% in hand cream, 1% in fine fragrances, 0.15% in deodorants (including non-spray deodorants), or 3% in other household, cosmetic or personal care products, the notified chemical is 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 notified chemical is not manufactured in Australia. Release of the notified chemical at sites is expected to be < 1% and will be from the transport, storage and product reformulation of the notified chemical. Accidental spills and equipment washings are to be collected and disposed of either to an on-site wastewater treatment plant or a licenced waste disposal contractor.

RELEASE OF CHEMICAL FROM USE
Most of the notified chemical will be washed down the sewer from its use as a fragrance in cosmetic, personal care and household cleaning products.

RELEASE OF CHEMICAL FROM DISPOSAL
Residues of the notified chemical may remain in the end use product containers which will be collected for recycling. Wash water from the recycling process containing the notified chemical is expected to be released to surface waterways. Some of the notified polymer is also expected to be disposed of to landfill through the disposal of empty containers.

7.1.2. Environmental Fate
Following its use as a fragrance in cosmetic and cleaning agents, the notified chemical is expected to be primarily released into the sewer system and treated at the sewage treatment plants (STP) before release to surface waters nationwide.

Ready biodegradability studies were conducted on the notified chemical which determined that the notified chemical is not biodegradable (< 1% in 28 days). A bioaccumulation study was also conducted which determined that the notified chemical is not bioaccumulative (540-570 L/kg wet-wt.). For details on the environmental fate studies see Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)
The Predicted Environmental Concentration (PEC) has been calculated based on 100% release rate into the sewer system over 365 days per year. Based on the physical and chemical properties of the notified chemical 94% of its introduction volume is expected to be removed during the sewage treatment process (Struijs et al. 1991). The resulting PEC in receiving waters is displayed in the table below.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	1,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	94%	Mitigation
Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.03	µg/L
PEC - Ocean:	0.003	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 3.988 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 1,500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.027 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 0.135 mg/kg and 0.27 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	LC50 (96hr) = 0.491 mg/L	Toxic to fish
Daphnia Toxicity	EC50 (48hr) = 0.014 mg/L	Very toxic to aquatic invertebrates
Algal Toxicity	EC50 (72hr) = 0.1 mg/L	Very toxic to algae
Inhibition of Bacterial Respiration	EC50 (3hr) > 1,000 mg/L	Not harmful to bacterial respiration
Earthworm Toxicity	LC50 (14d) = 63.0 mg/kg	Toxic to earthworms

Based on the above ecotoxicological endpoints for the notified chemical, it is expected to be very 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 Hazardous to the aquatic environment, Long-term hazard (Category 1).

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) of the notified chemical was calculated using the most sensitive ecotoxicity endpoint provided (48 h daphnia EC50 = 0.014 mg/L). An assessment factor of 100 was used as three aquatic endpoints were available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	0.014	mg/L
Assessment Factor	100.00	
Mitigation Factor	1.00	
PNEC:	0.14	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	***PEC µg/L***	***PNEC µg/L***	***Q***
Q - River:	0.03	0.14	0.241
Q - Ocean:	0.003	0.14	0.024

The risk quotient (Q = PEC/PNEC) has been calculated based on the assumption of release of 100% of the notified chemical into the sewers. Since the Q value determined was less than 1 for both river and ocean compartments the notified chemical is unlikely to reach ecotoxicologically significant concentrations based on the proposed annual importation and use patterns.

A worst case scenario for release to soil from the STP sludge indicates that the notified chemical will reach concentrations of 0.027 mg/kg, over 1 year. Even under the most conservative assessment factor of 1,000, the worst case 1 year soil concentration, is below the PNEC of 0.063 mg/kg derived from the earthworm toxicity LC50 (14 d). Although the 10 year PEC is slightly above the PNEC, it does not take account of degradation or dissipation of the notified chemical from soil and hence is an overestimate.

Therefore, on the basis of the predicted PEC/PNEC ratio the notified polymer is not expected to pose an unreasonable risk to the aquatic or terrestrial environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -20 °C

Method	OECD TG 102 Melting Point/Melting Range EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	None
Test Facility	Envigo (2017a)

Boiling Point 171 °C at 5.33 kPa

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Siwoloboff method; boiling range 166 – 171 °C at 5.33 kPa (40 mm Hg)
Test Facility	JRF (2017a)

Density 907.8 ± 0.6 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids US EPA OCSPP 830.7300 "Density/Relative Density/Bulk Density
Remarks	Pycnometer method
Test Facility	JRF (2017b)

Vapour Pressure 0.0013 kPa at 25 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Vapour pressure balance method
Test Facility	Envigo (2018a)

Water Solubility 0. 312 mg/L at 20 °C

Method	OECD TG 105 Water Solubility EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks	Flask Method
Test Facility	Envigo (2017a)

Partition Coefficient (n-octanol/water) log Pow = 5.67 at 25 °C

Method	OECD TG 123 Partition Coefficient (1-Octanol/Water): Slow-Stirring Method
Remarks	Slow-stirring method
Test Facility	Envigo (2018b)

Partition Coefficient (n-octanol/water) log Pow > 6.5 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks	HPLC Method
Test Facility	Envigo (2017a)

Adsorption/Desorption log K_{oc} = 4.19

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks	Screening test HPLC method
Test Facility	Envigo (2018c)

Flash Point 160 °C at 101.3 kPa

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

Autoignition Temperature 190 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks	Test item (100 µl) was placed in a pre-heated round flask (base was wrapped in aluminium foil) and placed in an electrically heated furnace.
Test Facility	JRF (2017d)

Explosive Properties Non-explosive

Method	US EPA OCSPP 830.6316 Explodability
Remarks	Differential scanning calorimeter. Test substance heated from 30 °C to 430 °C at 10 °C/minute in the presence of nitrogen. Three replications were performed. One endothermic peak was observed. No exothermic decomposition peak was observed at up to 430 °C.
Test Facility	JRF (2017e)

Oxidizing Properties Non-oxidising

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks	Mean pressure rise time for the test substance (notified chemical and cellulose) was 16.213 seconds and for the reference sample (cellulose and 65% nitric acid) was 1.082 seconds. The mean pressure rise time was significantly higher for the test substance.
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 EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	Arachis oil BP
Remarks – Method	GLP compliant No significant deviations from the protocol

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity	None
Effects in Organs	None
Remarks – Results	All animals made the expected body weight gains. However, the animal in group 2 gained no weight in the first week following exposure, but showed the expected body weight gain in the second week.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Envigo (2017b)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal)
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	None
Type of dressing	Semi-occlusive
Remarks – Method	GLP compliant No deviations from the protocol

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 F, 5 M	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	None
Signs of Toxicity – Systemic	None
Effects in Organs	No abnormalities observed
Remarks – Results	All animals made the expected body weight gains.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Envigo (2017c)

B.3. Acute Inhalation Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation)
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	None
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Remarks – Method	GLP compliant No significant deviations from the protocol Particle Size Distribution: Mean Mass Median Aerodynamic Diameter: 2.65 µm Inhalable Fraction: 69.2% of particles < 4 µm Geometric standard deviation: 2.28

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 F, 5 M	19.04	5.11	0/10

LC50	> 5.11 mg/L/4 hours
Signs of Toxicity	All animals exhibited wet fur and a decreased respiratory rate during the exposure period. Following removal from the test chamber and at the one-hour post-exposure observation, the symptoms persisted with the additions of hunched posture and pilo-erection. Sneezing was observed in two animals (females) following removal from the exposure chamber. Hunched posture and pilo-erection was observed in all animals one day after exposure, with two animals also exhibiting sneezing (1 male, 1 female) and noisy respiration (male only). Recovery was observed in four animals (3 males and 1 female) on day 2 with remaining animals continuing to exhibit hunched posture. Sneezing and noisy respiration persisted in those animals affected on day 1. Recovery was indicated in all animals except one on day 3. One male that had shown recovery at day 2 exhibited noisy respiration until day 6.
Effects in Organs	No abnormalities were detected in organs including the upper respiratory tract.
Remarks – Results	All males and 1 female made the expected body weight gains. All animals exhibited either a loss in body weight (all males and 3 females) or no change in body weight (2 females) on the day of exposure. One female exhibited body weight loss from days 1 to 3 post-exposure and 3 females exhibited body weight loss from days 3 to 7 post exposure. All animals gained weight over the course of the study period.
CONCLUSION	The notified chemical is of low acute toxicity via inhalation.
TEST FACILITY	Envigo (2018d)

B.4. Skin Irritation – *In Vitro* Reconstructed Human Epidermis Model (EpiDerm™)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 431 *In vitro* Skin Corrosion – Human Skin Model Test

EpiDerm™ Reconstructed Human Epidermis Model

Vehicle

Remarks – Method GLP compliant
No significant deviations from the protocol

Positive control: 8.0 N Potassium hydroxide
Negative control: Deionised water

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*		*Relative Mean Viability (%)*		*Coefficient of Variation (%)*	
	3 minutes	*1 hour*	*3 minutes*	*1 hour*	*3 minutes*	*1 hour*
Negative control	1.502	1.469	100	100	0.2	3.5
Test substance	1.448	1.512	96.4	103.0	3.1	4.6
Positive control	0.339	0.047	22.6	3.2	3.3	8.1

OD = optical density

Remarks – Results Positive and negative controls performed as expected.
All acceptance criteria were met.

CONCLUSION The notified chemical was considered non-corrosive to the skin under the conditions of the test.

TEST FACILITY Envigo (2017e)

B.5. Skin Irritation – *In Vitro* Reconstituted Human Epidermis Model (EpiSkin™)

TEST SUBSTANCE Notified chemical

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

Vehicle None

Remarks – Method GLP compliant
No significant deviations from the protocol

Positive control: 5% Sodium lauryl sulphate
Negative control: Phosphate buffered saline

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	1.168	84.3	15.2
Positive control	0.017	1.2	28.7

OD = optical density; SD = standard deviation

Remarks – Results Positive and negative controls performed as expected.

The relative standard deviation for tissue replicates exposed to the positive control was higher (28.7%) than the acceptance value of 18%.

The acceptance criteria for standard deviation between tissue replicates exposed to the test substance and the negative control were met for simple and relative standard deviation calculations.

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

Based on the mean tissue viability of > 50%, the notified chemical is not classified as a skin irritant according to the GHS criteria.

TEST FACILITY Envigo (2017d)

B.6. Skin Irritation – Rabbit

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion
EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation)

Species/Strain	Rabbit/New Zealand White (Hsdlf:NZW)
Number of Animals	Two (female)
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks – Method	GLP compliant No deviations from the protocol

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Erythema/Eschar	2	2	2	> 14 days	2
Oedema	1.3	1.3	2	> 14 days	1

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

Remarks – Results

Very slight erythema and oedema was observed in both animals 1 hour after exposure. Well defined erythema beyond the exposure site (25 mm) was observed in both animals at the 24 hour observation and persisted to the end of the observation period (14 days) in one animal (10 mm beyond the exposure site), while in the second animal, crust formation at the exposure site prevented accurate evaluation of erythema. Both animals exhibited a loss of skin elasticity and flexibility up to the 7 day observation, with one animal exhibiting haemorrhage of the dermal capillaries at the 72 hour observation. At the end of the 14 day observation period, one animal exhibited glossy skin, while the second animal exhibited crust formation.

Very slight oedema persisted in in both animals over the 14 day observation period, although its presence could not be confirmed at the end of the observation period in one animal based on adverse reactions at the exposure site. Both animals exhibited slight oedema over the course of the study period at different observation points (one animal at 24 hours, and the second animal at 72 hours). However, the oedema decreased in severity at the next observation period (48 and 7 days respectively).

Recovery from erythema and oedema was not indicated at the end of the 14-day observation period.

CONCLUSION The notified chemical is irritating to the skin.

TEST FACILITY Envigo (2018e)

B.7. Eye Irritation – *In Vitro* Bovine Corneal Opacity and Permeability Test

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
Vehicle	None
Remarks – Method	GLP compliant No deviations from the study plan Positive control: 2-Ethoxyethanol (99%) Negative control: 0.9% (w/v) Sodium chloride

RESULTS

Test Material	*Mean Opacities of Triplicate Tissues (SD)*	*Mean Permeabilities of Triplicate Tissues (SD)*	*IVIS (SD)*
Vehicle control	0 (± 0.00)	0.050 (± 0.001)	0.75 (± 0.03)
*Test substance**	0.67 (± 1.53)	0.03 (± 0.02)	1.09 (± 1.60)
*Positive control**	62.67 (± 5.86)	0.89 (± 0.21)	76.00 (± 4.09)

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

Remarks – Results	All acceptance criteria were met. Positive and negative controls performed as expected. The IVIS for the test substance was ≤ 3 indicating it is not a Category 1 eye irritant according to the test guideline.
CONCLUSION	The notified chemical was not considered corrosive or a severe eye irritant under the conditions of the test.
TEST FACILITY	Envigo (2017f)

B.8. Eye Irritation – *In Vitro* Reconstructed Human Corneal Epithelium Model

TEST SUBSTANCE	Notified chemical
METHOD	Determination of Ocular Irritation Potential Using the EpiOcular™ Human Cornea Model Test
Vehicle	
Remarks – Method	GLP compliant The test substance (50 µL) was applied to the tissues in duplicate. Following a 30 minute exposure period at ~37 °C, the tissues were rinsed and incubated at ~37 °C in fresh medium for 2 hours. The tissues were then treated with MTT and incubated at ~37 °C for 3 hours. Following extraction, the optical densities were determined at 570 nm. The study authors indicated that a preliminary test had been conducted, which indicated that the test substance does not directly reduce MTT. The test substance was considered by the study authors to be an irritant if the relative mean tissue viability was ≤ 60%. Positive and negative controls were run in parallel with the test substance: Negative control: deionised water Positive control: methyl acetate

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*	*Relative Mean Viability (%)*
Negative Control	1.846	100.0
Test Substance	1.706	92.4
Positive Control	0.379	20.6

OD = optical density

Remarks – Results
All acceptance criteria were met.
Positive and negative controls performed as expected.

CONCLUSION
The notified chemical was considered non-irritating to the eye under the conditions of the test.

TEST FACILITY
Envigo (2017g)

B.9. Eye Irritation – Rabbit

TEST SUBSTANCE
Notified chemical

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

Species/Strain
Rabbit/New Zealand White (Hsdlf:NZW)

Number of Animals
Two

Observation Period
72 hours

Remarks – Method
GLP compliant
No deviations from the study plan

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva – Redness	0.6	0.6	1	48 hours	0
Conjunctiva – Chemosis	0.3	0.3	1	24 hours	0
Conjunctiva – Discharge	0	0.3	1	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
Both animals exhibited slight conjunctival redness and chemosis 1 hour following exposure. Both animals had fully recovered from these effects at the 72 hour observation. Slight (animal 1) to moderate (animal 2) conjunctival discharge was observed in both animals 1 hour following exposure with full recovery at 24 hour for animal 1 and 48 hour for animal 2.

No reactions were observed in the cornea or iris of the two animals.

Both animals lost body weight slightly over the three day observation period.

CONCLUSION
The notified chemical is slightly irritating to the eye.

TEST FACILITY
Envigo (2017h)

B.10. Skin Sensitisation – LLNA

TEST SUBSTANCE
Notified chemical

METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay Method B.42 Skin Sensitisation (Local Lymph Node Assay) of Commission Regulation (EC) No. 440/2008
Species/Strain	Mouse/CBA/Ca (CBA/CaOlaHsd)
Vehicle	Acetone/Olive Oil (4:1)
Preliminary study	Yes
Positive control	α-hexylcinnamaldehyde
Remarks – Method	GLP compliant No significant deviations from the protocol In a preliminary study, > 25% increase in mean ear thickness was recorded in an animal exposed to the undiluted test substance but not the animal exposed to the test substance at 50% concentration. Concentrations of 10%, 25% and 50% were selected for the main study. However, due to technician error, the actual concentrations tested in the main study were 25%, 50% and 100%.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (DPM/animal)*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5 F	1,239.01 ± 435.05	-
25	5 F	2,993.92 ± 911.05	2.42
50	5 F	5,878.54 ± 3,059.89	4.74
100	5 F	6,114.30 ± 1,435.47	4.93
Positive Control			
25 (HCA)	5 F	9,196.57 ± 4,239.28	7.42

HCA = α-hexylcinnamaldehyde

EC3	31%
Remarks – Results	No signs of systemic toxicity or local skin irritation were observed in animals exposed to the test substance in the preliminary or main studies. Positive and vehicle controls performed as expected.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Envigo (2017i)

B.11. Skin Sensitisation – Human Volunteers (5% concentration)

TEST SUBSTANCE	Notified chemical at 5% w/w concentration
METHOD	Repeated insult patch test with challenge
Study Design	Induction procedure: patches infused with 0.15 mL test substance were applied 3 times per week on Mondays, Wednesdays and Fridays for a total of 9 applications. Patches were removed after 24 h and sites were graded after an additional 24 h (or 48 h for patches applied on Friday). Rest period: 10 - 21 days Challenge procedure: identical patches were applied to a naïve site next to the original induction patch site. Patches remained in place for 24 h. Sites were graded at 0, 24 and 48 h post-patch removal.
Study Group	90 F, 29 M; age range 18 - 70 years
Vehicle	Alcohol SD40B and Diethyl Phthalate
Remarks – Method	Occluded

	The test substance was spread on a 3.63 cm × 3.63 cm patch. Negative control containing 5% w/w distilled water was run concurrently. Three test subjects who were absent for grading during the challenge phase were graded at 96 h after patch removal.
RESULTS	
Remarks – Results	109/119 subjects completed the study. Nine subjects discontinued the experiment after 0 (one subject), 1 (five subjects), 2 (one subject), or 3 (two subjects) inductions for reasons unrelated to the test substance. One subject discontinued after 1 induction due to moderate diffuse erythema and oedema where the tape was placed both for the test substance and negative control. The study authors determined that the adverse reaction was due to a tape allergy and not a response to the test material. No other adverse responses were noted during induction or challenge. No adverse reactions were observed at the negative control patch sites.
CONCLUSION	The test substance at 5% concentration was non-sensitising under the conditions of the test.
TEST FACILITY	CRL (2018)

B.12. Skin Sensitisation – Human Volunteers (10% concentration)

TEST SUBSTANCE	Notified chemical at 10% concentration
METHOD	Repeated insult patch test with challenge
Study Design	Induction procedure: patches infused with 0.15 mL test substance were applied 3 times per week on Mondays, Wednesdays and Fridays for a total of 9 applications. Patches were removed after 24 h and sites were graded after an additional 24 h (or 48 h for patches applied on Friday). Rest period: 10 - 21 days Challenge procedure: identical patches were applied to a naïve site next to the original induction patch site. Patches remained in place for 24 h. Sites were graded at 0, 24 and 48 h post-patch removal.
Study Group	88 F, 32 M; age range 18 - 70 years
Vehicle	Alcohol SD40B and Diethyl Phthalate
Remarks – Method	Occluded The test substance was spread on a 3.63 cm × 3.63 cm patch. Negative control containing 6% w/w distilled water was run concurrently. Two test subjects who were absent for grading during the challenge phase were graded at 96 h after patch removal.
RESULTS	
Remarks – Results	107/120 subjects completed the study. Thirteen subjects discontinued the experiment for reasons unrelated to the test substance after 0 (two subjects), 1 (four subjects), 2 (two subjects), 5 (two subjects), or 6 (two subjects) inductions and one subject discontinued the experiment at the end of the induction phase. No adverse responses were noted during induction or challenge. No adverse reactions were observed at the negative control patch sites.

CONCLUSION	The test substance at 10% concentration was non-sensitising under the conditions of the test.
TEST FACILITY	Eurofins CRL (2019)

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 (Recovery Groups): 14 days
Vehicle	
Remarks – Method	GLP compliant No deviations from the study plan

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*		*Mortality*
		Nominal (ppm)	*Actual (mg/kg bw/day)*	
Control	5 M	0	0	0/5 M
	5 F			0/5 F
Low Dose	5 M	1,500	M: 121.9	0/5 M
	5 F		F: 120.9	0/5 F
Mid Dose	5 M	4,500	M: 358.1	0/5 M
	5 F		F: 363.6	0/5 F
High Dose	5 M	12,000	M: 942.7	0/5 M
	5 F		F: 970.1	0/5 F
Control Recovery	5 M	0	0	1/5 M
	5 F			0/5 F
High Dose Recovery	5 M	12,000	M: 942.7	0/5 M
	5 F		F: 970.1	0/5 F

Mortality and Time to Death

There were no unscheduled deaths of animals exposed to the test substance. One male in the control recovery group died prior to necropsy on Day 43.

Clinical Observations

No clinical signs of toxicity were observed. No treatment related changes were observed in behavioural parameters, functional performance or sensory reactivity.

When compared to control animals, males in all dose groups exhibited a reduction in body weight gain over the study period reaching statistical significance at some stages. Females in the high-dose and high-dose recovery groups also exhibited non-statistically significant reduction in body weight gain during weeks 1, 2 and 4. At the end of the exposure period, full recovery in body weight gain occurred in female animals in the high-dose recovery group, while male animals in the high-dose recovery group exhibited a reduction in body weight gain during the first week of recovery followed by signs of recovery in the second week. Females in the low- and mid-dose groups exhibited no significant effects on body weight gain.

Males in the high-dose and high-dose recovery groups exhibited a reduction in food consumption in weeks 1 and 2 when compared to control animals, and recovered over the remainder of the study period. No effects on food consumption were observed in females (all groups) or males in the low- and mid-dose groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No toxicologically significant effects were detected in haematological parameters. When compared to animals in the control groups, statistically significantly higher reticulocyte counts (females and males, all exposure groups), lymphocyte counts (high-dose recovery males), erythrocyte count (high-dose females) and activated

partial thromboplastin time (high-dose recovery females) and statistically significantly lower neutrophil counts (mid- and high-dose group males), eosinophil counts (high-dose group males) and mean corpuscular haemoglobin concentration (low-, mid- and high-dose females and males in the high-dose recovery group) were observed. Dose dependant decreases (not statistically significant) were observed in white blood cell counts (females and males) and in neutrophils and lymphocyte counts (females) of exposed animals when compared to controls. Higher (not statistically significant) lymphocyte counts (low-dose males), eosinophil counts (observed in all exposed females in a non-dose dependant manner) and activated partial thromboplastin time (females in low-and mid-dose groups) and lower lymphocyte counts (high-dose males) were observed when compared to animals in control groups.

The study authors did not consider these effects to be toxicologically significant due to the following reasons:

- the individual values were within the historical control range with the exception of one mean corpuscular haemoglobin concentration (male) and one lymphocyte value (female);
- a dose-related response was not always observed (especially where the observations were statistically significantly different to those observed in control animals);
- the effects were not always seen in both sexes;
- recovery was indicated as the effect was not observed in animals in the high-dose recovery groups; or
- no supporting histopathological effects were observed.

No toxicologically significant effects were detected in blood chemistry parameters. When compared to animals in the control groups, statistically significant increases in inorganic phosphorus and potassium concentration (mid-dose males), creatinine and calcium concentration (high-dose males), alanine aminotransferase and total protein (high-dose recovery males), and a statistically significant decrease in chloride concentration (high-dose and high-dose recovery males) were observed. Creatinine levels in males increased in a dose dependant manner. Dose dependant increases (not statistically significant) in total protein, albumin and calcium levels, and a dose dependant decrease (not statistically significant) in aspartate aminotransferase were also observed in females. Statistically significant higher levels of urea (19% higher than animals in the control group) were observed in high-dose females and statistically significant lower levels of triglycerides were observed in high-dose recovery females. The study authors did not consider these effects to be toxicologically significant.

Effects in Organs

No toxicologically significant macroscopic abnormalities were detected. One female (1/5) in the mid-dose group exhibited increased pelvic space and slight pelvic dilation in the left kidney. An enlarged spleen was observed in one male (1/5) in the high-dose recovery group. The study authors did not consider these effects to be a result of exposure to the notified chemical as they are common to the age and strain of the animals and were only observed once. Similar effects were not seen in high-dose animals in the non-recovery groups.

Males and females in the mid- and high-dose groups exhibited a statistically significant increase in absolute and relative liver weight compared to animals in the control group. Full recovery from this effect was observed in high-dose recovery groups. Minimal to slight centrilobular hypertrophy was observed in animals in the mid-dose group (males) and high-dose group (males and females).

Females in the high-dose and high-dose recovery groups exhibited statistically significantly higher absolute and relative kidney weights compared to control animals (absolute kidney weights were 8% higher in both the high-dose and high-dose recovery groups, and relative kidney weights were 10% higher in both the high-dose and high-dose recovery groups). The majority of individual values were within the historical control range, no dose-related response was observed and there were no supporting histopathological effects. Males in the low-dose group also exhibited statistically significantly higher absolute and relative kidney weights compared to control animals. The study authors did not consider the observations on the kidneys to be due to exposure to the test substance.

When compared to control animals, statistically significantly higher relative and absolute mean ovary weights were observed in females in the high-dose recovery group. However, the study authors did not consider these effects to be toxicologically significant as the majority of individual values were within the historical control range, there were no supporting histopathological effects, and females in the high-dose (non-recovery) group did not exhibit a similar effect.

Minimal to slight accumulation of hyaline droplets was observed in males in the mid- and high-dose groups. The study authors attributed this effect to an excessive accumulation of alpha-2u-globulin. This phenomenon is male rat specific and therefore not relevant to humans.

Remarks – Results

No clinical signs of toxicity were observed. No treatment related changes were noted in behavioural parameters, functional performance or sensory reactivity. No toxicologically significant changes were observed in females in the low- and mid-dose groups, or in males in the low-dose group.

Changes in blood chemistry and haematology were not considered to be toxicologically significant by the study authors based on individual values being within the historical control range, no clear dose-dependent relationships, an absence of effects in both sexes, or a lack of supporting histopathological findings.

The study authors considered that the observed reduction in body weight gain throughout the exposure period and food consumption in the first two weeks of the exposure period in the high-dose and high-dose recovery groups to be a response to the eating behaviour towards the dietary formula containing the test substance. A reduction in body weight gain was also observed in low- and mid-dose males in the final week of the exposure period.

CONCLUSION

The No Observed Effect Level (NOEL) was established by the study authors as as 363.6 mg/kg bw/day (4,500 ppm in diet) for females and 121.9 mg/kg bw/day (1,500 ppm in diet) for males in this study.

The No Observed Adverse Effect Level (NOAEL) was established as 970.1 mg/kg bw/day (12,000 ppm in diet) for females and 942.7 mg/kg bw/day (12,000 ppm in diet) for males in this study.

TEST FACILITY Envigo (2017j)

B.14. Genotoxicity – Bacteria

TEST SUBSTANCE Notified chemical

METHOD

	OECD TG 471 Bacterial Reverse Mutation Test EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria Experiment 1: Plate incorporation procedure Experiment 2: Pre-incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2*uvrA*
Metabolic Activation System	S9 fraction from Phenobarbital/β-naphtha flavone induced rat liver
Concentration Range in Test 1	a) With metabolic activation: 1.5 – 5,000 µg/plate b) Without metabolic activation: 1.5 – 5,000 µg/plate
Concentration Range in Test 2	a) With metabolic activation: 15 – 5,000 µg/plate b) Without metabolic activation: 15 – 5,000 µg/plate
Vehicle	Acetone
Remarks – Method	GLP compliant No deviations from the protocol Positive controls: without metabolic activation – N-ethyl-N'-nitro-N-nitrosoguanidine (WP2uvrA, TA100, TA1535), 9-Aminoacridine (TA1537), 4-nitroquinoline-1-oxide (TA98); with metabolic activation – 2-aminoanthracene (WP2uvrA, TA100, TA1535, TA1537), Benzo(a)pyrene (TA98)

RESULTS

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

Present				
Test 1	> 5,000	> 5,000	≥ 5,000	negative
Test 2		> 5,000	≥ 5,000	negative

Remarks – Results

In Test 1 and Test 2, no reduction in bacterial lawn was observed at any of the doses tested in the presence or absence of metabolic activation. Precipitate was observed at the highest dose tested (5,000 µg/plate) in both tests in the presence or absence of metabolic activation.

No significant dose-related increase in the number of revertants in either the presence or absence of metabolic activation was observed in Test 1 or Test 2.

Positive and negative controls performed as expected.

CONCLUSION

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

TEST FACILITY

Envigo (2017k)

B.15. Genotoxicity – *In Vitro* Chromosome Aberration Test

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 fraction from phenobarbital/β-naphtha flavone induced rat liver

Vehicle: Acetone

Remarks – Method: GLP compliant
No deviations from the protocol

Positive controls:
without metabolic activation – mitomycin C;
with metabolic activation – cyclophosphamide.

A preliminary toxicity test was performed using the same exposure and harvest periods as the main test. A dose range of 8.2 – 2,100 µg/mL was tested.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 7.5, 15*, 22.5*, 30*, 60, 180	4 h	24 h
Test 2	0*, 7.5, 15, 30*, 45*, 60*, 75	24 h	24 h
Present			
Test 1	0*, 15, 30*, 45*, 60*, 90, 120, 135	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	≥ 65.63	≥ 22.5	> 180	none
Test 2	≥ 131.25	≥ 75	> 75	none
Present				
Test 1	≥ 65.63	≥ 45	> 135	none

Remarks – Results	The test item is cytotoxic to cultured human lymphocytes inducing haemolysis at ≥ 22.5 µg/mL in the absence of metabolic activation, and at ≥ 45 µg/mL in the presence of metabolic activation. No statistically significant or biologically relevant increase in the number of cells with chromosome aberrations was observed in the presence or absence of metabolic activation. No increase in the number of polyploid cells and cells with endoreduplicated chromosomes were observed in the presence or absence of metabolic activation under the conditions of tests 1 and 2. Positive and negative controls performed as expected.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Envigo (2017l)

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	Sewage effluent
Exposure Period	28 days
Auxiliary Solvent	Acetone
Analytical Monitoring	Inorganic carbon
Remarks – Method	The following deviation from the test guideline is noted: pH of the stock solution was not determined prior to the addition of the filter paper due to insolubility of the test substance. This deviation is not expected to have affected the reliability of the study.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	0	2	49
8	0	8	66
14	0	14	82
21	0	21	75
28	0	28	72

Remarks – Results	All validity criteria were met. Total Inorganic Carbon (TIC) was < 3 mg C/L in the blank controls and the reference test reached 66% degradation at day 8. A toxicity study was also conducted which determined that the test substance was not inhibitory (42% biodegradation).
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	Envigo (2017m)

C.1.2. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD
Remarks – Method	No deviations reported

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	1.49	1	16.83
10	2.15	10	78.78
14	2.87	14	81.57
20	1.43	20	84.43
28	0.05	28	86.29

Remarks – Results	All validity criteria were met. Oxygen demand in the blanks was 22.5-28.2 mg/L, the differences of extremes was less than 20% and the substance was not considered inhibitory (> 35% biodegradation in the toxicity test).
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	Suzhou (2016a)

C.1.3. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305-I Aqueous Exposure Bioconcentration Fish Test.
Species	*Cyprinus carpio* (common carp)
Exposure Period	Exposure: 28 days Depuration: 6 days
Auxiliary Solvent	N,N-dimethylformamide
Concentration Range	Nominal: 0.4, 4 µg/L Actual: 0.299, 3.03 µg/L
Analytical Monitoring	GC-MS
Remarks – Method	No deviations reported.
RESULTS	
Bioconcentration Factor	470 - 500 L/kg (steady state, lipid normalised)
Remarks – Results	All validity criteria were met. The temperature was maintained at 24.2 – 25.1 ℃, dissolved oxygen was maintained at > 60% saturation, the concentration was maintained at ±20% of the mean measured concentration and there was no mortality or adverse effects in the control group.
CONCLUSION	The test substance is not bioaccumulative.
TEST FACILITY	CERI (2018)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE	Notified chemical
METHOD	The guidelines for the testing of chemicals, effects on biotic systems, Version 2, 203 Fish acute toxicity test, China Environmental Press, 2013 – Semi-static
Species	*Gobiocypris rarus* (rare minnow)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	75 mg $CaCO_3$/L
Analytical Monitoring	GC
Remarks – Method	The method is comparable to the OECD TG 203. However, the test species used is not included in the list of OECD test guideline recommended species. To avoid loss of test substance, wide-mouth bottles with glass stopper sealed with sealing film were used to prepare test solutions.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	7	0	0	0	0	0

0.80	0.236	7	0	0	0	0	0
1.61	0.375	7	0	0	0	0	1
3.22	0.426	7	0	0	1	2	2
6.47	0.490	7	0	0	1	2	3
13.00	0.614	7	0	0	2	4	6

LC50 0.491 mg/L at 96 hours

Remarks – Results All validity criteria were met. The dissolved oxygen content ranged from 68 - 100.4%. All the measured values of the test substance were outside of the 80 - 120% nominal values, therefore the reported LC50 was based on measured concentrations.

CONCLUSION The test substance is toxic to fish.

TEST FACILITY Suzhou (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

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent None
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 0.10, 1, 10 and 100 % v/v saturation.

A positive control test was also conducted using potassium dichromate.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual (geometric mean)*		*24 h*	*48 h*
Control	0	20	0	0
1.0	ND	20	0	0
3.2	< LOQ	20	0	0
10	0.017	20	2	16
32	0.056	20	6	20
100	0.14	20	14	20

LOQ = 0.0091 mg/L

LC50 0.014 mg/L at 48 hours
NOEC 0.0046 mg/L at 48 hours
LOEC 0.017 mg/L at 48 hours

Remarks – Results All of the validity criteria were met. The dissolved oxygen was > 8 mg/L, pH was maintained between 7.8 and 8.0 and temperature was maintained at 22 °C.

Results from the positive control test was within the normal range for potassium dichromate (48 hr EC50 = 1.2 mg/L).

CONCLUSION	The test substance is very toxic to aquatic invertebrates.
TEST FACILITY	Envigo (2018f)

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	Nominal: 10 – 100 mg/L Actual: 0.0013 – 0.19 mg/L
Auxiliary Solvent	None
Analytical Monitoring	GC
Remarks – Method	The study was conducted using sealed test vessels instead of vessels with air permeable stoppers. To account for the lack of air flow, each sample was treated with calcium carbonate. 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 0.10, 1.0, 3.2, 10, 32 and 100 % v/v saturation. A positive control test was also conducted using potassium dichromate.

RESULTS

Growth rate		*Yield*	
ErC50 (mg/L at 72 h)	*NOEC (mg/L)*	*EyC50 (mg/L at 72 h)*	*NOEC (mg/L)*
0.1	0.013	0.047	0.013

Remarks – Results	In all test samples there was a pH increase greater than 1.5 units over the course of the study, as all of the validity criteria were met this is not considered to have adversely impacted the study. The growth factor in the control test was greater than 16, the coefficient of variation for section by section growth was 24% and the variation of average specific growth rate was 3%. Results from the positive control test was within the normal range for potassium dichromate (72 hr EyC50 = 0.6 mg/L).
CONCLUSION	The test substance is very toxic to algal growth.
TEST FACILITY	Envigo (2018g)

C.2.4. 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: 10 – 1,000 mg/L
Remarks – Method	3, 5-dichlorophenol was used as the reference substance in this study.

RESULTS

NOEC	1,000 mg/L
Remarks – Results	All validity criteria were met. The oxygen uptake in the control vessels was 4.26% and the respiration of the controls was 28.55 mg O_2/g of sludge. The reference sample showed an EC50 of 7.5 mg/L for 3, 5-dichlorophenol which is within the expected range.
CONCLUSION	The notified chemical is not harmful to microbial respiration.
TEST FACILITY	Envigo (2017n)

C.2.5. Acute Toxicity to Earthworms

TEST SUBSTANCE	Notified chemical
METHOD	The guidelines for the testing of chemicals, effects on biotic systems, Version 2, 207 Earthworm, acute toxicity test, China Environmental Press, 2013.
Species	*Eisenia foetida*
Exposure Period	14 days
Concentration Range	14.9 – 480.2 mg/kg (dry weight)
Remarks – Method	The method is equivalent to OECD TG 207 Earthworm, Acute Toxicity Tests (artificial soil test).

RESULTS

Concentration (mg/kg dry weight)	*Total number of test earthworms*	*Exposure duration and cumulative mortality*	
		7 d	*14 d*
		Cumulative mortality (%)	*Cumulative mortality (%)*
Control	40	0	0
14.9	40	0	5
30.2	40	10	25
59.9	40	32.5	45
119.8	40	52.5	65
240.1	40	77.5	100
480.2	40	100	100

EC50	63.016 mg/kg (dry weight)
Remarks – Results	No mortalities or abnormalities were observed in the control sample.
CONCLUSION	The test substance is harmful to earthworms.
TEST FACILITY	Suzhou (2016b)

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