File No.: LTD/2144

August 2020

AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

5-Hexen-2-one, 1-bicyclo[2.2.1]hept-2-yl-

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 our website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2144	Firmenich Limited	5-Hexen-2-one, 1-bicyclo[2.2.1]hept-2-yl-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Skin Irritation (Category 2)	H315 – Causes skin irritation
Skin sensitisation (Category 1B)	H317 – May cause an allergic skin reaction
Eye irritation (Category 2A)	H319 – Causes serious eye irritation

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

Hazard Classification	*Hazard Statement*
Chronic (Category 2)	H411 – Toxic to aquatic life with long lasting effects

Human Health Risk Assessment
Under the conditions of the occupational settings described, the assessed chemical is not considered to pose an unreasonable risk to the health of workers.

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The assessed chemical should be classified as follows:
  - Skin Irritation (Category 2): H315 – Causes skin irritation
  - Skin sensitisation (Category 1B): H317 – May cause an allergic skin reaction
  - Eye irritation (Category 2A): H319 – Causes serious eye irritation

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

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 chemical during reformulation:
  - Enclosed/automated processes, where possible
  - Local exhaust ventilation and/or appropriate extraction systems, where 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 assessed chemical during reformulation:
  - Avoid contact with skin and eyes
  - Avoid inhaling 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 chemical during reformulation:
  - Impervious gloves
  - Safety glasses
  - Protective clothing
  - Respiratory protection if inhalation exposure may occur

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

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

- If products and mixtures containing the assessed 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.

Health Surveillance

- As the assessed 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 sensitisation.

Storage

- The handling and storage of the assessed 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 assessed chemical should be handled by adequate ventilation, physical collection and subsequent disposal.

Disposal

- Where reuse or recycling are not appropriate, dispose of the assessed chemical 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 chemical based on changes in certain circumstances. Under Section 101 of the IC Act the applicant of the assessed chemical has post-assessment regulatory obligations to provide information to AICIS when any of these circumstances change. These obligations apply even when the assessed chemical is 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 assessed chemical;
- the final use concentration of the assessed chemical exceeds 0.04% in leave-on/rinse-off cosmetics, 0.39% in fine fragrances, 0.4% in household cleaning products, or 2% in air fresheners;
- the function or use of the chemical has changed from a fragrance ingredient, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia; and
- 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 chemical 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)
Firmenich Limited (ABN: 86 002 964 794)
73 Kenneth Road
BALGOWLAH NSW 2093

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

PROTECTED INFORMATION (SECTION 38 OF THE TRANSITIONAL ACT)
Data items and details taken to be protected information include: specific other name(s), analytical data, degree of purity, impurities and additives/adjuvants.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are varied for flammability, explosive properties and oxidising properties.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT(S)
None

APPLICATION IN OTHER COUNTRIES
EU (2019)

2. IDENTITY OF CHEMICAL

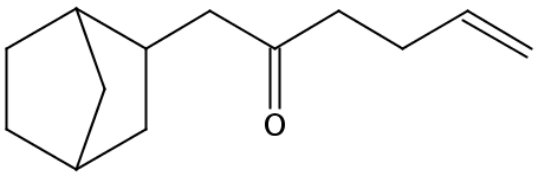
MARKETING NAME(S)
1-(3-bicyclo[2.2.1]heptanyl)hex-5-en-2-one

CAS NUMBER
1352216-91-1

CHEMICAL NAME
5-Hexen-2-one, 1-bicyclo[2.2.1]hept-2-yl-

MOLECULAR FORMULA
$C_{13}H_{20}O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
192.3 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless liquid

Property	*Value*	*Data Source/Justification*
Melting Point/Freezing Point	-111.6 °C	Measured
Boiling Point	268.2 °C at 101.3 kPa	Measured
Density	954 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.3×10^{-3} kPa at 20 °C 2.4×10^{-3} kPa at 25 °C	Measured
Water Solubility	4×10^{-2} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Stable at pH 2, 5, 7, 8.5 and 12	Measured*
Partition Coefficient (n-octanol/water)	log Pow = 3.89 at 25 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.05	Measured
Dissociation Constant	Not determined	Contains no dissociable functionality
Surface tension	55.4 mN/m at 20 °C	Measured
Flash Point	122 °C at 101.3 kPa	Measured
Flammability	Not determined	Not expected to be highly flammable based on the flash point
Autoignition Temperature	240 °C	Measured
Explosive Properties	Predicted negative	Based on the chemical structure
Oxidising Properties	Predicted negative	Based on the chemical structure

* The full study report was not provided.

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

Reactivity
The assessed 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 assessed 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 assessed chemical has a flash point of 122 ºC which is greater than 93 °C. Based on *Australian Standard AS1940* definitions for combustible liquid, the assessed 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 ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
The assessed chemical will be imported into Australia either in the neat form or as a component in fragrance formulations (at ≤ 2% concentration) or finished consumer products (≤ 2% concentration).

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

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

PORT OF ENTRY
Sydney

TRANSPORTATION AND PACKAGING
The imported assessed chemical or products containing it will be transported by road via truck to the applicant's warehouse or customers' facilities for storage or reformulation. Fragrance formulations containing the assessed chemical will be imported and distributed in tightly closed lacquered drums of varying sizes: 180, 100, 50, 25, 10 or 5 kg. End-use products will be packaged in containers suitable for retail sale.

USE
The assessed chemical will be used as a fragrance component in a variety of cosmetic and household products at typical final use concentrations of ≤ 0.04% in leave-on/rinse-off cosmetics, ≤ 0.39% in fine fragrances, ≤ 0.4% in household cleaning products, and ≤ 2% in air fresheners.

OPERATION DESCRIPTION
The reformulation procedures for incorporating the assessed chemical into end-use products will likely vary depending on the nature of the cosmetic and personal care/household cleaning products formulated. This may involve both automated and manual processes including transferring and blending the assessed chemical with other formulations. However, a typical blending operation will be highly automated and occur in a fully enclosed/contained environment, followed by automated filling using sealed delivery systems into containers of various sizes.

The end-use products containing the assessed chemical may be used by consumers and professionals such as hairdressers, workers in beauty salons 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 workers	unknown	unknown
Mixing	4	2
Drum handling	4	2
Drum cleaning/washing	4	2
Maintenance	4	2
Quality control	0.5	1
Packaging	4	2
Professional end users	not specified	not specified

EXPOSURE DETAILS

Transport and storage
Transport, storage and warehouse workers may come into contact with the assessed chemical in neat form or as a component of the imported preparations, only in the event of accidental rupture of containers.

Formulation of end use products
During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the assessed chemical (at up to 100% concentration) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The applicant states that exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems, and through the use of personal protective equipment (PPE) such as protective clothing, goggles, impervious gloves and respiratory protection if required.

Beauty care and cleaning professionals
Exposure to the assessed chemical in end-use products (at ≤ 2% concentration) may occur in professions where the services provided involve the application of cosmetics to clients (e.g. hairdressers and workers in beauty salons), or the use of household products in the cleaning industry. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the assessed chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the assessed chemical at ≤ 2% concentration through the use of a wide range of cosmetic and household products. The main route of exposure will be dermal, while ocular and inhalation exposure are also possible, particularly if products are applied by spray.

Data on typical use patterns of product categories in which the assessed chemical may be used are shown in the following tables and these are based on information provided in various literatures (SCCS, 2012; Cadby *et al.*, 2002; ACI, 2010; Loretz *et al.,* 2006). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. A dermal absorption (DA) rate of 100% was assumed for the assessed chemical for calculation purposes. For the inhalation exposure assessment, a 2-zone approach was used (Steiling *et al*., 2014; Rothe *et al*., 2011; Earnest, Jr, 2009). An adult inhalation rate of 20 m^3/day (enHealth, 2012) was used and it was conservatively assumed that the fraction of the assessed chemical inhaled is 50%. 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 (unitless)	Daily systemic exposure (mg/kg bw/day)
Body lotion	7,820	0.04	1	0.0447
Face cream	1,540	0.04	1	0.0088
Hand cream	2,160	0.04	1	0.0123
Fragrances	750	0.39	1	0.0418
Deodorant (non-spray)	1,500	0.04	1	0.0086
Shampoo	10,460	0.04	0.01	0.0006
Hair conditioner	3,920	0.04	0.01	0.0002
Shower gel	18,670	0.04	0.01	0.0011
Hand wash soap	20,000	0.04	0.01	0.0011
Hair styling products	4,000	0.04	0.1	0.0023
Total				**0.1215**

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

Household products (Indirect dermal exposure – from wearing clothes)

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	0.4	0.95	10	0.0125
Fabric softener	90	0.4	0.95	10	0.0049
Total					**0.0174**

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

Household products (Direct dermal exposure)

Product type	Frequency (use/day)	C (%)	Contact area (cm^2)	Product use C (g/cm^3)	Film thickness (cm)	Time scale factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	0.4	1,980	0.01	0.01	0.007	0.0001
Dishwashing liquid	3	0.4	1,980	0.009	0.01	0.03	0.0009
All-purpose cleaner	1	0.4	1,980	1	0.01	0.007	0.0079
Total							**0.0089**

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

Hairspray (Inhalation exposure):

Product type	Amount (g/use)	C (%)	Inhalation rate (m^3/day)	Exposure duration zone 1 (min)	Exposure duration zone 2 (min)	Fraction inhaled (%)	Volume zone 1 (m^3)	Volume zone 2 (m^3)	Daily systemic exposure (mg/kg bw/day)
Hairspray	9.89	0.04	20	1	20	50	1	10	**0.0012**

C = maximum intended concentration of assessed chemical

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

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the assessed chemical at the maximum intended concentrations specified by the applicant in various product types. This would result in a combined internal dose of 0.1490 mg/kg bw/day for the assessed chemical. It is acknowledged that inhalation exposure to the assessed chemical from use of other cosmetic and household products (in addition to hair spray) may occur. However, the combination of conservative hair spray inhalation exposure assessment parameters used and the aggregate exposure form use of the dermally applied products (using a conservative 100% dermal absorption rate), are sufficiently protective to cover additional inhalation exposure to the assessed chemical from use of other spray cosmetic and household products containing it with low exposure (e.g. air fresheners).

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the assessed chemical and an analogue chemical (4-penten-1-one, 1-(5-ethyl-5-methyl-1-cyclohexen-1-yl)-, CAS No. 1393645-32-3) 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 > 2000 mg/kg bw; low toxicity
Acute dermal toxicity – rat*	LD50 > 2000 mg/kg bw; low toxicity
Skin irritation – *in vitro* EpiSkin™ reconstructed human epidermis test	not classified as a skin irritant
Skin irritation – rabbit	irritating
Eye irritation – *in vitro* reconstructed human cornea-like epithelium test	not classified for eye irritation or serious eye damage
Eye irritation – rabbit	irritating
Skin sensitisation – mouse local lymph node assay	evidence of sensitisation (EC3 = 18.4%)
Repeat dose oral toxicity – rat, 28 days*	NOAEL = 500 mg/kg bw/day (established by study authors)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* mammalian cell micronucleus test	non genotoxic

* Analogue data

Toxicokinetics

Given the low molecular weight (192.3 g/mol) and log Pow (3.89 at 25 °C) of the assessed chemical, absorption across biological membranes may occur.

Acute Toxicity

The assessed chemical is of low acute oral toxicity based on a study conducted in rats.

No acute dermal or inhalation toxicity date were provided for the assessed chemical. The analogue chemical is of low dermal toxicity based on a study conducted in rats.

Irritation

In an *in vitro* study using the EpiSkin™ reconstructed human epidermis test model, the assessed chemical was determined not to require classification for skin irritation under the GHS according to the test guideline. However, the assessed chemical was found to be irritating to the skin in a study conducted in rabbits, warranting hazard classification (Cat 2).

In an *in vitro* reconstructed human cornea-like epithelium test, the assessed chemical was determined not to require classification for eye irritation or serious eye damage under the GHS according to the test guideline. However, the assessed chemical was found to be irritating to eyes in a study conducted in rabbits, warranting hazard classification (Cat 2A).

Sensitisation

The assessed chemical was determined to be a weak skin sensitiser in a mouse local lymph node assay (LLNA) with stimulation indices of 1.7, 4.8 and 5.4 at 10%, 30% and 100%, respectively. The EC3 value (i.e. the estimated concentration of a test substance needed to produce a stimulation index of three) was calculated to be 18.4% and the assessed chemical warrants classification as a weak skin sensitiser (Cat 1B).

Repeated Dose Toxicity
No repeat dose toxicity data were provided for the assessed chemical.

A 28 day repeated dose oral toxicity study was conducted in rats with the analogue chemical at dose levels of 0, 100, 300 and 500 mg/kg bw/day. The dose selection of this study was based on the results of a previous 14-day preliminary study in Sprague Dawley rats (treated at 1000 mg/kg/day showed abnormal gait, breathing irregularities, under activity, abnormal posture, and two male and two female animals needed humane sacrifice). In the 28-day study, test substance-related macroscopic changes (dark content in the caecum, dark renal medulla in kidneys, pale kidneys with a pale renal medulla, and pale liver) were observed after 4 weeks of treatment, and the findings remained in the kidney of 1 male animal following the 14-day recovery period. Test substance-related microscopic changes (tubular basophilia/vacuolation, granular casts and minimal accumulation of hyaline droplets in kidneys, centrilobular hypertrophy in the liver, and minimal follicular cell hypertrophy in the thyroid gland) were observed after 4 weeks of treatment. Following the recovery, these changes (except for hyaline droplets) were still present in the kidneys, liver and thyroids, with evidence of partial recovery in all organs. Statistically significantly increased mean absolute and relative liver weights were observed in all treated groups and the mean absolute liver weights of female animals treated at 500 mg/kg/day remained statistically higher than the control (16.16% increase compared to the control group) following the 14-day recovery period. The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 500 mg/kg bw/day as the liver, kidney and thyroid findings were considered to be either adaptive or have no relevance to human health. However, based on the statistically higher liver weight and microscopic changes following the recovery period, the NOAEL could be lower than 500 mg/kg bw/day.

Mutagenicity/Genotoxicity
The assessed chemical was tested negative in a bacterial reverse mutation assay and in an *in vitro* mammalian cell micronucleus test with human lymphocytes.

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

Hazard Classification	***Hazard Statement***
Skin Irritation (Category 2)	H315 – Causes skin irritation
Skin sensitisation (Category 1B)	H317 – May cause an allergic skin reaction
Eye irritation (Category 2A)	H319 – Causes serious eye irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the toxicological information provided, the assessed chemical is a weak skin sensitiser and a skin and eye irritant. No inhalation toxicity data were provided. Effects following repeated exposure at high doses could not be ruled out based on the information available on the analogue chemical.

Reformulation
Workers may experience dermal, ocular and perhaps inhalation exposure to the assessed chemical up to 100% concentration during reformulation. Given the assessed chemical is a skin sensitiser and a skin and eye irritant, control measures to prevent worker exposure are required when handling the assessed chemical during reformulation processes.

Provided that control measures are in place to minimise worker exposure, including the use of enclosed, automated processes and PPE such as impervious gloves, safety glasses, protective clothing and respiratory protection (if inhalation exposure may occur), the risk to the health of workers during the handling of the assessed chemical is not considered to be unreasonable.

End-use
Cleaners and beauty care professionals will handle the assessed chemical at ≤ 2% concentration, similar to public use. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to

be in place. Therefore, the risk to workers who use products containing the assessed chemical is expected to be of a similar or lesser extent than consumers who use such products on a regular basis. For details of the public health risk assessment see section 6.3.2 below.

6.3.2. Public Health

Members of the public may experience repeated exposure to the assessed chemical through the use of cosmetic and household products containing the assessed chemical at ≤ 2% concentration.

Sensitisation

Based on the results of an LLNA, the assessed chemical is a skin sensitiser with an EC3 value of 18.4%. Using fine fragrance as a worst-case example of leave-on cosmetic products that may contain the assessed chemical at ≤ 0.39% concentration, the Consumer Exposure Level (CEL) is estimated to be 14.63 μg/cm^2/day. Consideration of available information and application of appropriate safety factors, an Acceptable Exposure Level (AEL) of 14.63 μg/cm^2/day is estimated for the assessed chemical. In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16), use/time factor (3.16) and database factor (1), giving an overall safety factor of 300.

As the AEL = CEL, the risk to the public of the induction of sensitisation that is associated with the use of the assessed chemical in deodorants at ≤ 0.39% concentration (a worst-case example of leave-on cosmetic products) is not considered to be unreasonable. Based on lower expected exposure level from other cosmetic and household products, by inference, the risk of induction of sensitisation associated with the use of these products is also not considered to be unreasonable. However, it is acknowledged that consumers may be exposed to multiple products containing the assessed chemical, and a quantitative assessment based on aggregate exposure has not been conducted.

Repeated use

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the assessed chemical using the worst case exposure scenario from use of multiple products by an individual with total exposure of 0.1490 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 300 mg/kg bw/day for the assessed chemical (derived from a 28 day repeated dose toxicity study in rats on an analogue chemical), the margin of exposure (MoE) was estimated to be 2013. A MoE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences.

Overall, based on the information available, the risk to the public associated with use of the assessed chemical at ≤ 0.04% in leave-on/rinse-off cosmetics, ≤ 0.39% in fine fragrances, ≤ 0.4% in household cleaning products, and ≤ 2% in air fresheners is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The assessed chemical will be imported into Australia either in finished consumer products, or in the neat form or as a component in fragrance formulations for reformulation into finished products. In general, the reformulation processes are expected to involve blending operations that will be highly automated and occur in an enclosed system, followed by automated filling of the finished products into end-use containers. According to the applicant, the liquid waste containing the assessed chemical from reformulation equipment washing will be reused. Empty import containers containing residual assessed chemical up to 0.1% of the import volume as estimated by the applicant, will either be recycled or disposed of through an approved waste management facility. Accidental spills of the assessed chemical during import, transport, storage or reformulation are expected to be collected for disposal, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The majority of the assessed chemical are expected to be released to sewers across Australia as a result of its use in shampoo, fabric softener, laundry detergent, air fresheners and cleaning formulations, which are washed off hair and skin of consumers as well as from cleaning activities.

RELEASE OF CHEMICAL FROM DISPOSAL

Empty end-use containers are disposed of through domestic garbage disposal and are expected to enter recycling facility or landfill. Empty drums used to transport the pure assessed chemical or fragrance formulations will be rinsed and re-used by an approved waste management facility, sent to a recycler, or sent to landfill for disposal.

7.1.2. Environmental Fate

Following its use in cosmetic and household products, the majority of the assessed chemical will enter the sewers and be treated at sewage treatment plants (STPs) before potential release to surface waters nationwide. A proportion of the assessed chemical may volatilise to air. The half-life of the assessed chemical in air is calculated to be 6.5 hours (US EPA, 2012; calculated using AOPWIN v1.92). Therefore, the assessed chemical is not expected to persist in the air compartment.

A ready biodegradation test conducted on the assessed chemical indicates that it is not readily biodegradable (6.7% and 36% degradation over 28 days). However, the assessed chemical degraded in river water (36% in 28 days). For details of the biodegradation studies, refer to Appendix C. The assessed chemical is expected to partially sorb to sludge at STPs based on its low water solubility and moderate partition coefficient (log Pow = 3.89). Therefore, the assessed chemical is expected to be partly removed at STPs through adsorption to sludge. A proportion of the assessed chemical 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 assessed chemical as residues in landfill and soils is expected to have low mobility based on its soil adsorption coefficients (log K_{oc} = 3.05).

The assessed chemical is not expected to bioaccumulate based on its octanol-water partition coefficient value (log Pow = 3.89). In the aquatic and soil compartments, the assessed chemical is expected to eventually 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 assessed chemical 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 chemical into sewer systems nationwide over 365 days per annum. The extent to which the assessed chemical is removed from the effluent in STP processes based on the properties of the assessed chemical has not been considered for this scenario, and therefore no removal of the assessed chemical 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/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	200.0	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0%	**Mitigation**
Daily effluent production:	4,877	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC - River:	0.56	µg/L
PEC - Ocean:	0.06	µg/L

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 chemical in this volume is 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 0.377 µg/L may potentially result in a soil concentration of approximately 2.51 µg/kg.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h EC50 = 8.3 mg/L	toxic to fish

Daphnia Toxicity	48 h EC50 = 2.3 mg/L	toxic to invertebrates
Algal Toxicity	72 h EC50 = 4.845 mg/L	toxic to algae
Inhibition of Bacterial Respiration	3 h IC50 > 1000 mg/L	not harmful to bacterial respiration

Based on the above ecotoxicological endpoint for the assessed chemical, it is expected to be toxic to fish, aquatic invertebrates and algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the assessed chemical is formally classified as "Acute Category 2: Toxic to aquatic life". On the basis of acute toxicity and lack of rapid biodegradability criteria, the assessed chemical is formally classified as 'Chronic Category 2: Toxic to aquatic life with long-lasting effects".

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated based on the endpoint for Daphnia as shown in the table below. A conservative safety factor of 100 was used given the acute endpoint for three trophic level is available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 for Daphnia	2.3	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	23	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has been calculated based on the PEC and PNEC.

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.56	23	**0.024**
Q - Ocean	0.06	23	**0.002**

The conservative risk quotients (Q = PEC/PNEC) for the worst-case discharge scenario have been calculated to be less than 1 for both riverine and ocean compartments which indicates that the assessed chemical is unlikely to reach ecotoxicologically significant concentrations based on its annual importation quantity and use pattern. Therefore, based on the calculated risk quotient, the assessed chemical is not considered to pose an unreasonable risk to the aquatic environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point -111.6 °C

Method	OECD TG 102 Melting Point/Melting Range EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks	Determined using a differential scanning calorimeter
Test Facility	LPL (2018a)

Boiling Point 268.2 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Determined using a differential scanning calorimeter
Test Facility	LPL (2018a)

Density 954 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Determined using a pycnometer
Test Facility	LPL (2018b)

Vapour Pressure 1.3×10^{-3} kPa at 20 °C
2.4×10^{-3} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks	Dynamic method
Test Facility	LPL (2018c)

Water Solubility 4×10^{-2} g/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	LPL (2019a)

Partition Coefficient (n-octanol/water) log Pow = 3.89 at 25 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water) EC Council Regulation No 440/2008 A.8 Partition Coefficient
Remarks	Flask Method
Test Facility	LPL (2019b)

Surface Tension 55.4 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions
Remarks	Concentration: 90% of the saturation level; the test item is surface active.
Test Facility	LPL (2018d)

Adsorption/Desorption log K_{oc} = 3.05

Method	OECD TG 121 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using HPLC
Remarks	The test was conducted at neutral pH; the column temperature was 30 °C.
Test Facility	LPL (2019c)

Flash Point 122 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Closed cup
Test Facility	LPL (2018e)

Autoignition Temperature 240 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Test Facility	LPL (2018f)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

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

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method
Species/Strain	Rat/Wistar
Vehicle	Corn oil
Remarks – Method	No significant protocol deviations

RESULTS

Sighting Study

Dose (mg/kg bw)	*Administered*	*Evident Toxicity*	*Mortality*
300	1 F	no	0/1
2000	1 F	yes	0/1

Main Study

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	4 F	2000	0/4

LD50	> 2000 mg/kg bw
Signs of Toxicity	No clinical signs were seen in the animal treated at 300 mg/kg bw. In animals treated at 2000 mg/kg bw, clinical signs of toxicity included red extremities and elevated gait seen in all animals in the main and sighting studies, underactivity, piloerection, hunched posture and yellow staining in the ventral surface seen in 4 animals in the main study, hindlimbs splayed seen for 1 animal in the sighting study, and unsteady gait seen for 2 animals in the main study and 1 female in the sighting study. Recovery of animals from the observed effects was complete by Day 3.
Effects in Organs	No abnormalities were noted in any animals at macroscopic examination.
Remarks – Results	All animals showed expected body weight gains.
CONCLUSION	The assessed chemical is of low toxicity via the oral route.
TEST FACILITY	Covance (2019)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Analogue chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (1987)
Species/Strain	Rat/Wistar
Vehicle	None
Type of dressing	Semi-occlusive
Remarks – Method	No significant protocol deviations.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Crust formation was noted at the test site of 1 male 2 to 5 days after dosing. No signs of local toxicity were observed in the remaining animals.
Signs of Toxicity – Systemic	No signs of systemic toxicity were observed.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks – Results	Normal bodyweight gain was observed during the study.

CONCLUSION The test substance is of low acute toxicity via the dermal route.

TEST FACILITY Envigo (2015)

B.3. Skin Irritation – *In Vitro* Reconstructed Human Epidermis Test Method

TEST SUBSTANCE Assessed chemical

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

Vehicle None

Remarks – Method No significant protocol deviations. As the test substance was found to react with MTT, an additional MTT reduction control KC (water-killed control) was introduced. However, the result of the KC did not indicate an increased MTT reduction. Thus the KC was not used for viability calculation for corrosion.

RESULTS

Test Material	*Mean OD_{570} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.761	100	11.2
Test substance	0.562	73.9	15.7
Positive control	0.053	7	2.7

OD = optical density; SD = standard deviation

Remarks – Results The mean viability of the test-substance treated tissues determined after an exposure period of 15 minutes with about 42 hours post-incubation was 73.9%.

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

TEST FACILITY Envigo (2019a)

B.4. Skin Irritation – Rabbit

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion

Species/Strain Rabbit/New Zealand White

Number of Animals 3 M

Vehicle None

Observation Period 14 days

Type of Dressing Semi-occlusive

Remarks – Method No significant 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	3	3	3	< 14 days	0
Oedema	2.7	2	2	3	< 7 days	0

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

Remarks – Results Well-defined to moderate erythema were noted 1 hour after the patch removal in all animals and was reversible between Days 7 and 14.

	Moderate oedema was noted at the 1- or 24-hour observation in all animals and was reversible between on Day 7. Dryness of the skin was noted on Day 7 in all animals and the skin was recovered on Day 14.
CONCLUSION	The assessed chemical is irritating to the skin.
TEST FACILITY	Phycher (2019a)

B.5. Eye Irritation – *In Vitro* Reconstructed Human Cornea-like Epithelium (RhCE) Test Method

TEST SUBSTANCE	Assessed chemical
METHOD	OECD Guideline 492 Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage Reconstructed Human EpiOcular™ Model
Vehicle	None
Remarks – Method	No significant protocol deviations. As the test substance was found to react with MTT, an additional MTT reduction control KC (freeze-killed control) was introduced and the KC was used for viability calculation for corrosion.

RESULTS

Test Material	*Mean OD_{570} of Duplicate Tissues*	*Relative Mean Viability (%)*
Negative Control	2.24	100
Test Substance	2.681	121.07*
Positive Control	0.874	39.03

* Corrected mean viability; OD = optical density

Remarks – Results	The relative mean viability of the tissues treated with the test substance was > 60%.
CONCLUSION	The assessed chemical does not require classification for eye irritation or serious eye damage.
TEST FACILITY	Envigo (2019b)

B.6. Eye Irritation – Rabbit

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 M
Observation Period	21 days
Remarks – Method	No significant 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			
Conjunctiva – Redness	2	0.7	0.3	2	< 7 days	0
Conjunctiva – Chemosis	2	0	0	2	< 14 days	0
Corneal Opacity	1.7	1.3	0	2	< 72 days	0
Iridial Inflammation	0.7	0	0	1	< 21 days	0

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

Remarks – Results	Moderate redness was noted 1 hour after application in all animals and was reversible between Days 2 and 14. Moderate chemosis was noted 1 or 24 hours after application in all animals and was reversible between Days 1 and 7. Iridial irritation was noted 1 hour after application in 1 animal and was reversible on Day 3. Moderate opacity was noted 24 hours after application in 2 animals and was reversible between Days 14 and 21.
CONCLUSION	The assessed chemical is irritating to the eye.
TEST FACILITY	Phycher (2019b)

B.7. Skin Sensitisation – LLNA

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA/Ca
Vehicle	Acetone/olive oil 4:1
Preliminary study	Yes
Positive control	α-Hexyl cinnamaldehyde
Remarks – Method	No significant protocol deviations

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (DPM/lymph node)*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5 F	1245.3	1.0
10	5 F	2100.9	1.7
30	5 F	5926.3	4.8
100	5 F	6742.7	5.4
Positive Control			
25	5 F	9485.7	7.6

EC3	18.4%
Remarks – Results	Erythema of the ear skin was noted between Days 1 and 6. A statistically significant increase in ear thickness was observed for the animals treated with the test substance at 100% concentration and with the positive control item, respectively. This increase, however, was not considered by the study authors to be biologically relevant as it was well below the Guideline-recommended threshold of 25% increase. No mortalities or signs of systemic toxicity were observed in any of the test animals. The body weights of all animals were within the range commonly recorded for animals of this strain and age.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the assessed chemical.
TEST FACILITY	Envigo (2019c)

B.8. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Analogue chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Rat/Sprague Dawley
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week

	Post-exposure observation period: 14 days
Vehicle	Corn oil
Remarks – Method	No significant protocol deviations. The dose selection of this study was based on the results of a previous 14-day preliminary study in which Sprague Dawley rats treated at 1000 mg/kg bw/day showed abnormal gait, breathing irregularities, under activity, abnormal posture, abnormal eyes, piloerection and cold to touch after the 2nd administration and 2 males and 2 female animals needed humane sacrifice.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5 M/5 F	0	0/10
Low Dose	5 M/5 F	100	0/10
Mid Dose	5 M/5 F	300	0/10
High Dose	5 M/5 F	500	0/10
Control Recovery	5 M/5 F	0	0/10
High Dose Recovery	5 M/5 F	500	0/10

Mortality and Time to Death

There were no unscheduled deaths.

Clinical Observations

No test-substance related clinical signs of toxicity were observed. There was no effect on sensory reactivity, grip strength, motor activity, body weight gains and food consumption. Increased water consumption was observed in animals treated at 500 mg/kg/day during the last 9 days of treatment, which was reversed during the recovery period.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no test substance-related changes in haematological parameters.

Statistically significantly increased alanine amino transferase levels were observed in females treated at 300 or 500 mg/kg/day and statistically significantly decreased bile acids concentration was observed in all treated groups. These changes were reversed in females 2 weeks post-treatment; however, in males the bile acids concentration was still lower than the control group, but not statistically significant.

Statistically significantly decreased sodium and chloride ion concentrations and albumen/globulin ratios were observed in males and females treated at 500 mg/kg/day. Statistically significantly decreased chloride ion concentrations were also observed in males treated at 100 or 300 mg/kg/day. Treatment-related and statistically significantly increased phosphorous concentration was noted for males treated at 300 or 500 mg/kg/day, with an increased cholesterol level for females. These changes were reversed 15 days post-treatment.

Increased protein, glucose and sodium and chloride ion concentrations were observed in males and females treated at 300 or 500 mg/kg/day and increased glucose concentration was observed in males treated at 100 mg/kg/day. Slightly increased urine volume was observed in animals treated at 300 or 500 mg/kg/day. These changes were reversed 15 days post-treatment.

Effects in Organs

Organ weights

Statistically significantly increased mean absolute and relative liver weights were observed in all treated groups (26.73%, 38.45% and 50.67% increase in the mean absolute liver weight at 100, 300 and 500 mg/kg bw/day, respectively, compared to the control group in males and 25.15%, 67.05% and 36.03% increase in the mean absolute liver weight at 100, 300 and 500 mg/kg bw/day, respectively, compared to the control group in females). Partial recovery was observed 15 days post-treatment but the mean absolute liver weights of animals treated at 500 mg/kg/day remained statistically higher than the control (4.95% increase compared to the control group in males and 16.16% increase compared to the control group in females).

Statistically significantly increased absolute and relative kidney and thyroid/parathyroid weights were observed in males treated at 300 or 500 mg/kg/day and increased thyroid/parathyroid weights were observed in males

treated at 100 mg/kg/day and in females treated at 500 mg/kg/day (relative weights). Increased absolute and relative (statistically significantly increased) adrenal weights and absolute and adjusted uterus and cervix weights were observed in females treated at 500 mg/kg/day and absolute uterus and cervix weights were also increased in females treated at 300 mg/kg/day. These changes were reversed 15 days post-treatment.

Macropathology
Dark content was noted in the caecum of 2 males treated at 500 mg/kg/day and 2 males treated at 300 mg/kg/day.

One male treated at 500 mg/kg/day had pale kidneys with a dark renal medulla and 2 males treated at 300 mg/kg/day had pale kidneys with a pale renal medulla. Pale kidneys were seen in one female treated at 300 mg/kg/day and correlated with microscopic minimal tubular vacuolation, this was not considered by the study authors to be treatment-related and was not seen in any other animals.

Pale appearance of the liver was noted in 2 males and all females treated at 500 mg/kg/day, in 3 females and 2 males treated at 300 mg/kg/day and 2 females treated at 100 mg/kg/day.

Pale areas in the renal medulla were observed in 1 male recovery group animal at 500 mg/kg/day.

Microscopic Pathology
Treatment-related changes (tubular basophilia/vacuolation, granular casts and minimal accumulation of hyaline droplets) were noted in the kidneys of males treated at 100, 300 or 500 mg/kg/day, with a dose-relationship.

Centrilobular hypertrophy was noted in the liver of males treated at 300 or 500 mg/kg/day and in females treated at 100, 300 or 500 mg/kg/day. Minimal to moderate periportal vacuolation was noted in 1 male treated at 100 mg/kg/day, 1 male treated at 500 mg/kg/day and in females treated 100 mg/kg/day or above.

Minimal follicular cell hypertrophy in the thyroid gland was noted in males and females treated at 500 mg/kg/day and in females treated at 300 mg/kg/day.

In recovery group males, hyaline droplets were absent but tubular basophilia/vacuolation and granular casts were still present at a similar incidence to the treatment groups.

Periportal vacuolation and centrilobular hypertrophy were reduced in incidence or absent in recovery group animals.

Thyroid follicular cell hypertrophy was recorded in both sexes of the recovery animals at an incidence similar to the main study.

Remarks – Results
Test substance-related macroscopic changes were observed in caecum, kidney and liver after 4 weeks of treatment, and the findings remained in the kidney of 1 male animal following recovery.

Test substance-related microscopic changes were observed in the kidney of males and liver and thyroid of males and females. Following recovery, treatment-related changes were still present in the kidneys, liver and thyroids, with evidence of partial recovery in all organs.

Although the liver, kidney and thyroid findings were considered to be either adaptive or have no relevance to human health by the study authors, the mean absolute liver weights of animals treated at 500 mg/kg/day remained statistically higher than the control (16.16% increase compared to the control group in females) following the recovery period.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 500 mg/kg bw/day, the highest dose tested, by the study authors, based on the observed liver, kidney and thyroid findings were considered either adaptive or have no relevance to human health. However, based on the mean absolute liver weights of animals treated at 500 mg/kg/day remained statistically higher than the control (16.16% increase compared to the control group in females) following the recovery period, the NOAEL could be lower than 500 mg/kg bw/day.

TEST FACILITY Envigo (2016)

B.9. Genotoxicity – Bacteria

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix prepared from phenobarbital/β-naphthaflavone induced rat liver
Concentration Range in Main Test	Test 1: a) With metabolic activation: 1.5 - 5000 µg/plate b) Without metabolic activation: 0.05 - 5000 µg/plate Test 2: a) With metabolic activation: 0.15 - 5000 µg/plate b) Without metabolic activation: 0.05 - 150 µg/plate
Vehicle	Dimethyl sulphoxide (DMSO)
Remarks – Method	No significant protocol deviations. Negative control: DMSO Positive control: with S9-mix: 2-aminoanthracene (TA1535, TA1537, TA100) and benzo(a)pyrene (TA98); without S9-mix: N-ethyl-N'-nitro-N-nitrosoguanidine (TA1535 and TA100); 9-aminoacridine (TA1537); 4-Nitroquinoline-1-oxide (TA98)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	≥ 50	> 5000	negative
Test 2	-	≥ 15	> 150	negative
Present				
Test 1	-	≥ 150	> 5000	negative
Test 2	-	≥ 150	> 5000	negative

Remarks – Results	The test substance did not induce an increase in the frequency of revertant colonies in the test strains at any concentration, with or without metabolic activation. The negative and positive controls performed as expected, confirming the validity of the test system.
CONCLUSION	The assessed chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Envigo (2019d)

B.10. Genotoxicity – *In Vitro* Mammalian Cell Micronucleus Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 487 In vitro Mammalian Cell Micronucleus Test (2016)
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 mix prepared from phenobarbital/β-naphthaflavone induced rat liver
Vehicle	DMSO
Remarks – Method	No significant protocol deviations. Negative control: DMSO

Positive control: with S9-mix: cyclophosphamide; without S9-mix: mitomycin C and demecolcine

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 10, 20, 40*, 60*, 70*, 80, 90, 100, 120	4 h	28 h
Test 2	0*, 10, 20, 40*, 60*, 70*, 80*, 90, 100, 120	24 h	48 h
Present			
Test 1	0*, 10, 20, 40, 60*, 80*, 90*, 100, 110, 120	4 h	28 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	≥ 120	≥ 80	> 120	negative
Test 2	≥ 120	≥ 80	> 120	negative
Present				
Test 1	≥ 120	≥ 100	> 120	negative

Remarks – Results

The test substance did not induce any statistically significant increases in the frequency of cells with micronuclei at any concentrations tested, with or without metabolic activation.

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

CONCLUSION

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

TEST FACILITY

Envigo (2019e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability Study 1

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test
Inoculum	River water
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical Oxygen Demand (BOD)
Remarks – Method	No major deviations from the test guidelines were reported. The test substance (2 mg/L) was exposed to river water, which was spiked with nutrient medium, dosed in closed bottles and incubated in the dark at 22-24°C for 28 days.

RESULTS

Test Substance		*Sodium acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	8	7	79
14	24	14	92
21	32		
28	36		

Remarks – Results	All validity criteria were met. The differences of the replicate values at day 28 were less than 20%. The biodegradation percentage of the reference compound, sodium acetate, at day 14 was 92%. The oxygen concentrations were > 0.5 mg/L in all bottles during the test period.
CONCLUSION	The assessed chemical is not readily biodegradable.
TEST FACILITY	NOURYON (2019)

C.1.2. Ready Biodegradability Study 2

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated sludge from a sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD
Remarks – Method	No major deviations from the test guidelines were reported.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	75.4
14	3.5	14	83.6
21	4.5	21	83.4
28	6.7	28	85

Remarks – Results	All validity criteria were met. The differences of the replicate values at day 28 were less than 20%. The total oxygen intake in the inoculum blank was

30 mg O_2/L at the end of the study. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance.

CONCLUSION The assessed chemical is not readily biodegradable.

TEST FACILITY CTI (2019a)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE Assessed chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test – semi-static
EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish – semi-static

Species	Zebra fish *(Danio rerio)*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC)
Remarks – Method	A definitive test was conducted based on a preliminary test result with no major deviations from the test guidelines. The test concentrations were 18%, 23%, 30%, 38% and 50% of saturated concentration. Test solutions were renewed at 24 hours. A positive control was also conducted using potassium dichromate as part of a quality assurance program.

RESULTS

Concentration		*Number of Fish*	*Mortality*			
Nominal (% of saturated concentration)	*Actual (mg/L)*		*24 h*	*48 h*	*72 h*	*96 h*
Control	0	10	0	0	0	0
18	6.2	10	0	0	0	0
23	7.9	10	0	2	2	4
30	11	10	5	9	10	10
38	17	10	10	10	10	10
50	20	10	10	10	10	10

LC50 8.3 (95% CL of 7.6-9.1) mg/L at 96 hours

Remarks – Results Trimmed Spearman-Karber method version 1.5 was used to calculate the LC50 value.

All validity criteria were met. Dissolved oxygen was maintained above 60% and concentration of the test substance was maintained above 80% of the nominal concentration. The reference test concluded a 24 h EC50 value of 354 mg/L which is within the range of expected responses. The results are based on geometric mean measured concentrations.

CONCLUSION The assessed chemical is toxic to fish.

TEST FACILITY CTI (2019b)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Assessed chemical

METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – static EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia – semi-static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	High Performance Liquid Chromatography with A Diode Array Detector (HPLC-DAD)
Remarks – Method	A definitive test was conducted based on a range-finding test result with no major deviations from the test guidelines. A reference test was also conducted using potassium dichromate approximately 5 months prior to the definitive study.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
Control	0	20	0	0
0.75	0.7	20	0	0
1.5	1.58	20	0	0
3.0	3.08	20	2	19
6.0	6.12	20	6	20
12.0	12.61	20	14	20

EC50	2.3 (95% CL of 2.06 – 2.56) mg/L at 48 hours calculated ToxRat (8) using Probit analysis.
Remarks – Results	All validity criteria were met. Dissolved oxygen concentration was > 60% in all test vessels and control vessels. The reference test concluded a 24h EC50 value of 1.1mg/L which is within the range of expected responses. The results are based on geometric mean measured concentrations.
CONCLUSION	The assessed chemical is toxic to aquatic invertebrates.
TEST FACILITY	LPL Laboratories (2019d)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test EC Council Regulation No 440/2008 C.3 Algal Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L Actual: 0.81, 1.01, 2.08, 4.44,and 8.75 mg/L (Geometric mean of daily measurements)
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	HPLC-DAD
Remarks – Method	A definitive test was conducted based on a range-finding test result with no major deviations from the test guidelines. A reference test was conducted using potassium dichromate approximately 5 months prior to the current study.

RESULTS

Growth rate		*Yield*	
ErC50 (mg/L at 72 h)	*NOEC* (mg/L)*	*EyC50 (mg/L at 72 h)*	*NOEC (mg/L)*
4.845 (4.329-5.432)	N.D.	1.708 (1.256-2.20)	< 0.810

*A 72 h NOEC for growth rate could not be determined, due to statistical reasons.

Remarks – Results	At 48 hours the NOEC was 1.01 mg/L. All validity criteria were met. A 105-fold growth rate was observed in the control cultures. The coefficients of variation for section-by-section specific growth rate was 16% in the control cultures. The coefficients of variation of average specific growth rates was 3.5% in the replicate control cultures. The reference test concluded a 72 h ErC50 value of 1.11 mg/L which is within the range of expected responses. The results are based on geometric mean measured concentrations.
CONCLUSION	The assessed chemical is toxic to algae.
TEST FACILITY	LPL Laboratories (2019e)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test EC Council Regulation No 440/2008 C.11 Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 100 and 1000 mg/L
Remarks – Method	A definitive test was conducted based on a range-finding test result with no major deviations from the test guidelines. The test substance was added to the test medium and stirred for 24 hours before testing. A reference test with 3,5-dichlorophenol was run.
RESULTS	
IC50	> 1000 mg/L
Remarks – Results	The validity criteria for the test were satisfied. Mean oxygen uptake rate in the blank controls was 34.2 mg O_2/g dry weight activated sludge/h during the test. The coefficient of variation of oxygen uptake rate in control replicates was 8.4% at the end of the definitive test. The reference item gave a 3 h IC50 of 10.1 mg/L, which was within the historical range.
CONCLUSION	The assessed chemical does not practically inhibit microbial respiration
TEST FACILITY	LPL Laboratories (2019f)

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