File No: LTD/1934

May 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

4-Penten-1-one, 1-(5-ethyl-5-methyl-1-cyclohexen-1-yl)-

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

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

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.nicnas.gov.au

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/1934	Firmenich Limited	4-Penten-1-one, 1-(5-ethyl-5-methyl-1-cyclohexen-1-yl)-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Acute Toxicity, oral (Category 4)	H302 – Harmful if swallowed
Sensitisation, skin (Category 1)	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*
Acute toxicity (Category 1)	H400 – Very toxic to aquatic life
Chronic toxicity (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 in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Acute Toxicity, oral (Category 4): H302 – Harmful if swallowed
  - Sensitisation, skin (Category 1): 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 and the intended use/exposure scenario.

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

Health Surveillance

- As the notified chemical is a 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.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical during reformulation processes:
  - Enclosed, automated process 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 notified chemical during reformulation process:
  - Avoid inhalation of vapours and aerosols
  - Avoid contact with skin.

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation processes:
  - Coveralls, impervious gloves
  - Respiratory protection when handling the chemical undiluted

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

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

- If products and mixtures containing the notified 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.

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.

Storage

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

Emergency procedures

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

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;
- additional information has become available to the person as to the respiratory irritation or sensitisation potential of the notified chemical;
- the concentration of the notified chemical exceeds or is intended to exceed the following concentrations in end use products:
  - 0.12% in deodorants
  - 0.25% in fine fragrances
  - 0.34% in face creams
  - 0.37% in hand creams
  - 1% in other cosmetic and household products.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from 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.

(Material) Safety Data Sheet
The (M)SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Firmenich Limited (ABN: 86 002 964 794)
73 Kenneth Road
BALGOWLAH NSW 2093

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: other names, degree of purity, residual monomers, impurities and use details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
Taiwan TCSI (2015)

2. IDENTITY OF CHEMICAL

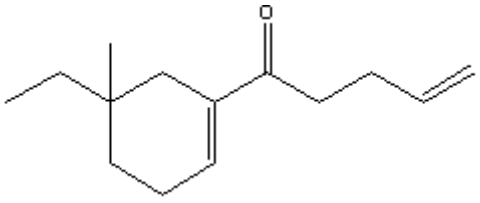
MARKETING NAME(S)
1-(5-ethyl-5-methylcyclohex-1-en-1-yl)pent-4-en-1-one

CAS NUMBER
1393645-32-3

CHEMICAL NAME
4-Penten-1-one, 1-(5-ethyl-5-methyl-1-cyclohexen-1-yl)-

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

STRUCTURAL FORMULA



MOLECULAR WEIGHT
206.33 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	< -80 °C	Measured
Boiling Point	284 °C at 101.9 kPa	Measured
Density	934 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.00036 – 0.00096 kPa at 25 °C	Measured
Water Solubility	0.013 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Hydrolytically stable at pH 2-12 at 40 °C	Measured
Partition Coefficient (n-octanol/water)	log P_{ow} = 4.39	Measured. However, the notified chemical is surface active and may therefore be present at the n-octanol/water interface.
Surface Tension	52.3 mN/m at 20 °C	Measured. The measured value is < 60 mN/m, and hence is indicative of potential surface activity.
Adsorption/Desorption	log K_{oc} = 3.8	Measured
Dissociation Constant	Not determined	No dissociable functionalities.
Flash Point	130 °C	Measured
Flammability	Predicted to be non-flammable.	Chemical is not expected to be a flammable liquid as its flash point is > 93 °C.
Autoignition Temperature	260 °C	Measured
Explosive Properties	Predicted negative	Contains no functional groups that would imply explosive properties.
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidative properties.

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia in its pure form or as a component in a fragrance formula (at a concentration ≤ 1%).

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
Sydney.

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited.

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia in either its pure form or as a component of fragrance preparations (containing the notified chemical at ≤ 1% concentration) in tightly closed lacquered drums of 180, 100, 50, 25, 10 or 5 kg 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 ≤ 1%) 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 usage concentrations will not exceed 0.1% in cosmetic products with the exception of fine fragrances where the concentration will not exceed 0.2%. In air fresheners the maximum concentration will not exceed 1% and in all other household products the concentration will not exceed 0.1%.

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
Air fresheners and other household products containing the notified chemical (at ≤ 1% and ≤ 0.1% concentration respectively) 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 ≤ 0.1% concentration or fine fragrances at ≤ 0.2% concentration 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 workers	Unknown	Unknown
Mixer	4	2
Drum handling	4	2
Drum cleaning	4	2
Maintenance	4	2
Quality Control	0.5	1
Packaging	4	2
Salon Workers	Unspecified	Unspecified
Cleaners	Unspecified	Unspecified

EXPOSURE DETAILS
Transport and storage
Transport and storage workers may come into contact with the notified chemical in its pure form, as a component of fragrance preparations or as a component of end-use products (at concentrations ≤ 1%) 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 ≤ 100% 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 ≤ 0.1% concentration in cosmetic products with the exception of fine fragrances where the concentration will be ≤ 0.2%) 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 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	0.1	1	0.12219
Face cream	1540	0.1	1	0.02406
Hand cream	2160	0.1	1	0.03375
Fragrances	750	0.2	1	0.0234
Deodorant (non-spray)	1500	0.1	1	0.02344
Shampoo	10460	0.1	0.01	0.00163
Hair conditioner	3920	0.1	0.01	0.00061
Shower gel	18670	0.1	0.01	0.00292
Hand wash soap	20000	0.1	0.01	0.00313
Hair styling products	4000	0.1	0.1	0.00625
Total				**0.2414**

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

Hair spray (inhalation exposure)

Product type	Amount (g/day)	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	0.1	20	1	20	50	1	10	**0.00322**

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	0.1	0.95	10	0.00341
Fabric softener	90	0.1	0.95	10	0.00134
Total					**0.00475**

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	0.1	1980	0.01	0.01	0.007	0.00003
Dishwashing liquid	3	0.1	1980	0.009	0.01	0.03	0.00025
All-purpose cleaner	1	0.1	1980	1	0.01	0.007	0.00217
Total							**0.00245**

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 0.2518 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 full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	300 mg/kg bw < LD50 < 2000 mg/kg bw; harmful
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 6.43 mg/L/4 hour; low toxicity
Skin irritation (in vitro)	non-irritating
Eye irritation (in vitro)	non-irritating
Guinea pig, skin sensitisation – adjuvant test	evidence of sensitisation
Human, skin sensitisation – RIPT (1.25%)	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL 500 mg/kg/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Mammalian Chromosome Aberration Test	non genotoxic

Toxicokinetics, metabolism and distribution

No information on the toxicokinetics of the notified chemical was provided. For dermal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption (ECHA, 2017). Dermal uptake is likely to be low to moderate if the water solubility is between 1-100 mg/L. Dermal uptake through the epidermis is expected if the partition coefficient (log P) values are

between -1 and 4 (ECHA, 2017). Gastrointestinal absorption and absorption across the respiratory tract are also likely to be high if the partition coefficient (log P) values are between -1 and 4. Absorption of the notified chemical through the skin, gastrointestinal tract and respiratory tract is expected based on the water solubility (13.2 mg/L) and moderately low molecular weight (206.33 g/mol). However, the high partition coefficient (4.39), may limit the rate of transfer between the stratum corneum and epidermis mitigating dermal penetration of the notified chemical.

Acute toxicity
The notified chemical was of low acute dermal toxicity based on a study conducted in rats.

In an acute oral toxicity study two animals were euthanised on day 2 of the study after exhibiting splayed hind limbs, unsteady gait, decreased activity, piloerection and hunched posture 5 hours after the initial exposure, and unresponsive behaviour, cold to touch, hunched posture and reduced body tone, flattened posture, lachrymation, shallow breathing and partially closed eye lids (both eyes) on day 2 of the study period. These animals exhibited congestion of the lungs and bronchi, as well as pallor of the lungs, liver and spleen. The remaining survivor exhibited piloerection and hunched posture with full recovery was observed on Day 11 of the study period. No adverse effects were observed in animals exposed to 300 mg/kg bw.

In an acute inhalation toxicity study, dark patches on the lungs were observed in three rats (2/5 males and 1/5 females) exposed to an aerosol containing the notified chemical at 2.14 mg/L, and in three rats (2/5 males and 1/5 females) exposed to an aerosol containing the notified chemical at 6.43 mg/L. Abnormally red lungs were also observed in one female in the high-dose group. No macroscopic effects were observed in the upper respiratory tract during necropsy. Based on historical macropathology data available for control animals (Envigo, 2019) provided by the notifier, the historical control range for dark patches in the lungs is 0 – 50% (short-term inhalation studies). The occurrence of dark patches in the lungs in this study is within the historical control range. No dark patches were observed in the lungs of those animals in the control or low-dose groups.

Persistent sneezing was observed in one animal (female) in the low dose group (recovery observed on Day 4), noisy respiration was observed in one animal (male) in the high-dose group (recovery observed on Day 4), while changes in respiration rate were observed in all animals following exposure to the notified chemical. Recovery from all irritant effects was observed on Day 5 (all animals (five males and five females) in the low dose group) and over Days 5 and 6 (all animals (five males and five females) in the high dose group). No significant changes to the function of the respiratory system were observed.

Based on the an absence of correlating clinical effects, dose-response relationship or functional impairments, the study authors did not consider these effects to be a response to exposure to the notified chemical.

Irritation
The notified chemical was non-irritating to the skin and not corrosive to the eye based on *in vitro* studies conducted on a human epidermis model (EpiSkin Reconstructed Human Epidermis Model) and a chicken eye test respectively.

Sensitisation
The notified chemical showed evidence of skin sensitisation in a Guinea Pig maximisation test with an induction concentration of 20% and at a challenge concentration of 50% (challenge phase was 21 days after exposure). In a human repeat insult patch test (HRIPT) completed on 103 test subjects (of 125 subjects that enrolled), the notified chemical at 1.25% concentration did not induce a skin sensitisation reaction.

No data are available to determine whether the chemical has the potential for respiratory sensitisation.

Repeated dose toxicity
In a 28 day repeat dose study by oral gavage with two week recovery period, rats were administered the notified chemical at 100, 300 and 500 mg/kg bw/day.

There was decreased mean haemoglobin levels in all treated rats compared to the control groups (statistically significant in females only). There was a slight dose response observed in males although only statistically significant in the recovery group males.

The liver, kidney and thyroid organs were most affected by the test substance in males and females. Some effects, such as the presence of hyaline droplets in the kidneys of males, the presence of follicular hypertrophy

(including in animals in the high-dose recovery group) and centrilobular hypertrophy in the thyroid and liver were considered species specific or adaptive by the study authors.

Recovery from adverse effects was indicated in animals in the high-dose recovery group. Partial recovery was observed in the kidneys where organ weights decreased to weights similar (although still higher) to those of animals not exposed to the test substance. However, no recovery at the microscopic level was indicated with the presence of tubular basophilia/vacuolation and granular casts occurring at a similar incidence and severity to those animals that were not in the recovery group. Accumulation of hyaline droplets was not observed in males in the high-dose recovery group, even though minimal accumulation was observed in treated males in the low-, mid- and high-dose groups.

While adverse effects were noted at both the mid- and high-dose levels, given these effects did not exhibit clear dose-response relationships, were not outside normal levels of variation, were limited to one sex, were not seen in the recovery group, or were considered to be either adaptive or not relevant to human health, the study authors established the No Observed Adverse Effect Level (NOAEL) as 500 mg/kg bw/day in this study.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation study and non-clastogenic in an *in vitro* mammalian chromosome aberration test.

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

Hazard classification	***Hazard statement***
Acute Toxicity, oral (Category 4)	H302 – Harmful if swallowed
Sensitisation, skin (Category 1)	H317 – May cause an allergic skin reaction

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is expected to be harmful following oral exposure and may cause an allergic skin reaction. Therefore, control measures are required to mitigate possible adverse health effects to the workers who may come into contact with the notified chemical.

Transport, Storage and Reformulation
Exposure of workers to the notified chemical (at ≤ 100% concentration) may occur during transport and blending operations. Provided that adequate control measures are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to workers from use of the notified chemical is not considered to be unreasonable.

End-use
Cleaners and beauty care professionals will handle the notified chemical at ≤ 1% 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 experienced 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.

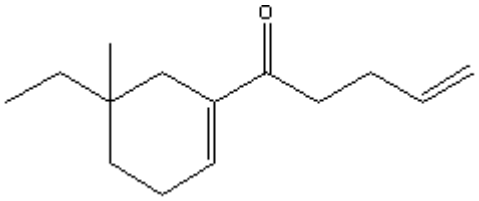
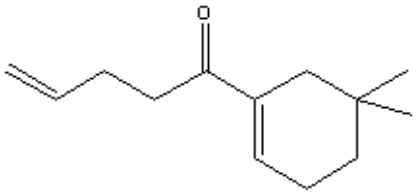
6.3.2. Public Health
Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic and household products (at ≤ 0.1% concentration in individual products and ≤ 1% concentration in air fresheners). The main route of exposure is expected to be dermal, while ocular and inhalation exposure are also expected where products are applied by spray.

Sensitisation
The main identified risk associated with use of the notified chemical at the proposed concentrations in cosmetic and household products, is its potential to cause sensitisation by skin contact.

When tested at 1.25% concentration in a human repeat insult patch study (0.3 mL applied to 2.54 cm^2 patches), the notified chemical was determined by the study authors to not be a skin sensitiser.

Although the data from the study conducted in guinea pigs is not suited to potency estimation, interpretation in terms of potency is possible (WHO, 2012 and ECHA, 2012). The notified chemical may be considered as a low to moderate sensitiser based on the number of animals that exhibited a sensitisation response (≥ 30% following intradermal induction with the notified chemical at > 1%), and the observed recovery in relation to the severity of the sensitisation response over the study period.

An EC3 value for the notified chemical can also be predicted based on information from LLNA studies on a structurally similar chemical [4-penten-1-one, 1-(5,5-dimethyl-1-cyclohexen-1-yl)-], which had an EC3 value of 3% (Scognamiglio *et al*., 2013).

	Notified chemical	*Analogue chemical*
Chemical name	4-Penten-1-one, 1-(5-ethyl-5-methyl-1-cyclohexen-1-yl)-	4-penten-1-one, 1-(5,5-dimethyl-1-cyclohexen-1-yl)-
CAS number	1393645-32-3	56973-85-4
Chemical structure		
Molecular weight (g/mol)	206.33	192.30
Density	934 kg/m^3 at 20 °C	906 kg/m^3 at 20 °C (predicted)
Log Pow	4.39	3.5 (predicted)

Methods for the quantitative risk assessment of dermal sensitisation have been proposed and been the subject of significant discussion (see for example, Api *et al.*, 2008 and RIVM, 2010). The Consumer Exposure Level (CEL) for various cosmetic and consumer products can also be estimated (Cadby *et al.*, 2002). Using the EC3 value of 3% for the analogue 4-penten-1-one, 1-(5,5-dimethyl-1-cyclohexen-1-yl)-, consideration of the details of the study, and application of appropriate safety factors, allowed the derivation of an Acceptable Exposure Level (AEL) of 9.34 $\mu g/cm^2$/day. As the AEL > CEL for all expected cosmetic product types, the risk to the public of the induction of sensitisation that is associated with the use of leave-on cosmetic products at ≤ 0.1% concentration or in fine fragrances at 0.2% concentration is not considered to be unreasonable.

The maximum safe concentrations at which the risk of sensitisation is not considered unreasonable are shown in the table below. Only examples of leave on cosmetic product categories are shown as the calculated allowable concentration was significantly higher in rise off cosmetics and other product categories.

Product type	**Concentration** (%)	**CEL (chemical)** ($\mu g/cm^2$/day)	**AEL** ($\mu g/cm^2$/day)	**Maximum allowable concentration** (%)
Body lotion	0.1	0.50	9.34	1.87
Face cream	0.1	2.73	9.34	0.34
Hand cream	0.1	2.51	9.34	0.37
Fine fragrances	0.2	7.50	9.34	0.25
Deodorant (non-spray)	0.1	7.50	9.34	0.1245

No information was provided on the potential for respiratory sensitisation following exposure to the notified chemical. Some (non-significant) irritation was observed in rats following acute inhalation exposure to the notified chemical (sneezing and changes to respiratory rate). However, based on the dispersive use pattern of air fresheners the risk of exposure (with respect to sensitisation) is expected to be lower than that of spray products such as hairspray.

Repeated dose toxicity

Members of the public may experience repeated exposure to the notified chemical through the use of the cosmetic and household products (containing the notified chemical at ≤ 1% concentration).

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 of 0.2518 mg/kg bw/day (see Section 6.1.2) and the NOAEL of 500 mg/kg bw/day, as determined by the study authors in a 28-day repeated dose toxicity study on the notified chemical. Using the abovementioned NOAEL, a MoE of 1,986 was estimated. A MoE value ≥ 100 is considered acceptable to account for intra- and inter-species differences, and to account for long-term exposure; therefore, the MoE is considered to be acceptable. However, if the treatment related effects such as the kidney effects in both sexes in the 500 mg/kg bw/day dose group are considered adverse and the next lowest dose of 300 mg/kg bw/day is used as the NOAEL the MOE estimate would still be > 100 (1,191).

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 0.2% in fine fragrances, ≤ 0.1% in all other cosmetic products, ≤ 1% in air fresheners and ≤ 0.1% in all other household products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia in its pure form or as a component in a fragrance formula for reformulation into end-use cosmetic and household 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 notifier, the liquid waste from cleaning of the reformulation equipment will be reused so no release is expected from this activity. Release of the notified chemical to the environment in the event of accidental spills or leaks during reformulation, storage and transport is expected to be collected for disposal of in accordance with local government regulations. Empty import containers containing residue notified chemical up to 0.1% of the import volume as estimated by the notifier, will either be recycled or disposed of through an approved waste management facility.

RELEASE OF CHEMICAL FROM USE

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

RELEASE OF CHEMICAL FROM DISPOSAL

The empty end-use containers are disposed of through domestic garbage disposal and are expected to enter recycling facility or landfill.

7.1.2. Environmental Fate

Following its use in cosmetic and household products, the majority of the notified chemical is expected to enter sewers across Australia. The ready biodegradation tests conducted on the notified chemical indicate that it is not readily biodegradable, but shows inherent biodegradability in aquatic environment (20-23% degradation over 28 days in OECD 301 C and OECD 301 F tests). The notified chemical is not expected to be bioaccumulative based on its relatively low bioaccumulation factor (BCF = 170 - 220). For details of the biodegradation and bioaccumulation studies, please refer to Appendix C. The notified chemical is expected to partly sorb to sludge at sewage treatment plants (STPs) based on its relatively low water solubility (0.013 g/L) and high partition coefficient (log P_{ow} = 4.39). As a result, the notified chemical is expected to be moderately removed at STPs through biodegradation and adsorption to sludge before potential release to surface waters nationwide. A proportion of the notified 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 notified chemical residues in sludge, landfill and soils are expected to have very low mobility based on its high soil adsorption coefficient (log K_{oc} = 3.8). In the aquatic and soil compartments, the notified chemical is expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon.

The half-life of the notified chemical in air is calculated to be < 2 hours, based on reactions with hydroxyl radicals (US EPA, 2012; calculated using AOPWIN v1.92). Therefore, the notified chemical is not expected to persist in the air compartment.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated to assume the worst case scenario with 100% release of the notified chemical into sewer systems nationwide over 365 days per annum. It is also assumed under the worst-case scenario that there is no removal of the notified chemical during sewage treatment processes. The resultant 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	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
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 notified 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 0.56 µg/L may potentially result in a soil concentration of approximately 3.74 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of the notified chemical in the applied soil in 5 and 10 years may be approximately 18.7 µg/kg and 37.4 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h EC50 = 2.09 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 0.799 mg/L	Very toxic to aquatic invertebrates
Algal Toxicity	72 h EC50 = 1.11 mg/L	Toxic to alga
Inhibition of Bacterial Respiration	3 h EC50 > 1,000 mg/L	Does not inhibit microbial activity in STPs

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS), the notified chemical is expected to be toxic to fish and alga, and very toxic to aquatic invertebrates. Therefore, the notified chemical is formally classified as “Acute Category 1; Very toxic to aquatic life” under the GHS. Based on the acute toxicity and lack of readily biodegradability, the notified chemical is formally classified as “Chronic Category 1; Very toxic to aquatic life with long lasting effects” under the GHS (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated based on the most sensitive endpoint for Daphnia as shown in the table below. An assessment factor of 100 was used given the acute endpoint for three trophic levels are available as a general indication of potential toxicity.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
48 h EC50 for Daphnia	0.799	mg/L
Assessment Factor	100	
Mitigation Factor	1	
PNEC:	7.99	µg/L

7.3. Environmental Risk Assessment

Based on the above predicted PEC and PNEC, the following Risk Quotient (Q = PEC/PNEC) has been calculated:

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.56	7.99	**0.070**
Q - Ocean	0.06	7.99	**0.007**

The conservative Risk Quotients (Q = PEC/PNEC) for the worst-case discharge scenario have been calculated to be much less than 1 for both river and ocean discharge. There is evidence that the notified chemical is inherently biodegradable, and is not expected to be bioaccumulative. Therefore, on the basis of the predicted PEC/PNEC ratio and the assessed use pattern as a component in cosmetic and household products, the notified chemical is not expected to pose an unreasonable risk to the aquatic environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point < - 80 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Differential scanning calorimetry and applying a storage experiment in freezer.
Test Facility WIL (2015a)

Boiling Point 284 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Differential scanning calorimetry.
Test Facility WIL(2015a)

Density 934 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 WIL(2015a)

Vapour Pressure 0.00036 – 0.00096 kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 761/2009, Part A: Methods for the Determination of Physico-Chemical properties, Guideline A.4: "Vapour Pressure".
Remarks Isothermal thermogravimetric effusion.
Vapour pressure also measured at 20 °C: 0.00017 – 0.00053 kPa
Test Facility WIL (2015b)

Water Solubility 0.013 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 Dr U Noack-Laboratorien (2015a)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ <year>*
2	40	> 1
5	40	> 1
7	40	> 1
8.5	40	> 1
12	40	> 1

Remarks The test substance is hydrolytically stable in environmental conditions
Test Facility Firmenich (2016a)

Partition Coefficient (n-octanol/water) log P_{ow} = 4.39

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks HPLC Method. The test substance is surface active.
Test Facility Firmenich (2011)

Surface Tension 52.3 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks	Ring method. Concentration: 90% saturated in water (1 mg/mL). The test substance is considered to be surface active.
Test Facility	WIL (2015c)

Adsorption/Desorption log K_{oc} = 3.8

Method	OECD TG 121 Adsorption Coefficient EC Council Regulation No 440/2008 C.19 Adsorption Coefficient
Remarks	HPLC method. The test substance is surface active.
Test Facility	WIL (2015d)

Flash Point 130 °C

Method	EC Council Regulation No 440/2008 A.9 Flash Point. ASTM International, ASTM D93: "Standard Test Methods for Flash Point by Pensky-Martens Closed Cup Tester", December 10, 2002
Remarks	Determined using an Eraflash flash-point tester (ASTM D93c)
Test Facility	WIL(2015a)

Autoignition Temperature 260 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Flask heater.
Test Facility	WIL (2015e)

Explosive Properties

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Predicted to be negative based on the chemical structure not having any structural alerts that would imply explosive properties.
Test Facility	WIL (2015e)

Oxidizing Properties

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	Predicted to be negative based on the chemical structure not having any structural alerts that would imply oxidising properties.
Test Facility	WIL (2015e)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method EC Council Regulation No 440/2008 B.1 tris Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Wistar (RccHan®:WIST)
Vehicle	Corn oil
Remarks - Method	GLP compliant. No deviations from the protocol.

RESULTS

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

LD50	300 – 2,000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were observed in the animals exposed to 300 mg/kg bw of the test substance. In the 2,000 mg/kg bw dose group, 1/3 animals exhibited piloerection (5 hours after exposure and on Days 3 – 10) and hunched posture (Day 3). Full recovery from these effects was observed on Day 11. Splayed hind limbs, unsteady gait, decreased activity, piloerection and hunched posture were observed in the remaining 2/3 animals in the 2,000 mg/kg bw dose group 5 hours following exposure. In addition to these effects, on Day 2 these animals exhibited unresponsive behaviour, cold to touch, hunched posture and reduced body tone, flattened posture, lachrymation (both eyes), shallow breathing and partially closed eye lids (both eyes). Both animals were euthanised on Day 2 due to poor clinical condition.
Effects in Organs	No abnormalities were recorded in animals exposed to the low dose or the surviving animal in the high dose group. Macroscopic examination of the two animals that were euthanized on Day 2 revealed congestion of the lungs and bronchi (both animals) and pallor of the kidneys (both animals), liver (one animal) and spleen (both animals).
Remarks - Results	All animals that survived to the end of the study made the expected body weight gains. The animals that were euthanized (2/3 high dose group) exhibited a loss (< 5%) in body weight.
CONCLUSION	The notified chemical is harmful via the oral route.
TEST FACILITY	Huntingdon (2015a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity 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 study protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 M	2,000	0/5
3	5 F	2,000	0/5

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	No signs of irritation were recorded in 5/5 females and 4/5 males. Crust formation at the test site was noted in 1/5 males on Day 2 persisting to Day 5. Recovery was observed on Day 6.
Signs of Toxicity - Systemic	There were no deaths or test-substance related clinical signs.
Effects in Organs	None detected.
Remarks - Results	Two females (2/5) showed no gain in body weight during the first week of the study, but did make the expected gain in body weight during the second week. All other animals made the expected body weight gains over the study period.

CONCLUSION The notified chemical is of low toxicity via the dermal route.

TEST FACILITY Envigo (2015a)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 403 Acute Inhalation Toxicity and 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.
Particle Size	Mean mass median aerodynamic diameter (µm): 2.41 & 2.86 for group 1 & 2 respectively. Inhalable fraction (% < 4 µm): 77.7 & 66.3 for group 1 & 2 respectively.
Remarks - Method	GLP compliant. The test guideline recommends that chamber atmosphere samples be taken at least twice during the four hour exposure period. In this study, three samples were outside the test guideline recommendation of ± 20% of the mean achieved atmosphere concentration (samples at 13 minutes and 152 minutes were lower; and the sample at 234 minutes was higher). Based on the increased frequency of sampling (n = 9) compared to that recommended and all other samples were within ± 20% of the mean, this deviation is not considered to have affected the validity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 M, 5 F	6.87	2.14	0/10
2	5 M, 5 F	21.7	6.43	0/10

LC50	> 6.43 mg/L/4 hours
Signs of Toxicity	Hunched posture and piloerection were observed in all animals (low- and high-dose groups) on day 1 post-exposure, with hunched posture observed in all animals on days 2 (low- and high-dose groups) and 3 (high-dose

group) post-exposure.

Persistent sneezing was observed in 1/5 females in the low-dose group, with recovery observed on Day 4. Noisy respiration was observed in 1/5 males in the high-dose group with recovery observed on Day 4.

Changes in respiratory rate were observed in all exposed rats.

Full recovery was observed in all animals on day 5 (all animals, low-dose group, 9/10 animals, high dose group) and day 6 (1/10 animals, high-dose group).

Effects in Organs	Dark patches on the lungs were observed in 2/5 males and 1/5 females in the low-dose group, and abnormally red lungs (1/5 females) or dark patches (2/5 males, 1/5 females) on the lungs were observed in animals in the high-dose group.
Remarks - Results	Body weight losses were observed in all males and 4/5 females in the low-dose group and all animals in the high-dose group on day 1 post-exposure. Body weight gains were observed in all animals (5/5 males, 4/5 females, low-dose group; all animals, high dose group) during the remainder of the recovery period. No body weight gains were observed in one female (low-dose group) from day 1 to day 3 post-exposure, although normal body weight gains were observed over the remainder of the recovery period.
CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	Harlan (2015a)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method EpiSkin Reconstructed Human Epidermis Model
Vehicle	None.
Remarks - Method	GLP compliant. No significant deviations from the protocol.

The test substance was applied undiluted (10 µl) directly on top of the 3 skin tissues for 15 minutes. After a 42 hour post-incubation period, determination of the cytotoxic (irritancy) effect was performed. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at the end of the treatment.

Viable cells have the ability to enzymatically reduce MTT into blue formazan. Skin irritation is expressed as the remaining cell viability after exposure to the test item. The amount of the extracted formazan was determined spectrophotometrically at 562 nm in duplicates with the Anthos 2001 microplate reader.

The test item was checked for colour interference in aqueous solutions and for possible direct MTT reduction by adding the test item to MTT medium. Non-specific MTT reduction by the test item was noted in this preliminary test. Therefore, in addition to the normal procedure, three killed tissues treated with test item and three killed untreated tissues were used for cytotoxicity evaluation with MTT.

Dulbecco's Phosphate Buffered Saline (DPBS) with Ca^{++} and Mg^{++} and 5% sodium dodecyl sulfate (SDS) were used as a negative and positive control test substances, respectively. The controls were also performed in

triplicates.

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability (%)*
Negative control	0.819 (± 0.03)	100	3.3
Test substance	0.486 (± 0.03)	59.4	3.4
Positive control	0.051 (± 0.004)	6.2	0.5

OD = optical density; SD = standard deviation

Remarks - Results

The test substance did not react with MTT indicating that the test substance did not directly reduce MTT. Therefore, no correction was applied to the ODs of the test substance treated tissues.

The relative mean tissue viability obtained after treatment with the test substance compared to the negative control tissues was 59.4%. Since the mean relative tissue viability for the test item was above 50% (and the standard deviation value of the percentage viability is ≤ 18), after treatment, the test substance is considered to be non-irritating.

The positive control had a mean cell viability of 6.2% after exposure. As this is ≤ 40% relative to the negative control treated tissues and the standard deviation value of the percentage viability is ≤ 18 the positive control data meets the acceptance criteria.

The mean OD_{562} (optical density at 562 nm) of the negative control tissues was 0.819 and the standard deviation value of the percentage viability was 3.3%. As this is ≥ 0.6% and the standard deviation value of the percentage viability is ≤ 18 the negative control data meets the acceptance criteria.

CONCLUSION

The test substance is non-irritating to the skin.

TEST FACILITY

Harlan (2015b)

B.5. Irritation – eye (in vitro)

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 438 Isolated Chicken Eye 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 significant deviations from the protocol.

Thirty microlitres of either the negative control (0.9% w/v Sodium Chloride), positive control (Benzalkonium Chloride) or test substance (undiluted) was applied in triplicates onto the epithelium of the cornea and incubated for 10 minutes at 32 °C. After exposure the cornea was thoroughly washed and incubated for 2 hours with fresh medium, followed by opacity and permeability measurement.

Corneal opacity, fluorescein retention and corneal swelling were evaluated and scored.

RESULTS

Test material	*Maximal mean score for corneal opacity*	*Mean score of Fluorescein retention*	*Corneal swelling (%)*
Vehicle control	0.25	0	10.4
*Test substance**	0	0.5	6.74
*Positive control**	3	2.67	71.41

*Corrected for background values

Remarks - Results

All eyes exposed to the test substance exhibited slight fluorescein adhering (minimal), and no opacity or cloudiness of the cornea. The responses observed for opacity, fluorescein retention and corneal swelling following exposure to the test substance were identified as GHS Non-Classified. Therefore, the test substance is not classified as corrosive to the eye.

Slight opacity was observed in 1/3 eyes treated with the negative control. The negative control responses for opacity, fluorescein retention and corneal swelling were identified as GHS Non-Classified. Therefore, the negative control meets the acceptance criteria.

The positive control responses for opacity, fluorescein retention and corneal swelling were identified as GHS Category 1. Therefore the positive control meets the acceptance criteria.

CONCLUSION

The test substance is not a corrosive to the eye and the results indicate the chemical as not requiring classification as an eye irritant.

TEST FACILITY

Harlan (2015c)

B.6. Skin sensitisation

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 406 Skin Sensitisation - GPMT.
EC Directive 440/2008 B.6 Skin Sensitisation – Magnusson and Kligman

Species/Strain: Guinea pig/Dunkin Hartley

PRELIMINARY STUDY: Maximum Non-irritating Concentration:
topical: 50%

MAIN STUDY

Number of Animals: Test Group: 10 Control Group: 5

Vehicle: Olive oil (intradermal) and liquid paraffin (topical)

Positive control: Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-Hexylcinnamaldehyde.

INDUCTION PHASE: Induction Concentration:
intradermal: 20%
topical: 100%

Signs of Irritation: None observed in animals exposed to the test substance intradermally or topically.

CHALLENGE PHASE

1st challenge: Topical: 50% (Day 21)

2nd challenge: Not conducted.

Remarks - Method: GLP compliant.
No significant deviations from the protocol.

Preliminary study: two animals received the test substance at 100%, 50%, 25% and 10% concentration to determine the maximal non-irritant concentration. Discrete erythema was observed in ½ animals exposed to the notified chemical at 100%.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*		
		24 h	*48 h*	*72 h*
Test Group	50%	8/10	4/10	4/10
Test Group negative control	0%	0/10	0/10	0/10
Control Group	50%	0/5	0/5	0/5
Control group negative control	0%	0/5	0/5	0/5

Remarks - Results

The negative controls performed as expected.

Following exposure to the test substance during the challenge phase, discrete to intense erythema was observed in animals exposed to the test substance at 24 (8/10), 48 (4/10) and 72 (4/10) hours.

The mean severity of the sensitising response was calculated to be 1.4 (24 hours), 1 (48 hours) and 0.5 (72 hours). Mean severity was based on the skin irritation effects observed using a Draize scale across all animals at the three observation points.

All animals made the expected body weight gains.

CONCLUSION

There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY

PBD (2016)

B.7. Skin sensitisation – human volunteers

TEST SUBSTANCE

Notified chemical at 1.25%

METHOD

Repeated insult patch test with challenge

Study Design

Induction Procedure: Patches [occlusive webril/adhesive patch (25 mm Hill Top Chamber System®)]containing 0.3 mL test substance were applied 3 times per week for a total of 9 applications. Patches were removed by the applicants after 24 h and graded after an additional 24 h (or 48 h for patches applied on Friday).
Rest Period: approx. 2 weeks
Challenge Procedure: An occlusive webril/adhesive patch (25 mm Hill Top Chamber System®) was applied to a naïve site two weeks after the last induction patch. Patches were removed by technicians and sites were graded at 24 h, 48 h, 72 h and 96 h following patch application.

Study Group

75 F, 50 M; age range 18 - 68 years

Vehicle

Unknown. Test substance was used as supplied (at 1.25% concentration)

Remarks - Method

Occluded. The test substance was spread on a 2.5 cm × 2.5 cm patch.

RESULTS

Remarks - Results

103/125 subjects completed the study. Of the subjects that withdrew, no details were provided regarding why they withdrew. Six subjects (4 M, 2 F) withdrew prior to the induction procedure, 14 subjects withdrew following two (1 M), three (1 M, 3 F), four (5M, 1 F), five (1 M, 1 F) and seven (1 F) applications, one subject failed to attend the challenge phase (1 F), and one subject withdrew after receiving one challenge application. One subject (M) did not attend the third challenge observation, but returned for the fourth challenge procedure and observation.

Faint, minimal erythema was noted in two subjects (1 M, 1 F) following

	the sixth induction observation. This effect was not observed in these individuals at any of the following induction or challenge observations. No adverse responses were noted at challenge.
CONCLUSION	The notified chemical at 1.25% concentration was non-sensitising under the conditions of the test.
TEST FACILITY	SGS (2018)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral). OPPTS 870.3050, Repeated Dose 28-Day oral Toxicity Study in Rodents
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14
Vehicle	Corn oil
Remarks - Method	GLP compliant. No significant deviations from the protocol. In a 14-day preliminary study the test substance was administered to rats at 250, 500 and 1,000 mg/kg/day. Four animals (2 males, 2 females) in the 1,000 mg/kg bw/day dose group were euthanised following exposure on day 2 while the remaining animals were no longer exposed to the test substance and were euthanised on day 4 based on the severity of clinical effects (abnormal gait, breathing irregularities, under activity, abnormal posture, abnormal eyes, piloerection and cold to touch). Animals in the 500 mg/kg bw/day dose group exhibited abnormal gait, hunched posture and rapid respiration on days 2 to 4. No clinical effects were observed in the 250 mg/kg bw/day dose group and the doses for the main study were selected based on the preliminary study.

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

There were no clinical signs attributed to exposure to the test substance. Variations in grip strength (slightly higher in females in 300 and 500 mg/kg bw/day dose groups) and motor activity (slightly to significantly higher in males in 300 and 500 mg/kg bw/day dose groups and 500 mg/kg bw/day dose recovery group; and slightly to significantly lower in females in the 300 mg/kg bw/day dose group) were not considered to be due to the test substance by the study authors as there was no dose-response, variations between the sexes, were within the range of historical control data, or the effect could be attributed to normal biological variation.

All animals made the expected body weight gains. Animals in the 500 mg/kg bw/day dose group exhibited an increase in water consumption during the last week of treatment.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Statistically significant lower haemoglobin concentration and mean cell haemoglobin concentration were observed in all treated females on day 29 (mean haemoglobin levels of 14.6, 14.2 and 14.9 g/dL at 100, 300 and 500 mg/kg bw/day treatment groups respectively, compared to 15.4 g/dL in the control group). No difference in the mean haemoglobin level was observed in the 500 mg/kg bw/day recovery group at the end of the recovery period. Although not identified as statistically significant, males in all treatment groups also had lower mean haemoglobin levels (14.4, 14.5 and 14.6 g/dL at 100, 300 and 500 mg/kg bw/day, respectively) compared to the control group (15.2 g/dL) and the mean decrease was statistically significant in the 500 mg/kg bw/day dose recovery males at the end of the recovery period (14 g/dL compared with 14.6 g/dL in the control group). The study authors reported these changes as fortuitous.

Dose-dependent, statistically significant increased mean alanine amino transferase (ALT) activities were evident in females in the 300 and 500 mg/kg bw/day dose groups (37 and 38 U/L respectively, compared with 28 U/L for the control group) but with no difference in the 500 mg/kg bw/day dose recovery group. Lower bile acids concentrations were observed in males (statistically significant) and females in all treatment groups with low mean concentrations in the 500 mg/kg bw/day dose recovery animals (8.6 in control females compared to 12.9 in 500 mg/kg bw/day dose recovery females where the control mean was 33.4 at the end of the study).

Males and females in the 500 mg/kg bw/day dose group showed some changes (statistically significant) in the clinical chemistry parameters such as low sodium and chloride ion concentrations and albumen to globulin ratios. These changes were not observed in animals in the 500 mg/kg bw/day dose recovery group.

High protein (statistically significant in 300 and 500 mg/kg bw/day dose males and females) and glucose (statistically significant in all treated males and 500 mg/kg bw/day dose females) levels in in urine were observed in males and females in all treatment groups (statistically significant) but there were no changes in the 500 mg/kg bw/day dose recovery groups, compared to the control groups.

Effects in Organs

Animals exposed to the test substance exhibited statistically significant higher absolute and adjusted liver weights (11%, 23% and 39% higher in males and 20%, 60% and 70% higher in females at 100, 300 and 500 mg/kg bw/day respectively when compared to the control group). Partial recovery from exposure was indicated in the 500 mg/kg bw/day dose recovery group where liver weights remained high, but not at a statistically significant level. Higher thyroid/parathyroid weights (36% higher in males at 100 and 300 mg/kg bw/day, 70% higher in males at the 500 mg/kg bw/day, and 36% higher in females at 500 mg/kg bw/day when compared to the control group) and absolute and adjusted kidney weights (30% and 23% higher in males at 300 and 500 mg/kg bw/day respectively.) were observed.

Females in the 500 mg/kg bw/day dose group exhibited high absolute and adjusted adrenal weights and absolute and adjusted uterus and cervix weights (adjusted weights were statistically significantly higher). Females in the 300 mg/kg bw/day dose group also exhibited increased absolute uterus and cervix weights. Increased adrenal, uterus and cervix weights were not observed in the high-dose recovery group indicating recovery.

Macroscopic changes were observed in the caecum, kidney and liver of some treated rats. These were dark content in the caecum of 300 and 500 mg/kg bw/day dose males (2/5 in each group) and pale coloured liver and kidneys.

Pale livers were observed in males of 300 and 500 mg/kg bw/day dose groups (2/5 in each group) and in females of all treatment groups (2/5, 3/5 and 5/5 at 100, 300 and 500 mg/kg bw/day respectively). Pale kidneys were observed in 2/5 males (with pale renal medulla) and in 1/5 females at 300 mg/kg bw/day and in 1/5 males at 500 mg/kg bw/day.

Centrilobular hypertrophy of the liver was observed in males at 300 and 500 mg/kg bw/day (2/5 and 5/5 respectively) and in females at all treatment groups (3/5, 3/5 and 5/5 at 100, 300 and 500 mg/kg bw/day respectively). Periportal vacuolation of the liver was observed in females (one at high dose with moderate degree and minimal to slight in 2/5, 5/5 and 3/5 females at 100, 300 and 500 mg/kg bw/day, respectively) and males (1/5 each with minimal degree at 100 and 500 mg/kg bw/day).

Microscopic changes were observed in kidneys of treated males and these included minimal to slight tubular basophilia/vacuolation indicating an early degenerative change (in 1/5 males in the 100 mg/kg bw/day dose group and all males in the 300 and 500 mg/kg bw/day dose groups), and minimal to slight granular cysts (5/5 in

the 300 mg/kg bw/day dose group and 2/5 in the 500 mg/kg bw/day dose group). These were still present at the end of the recovery period at a similar incidence and severity in males received the 500 mg/kg bw/day dose. Minimal accumulation of hyaline droplets was observed in males (2/5 in the 100 mg/kg bw/day dose group, 5/5 in the 300 mg/kg bw/day dose group and 5/5 in the 500 mg/kg bw/day dose group). These were not observed in males in the 500 mg/kg bw/day dose recovery group.

Minimal follicular cell hypertrophy was observed in the thyroid of some males (1/5 each in the control, 100 and 300 mg/kg bw/day dose groups and 4/5 in the 500 mg/kg bw/day dose group) and females (3/5 and 2/5 in the 300 and 500 mg/kg bw/day dose groups, respectively). This was also observed in 1/5 females and 3/5 males in the 500 mg/kg bw/day dose recovery groups compared, compared to 1/5 females in the control group at the end of the recovery period.

Remarks – Results

The presence of hyaline droplets in treated male rats was considered to be the result of reversible binding of the test substance and α2-microglobulin which is synthesised by the parenchymal cells of the liver of the adult male rat. As α2-microglobulin is not found in immature male rats, female rats or humans, the presence of hyaline droplets in the kidneys is not considered to be relevant to human health.

Within the liver, the study authors considered the pale areas correlated with those areas of periportal vacuolation, while the dose-dependent increase in alanine amino transferase activities observed in 300 and 500 mg/kg bw/day dose females was considered a result of leakage caused by hepatocellular periportal vacuolation. In addition, the statistically significant increase in liver weights (15% higher compared to the control group) following exposure was associated with hepatocyte hypertrophy.

The study authors indicated that the presence of follicular hypertrophy and centrilobular hypertrophy in the thyroid and liver were the result of the chemical induction of microsomal hepatic enzymes in the enlarged liver causing an increase in the metabolism and excretion of thyroid hormones, as well as an increase in thyroid weight and follicular cell hypertrophy. In addition, the presence of follicular hypertrophy in 100 mg/kg bw/day dose females was considered as a secondary effect of a hepatocellular hypertrophy of minimal severity (making it undetectable by light microscopy).

While no recovery of follicular cell hypertrophy was observed in rats, the study authors considered that while the changes in the liver and thyroid could be correlated, the effects observed were indicative of species specific differences in thyroid hormone half-life and serum protein binding and transport in rats.

The liver, kidney and thyroid were most affected by the test substance in males and females. However, the study authors considered the effects to be either non-adverse or not relevant to human health.

CONCLUSION

The study authors established a No Observed Adverse Effect Level (NOAEL) of 500 mg/kg bw/day.

TEST FACILITY	Envigo (2016)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Commission regulation (EC) No 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. USA, EPA OCSPP harmonized guideline 870.5100 – Bacterial Reverse Mutation Test. Pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia. coli*: WP2uvrA
Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 0.5 – 5,000 µg/plate b) Without metabolic activation: 0.5 – 5,000 µg/plate
Vehicle	Dimethyl sulphoxide
Remarks - Method	GLP compliant.

No deviations from the protocol.

Positive controls: without metabolic activation – N-ethyl-N'-nitro-N-nitrosoguanidine (TA100, TA1535, WP2*uvrA*), 9-Aminoacridine (TA1537), 4-Nitroquinoline-1-oxide (TA98); with metabolic activation – 2-Aminoanthracene (TA100, TA1535, TA1537, WP2*uvrA*), 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	≥ 1,500	≥ 500	> 5,000	negative
Present				
Test 2	≥ 500	≥ 500	> 5,000	negative

Remarks - Results

A range finding test (test 1) determined a dose range of 0.5 to 5,000 µg/plate depending on the bacterial strain and the presence or absence of metabolic activation. A visible reduction in the bacterial background lawn was observed at 150 µg/plate (TA1535), 500 µg/plate (TA100) and 1,500 µg/plate (TA1537) in the absence of metabolic activation and at 500 µg/plate (TA100, TA1535) and 1,500 µg/plate (TA1537) in the presence of metabolic activation.

In the main test, a visible reduction in the bacterial background lawn was observed at 50 µg/plate (TA100), 150 µg/plate (TA1535), 500 µg/plate (TA1537) and 5,000 µg/plate (TA98) in the absence of metabolic activation and a weakened bacterial lawn at 150 µg/plate (TA100), 500 µg/plate (TA1535) and 1,500 µg/plate (TA1537) in the presence of metabolic activation.

No significant increases in the frequency of revertant colonies were recorded for any of the strains of bacteria, at any concentration either with or without metabolic activation.

All of the positive control chemicals used in the test induced significant increases in the frequency of revertant colonies with or without metabolic activation, confirming the sensitivity and activity of the S9-mix.

CONCLUSION

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

TEST FACILITY

Harlan (2015d)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Commission Regulation (EC) 440/2008 B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
US EPA OPPTS 870.5375 Guideline, August 1998
40 CFR 799.9537 TSCA *in vitro* mammalian chromosome aberration test.

Species/Strain: Human
Cell Type/Cell Line: Lymphocytes
Metabolic Activation System: S9 mix from phenobarbitone/β-naphthoflavone induced rat liver.
Vehicle: Dimethyl sulphoxide
Remarks - Method: GLP compliant.
No deviations from the protocol.

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

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 4.06, 8.13, 16.25*, 32.5*, 48.75*, 65	4	24
Test 2	0*, 4.06, 8.13, 16.25, 32.5, 43.5*, 54*, 65*	24	24
Present			
Test 1	0*, 8.13, 16.25, 32.5, 43.5*, 54*, 65*, 130	4	24
Test 2	-	-	-

*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	≥ 64.47	> 65	> 65	negative
Test 2	≥ 64.47	> 65	> 65	negative
Present				
Test 1	≥ 64.47	≥ 130	> 130	negative
Test 2	-	-	-	-

Remarks - Results

No statistically significant increases in the frequency of cells with aberrations were recorded in the presence of the test substance, at any dose level either with or without metabolic activation.

Positive and negative controls performed as expected confirming the sensitivity and activity of the S9-mix.

CONCLUSION

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

TEST FACILITY

Harlan (2015e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability 1

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I). Method for Testing the Biodegradability of Chemical Substances by Microorganisms, Japanese Government, 2011-2012.
Inoculum	Sludge sampled from 10 locations from rivers, lakes, inland sea and return sludge from STPs in Japan
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical oxygen demand (BOD) by oxygen measuring apparatus, and test substance by high performance liquid chromatography (HPLC)
Remarks - Method	No significant deviations from the test guidelines were reported. The test substance was directly added to the test vessels. A toxicity control was run.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	2	7	59
14	9	14	72
21	18	21	73
28	20	28	75

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound, aniline surpassed the threshold level of 60% within 14 days indicating the suitability of the inoculums. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days based on BOD was 20%. The degree of primary degradation based on the test substance measurement was 75% with eight converted products detected in the test vessels.
CONCLUSION	The test substance is not readily biodegradable, but shows evidence of inherent biodegradability.
TEST FACILITY	CERI (2016)

C.1.2. Ready biodegradability 2

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test EC Council Regulation No 440/2008 C.4 D Ready Biodegradability
Inoculum	Activated sludge from a local STP
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical oxygen demand (BOD) by oxygen measuring apparatus
Remarks - Method	No significant deviations from the test guidelines were reported. The test substance was directly added to the test vessels. A toxicity control was run.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation based on BOD*	*Day*	*% Degradation based on BOD*
7	-6	7	71
14	7	14	84
21	19	21	86
28	23	28	90

Remarks - Results: All validity criteria for the test were satisfied. The percentage degradation of the reference compound, sodium benzoate surpassed the threshold level of 60% within 14 days indicating the suitability of the inoculums. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days based on BOD was 23%.

CONCLUSION: The test substance is not readily biodegradable, but shows evidence of inherent biodegradability.

TEST FACILITY: Firmenich (2016b)

C.1.3. Bioaccumulation

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 305 Bioconcentration: Flow-through Fish Test.
Method for Testing the Degree of Accumulation of Chemical Substance in Fish Body, Japanese Government, 2011

Species: *Cyprinus carpio*
Exposure Period: Exposure: 28 days
Auxiliary Solvent: *N,N-dimethylformamide*
Concentration Range: Nominal: Level 1: 20 µg/L and Level 2: 2 µg/L
Initial measured: 19.6 µg/L and 1.98 µg/L
Analytical Monitoring: Liquid chromatography tandem mass spectrometry (LC-MS/MS)
Remarks - Method: No significant deviations from the test guidelines were reported. The concentrated stock of the test substance was prepared in *N,N-dimethylformamide* before adding to the test tanks. *N,N-dimethylformamide* stock without the test substance was added to the control. The test substance concentration in the test water were analysed before the uptake phase and at the same time as analysis of test fish at 6, 12, 15, 20 and 28 days.

RESULTS
Bioconcentration Factor: Level 1 BCF = 220 and Level 2 BCF = 170
Remarks - Results: All validity criteria for the test were satisfied. Dissolved oxygen in the test water was ≥ 7.4 mg/L at 23.5 – 25 °C (≥ 87%) during the test. The measured test substance of all samples was within ±20% of the nominal concentration during the test.

CONCLUSION: The test substance is not considered to be bioaccumulative.

TEST FACILITY: CERI (2017)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-Static GB/T 27861-2011, Chemical Fish Acute Toxicity Test, Beijing: Standards Press of China, 2012
Species	*Danio rerio*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	99.1 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography (GC)
Remarks – Method	No significant deviations from the test guidelines were reported. The test substance was added directly to the test water. The test water was replaced daily. The test substance in the test water was measured at 0 h, 96 h, before and after each renewal.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*
Nominal	*Measured*		*96 h*
Control	Control	10	0
1.80	1.33	10	10
2.32	1.82	10	40
3.00	2.34	10	50
3.87	3.10	10	90
5.00	3.97	10	100

LC50	2.09 mg/L (95% CL: 1.75-2.43 mg/L) at 96 hours (Calculated by Probit method)
Remarks – Results	All validity criteria for the test were satisfied. The dissolved oxygen concentration in the test solution during the test was ≥ 81%. The measured test substance concentration of all samples ranged from 65% to 95% of the nominal concentration so the test results were described based on geometric means of measured concentrations.

CONCLUSION	The test substance is toxic to fish.
TEST FACILITY	Guangdong Detection Center of Microbiology (2015)

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 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	260 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography mass spectrometry (GC-MS)
Remarks - Method	No significant deviations from the test guidelines were reported. A stock solution of 1.20 mg/L of the test substance was prepared in dilution water two days before the exposure starts. The lower test concentrations were prepared by further diluting the stock solution. The test water was replaced daily. The test substance in the test water was measured at 0, 24 and 48 h.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised (%)*
Nominal	*Measured*		*48 h*
Control	<LOQ*	20	0
0.075	0.058	20	0
0.150	0.117	20	0
0.300	0.242	20	5
0.600	0.469	20	15
1.20	0.903	20	60

**LOQ: limit of quantification of 0.005 mg/L*

LC50	0.799 mg/L (95% CL: 0.642 - > 0.903 mg/L) at 48 hours (LC50 was calculated by sigmoidal dose-response regression, and 95% confidence limits was calculated from the standard error and the t-distribution)
Remarks - Results	All validity criteria for the test were satisfied. The dissolved oxygen concentration in the test solution during the test was ≥ 8.33 mg/L at 19-20°C (≥ 90%). The measured test substance concentration of all samples ranged from 67% to 91% of the nominal concentration so the test results were described based on geometric means of measured concentrations.
CONCLUSION	The test substance is very toxic to aquatic invertebrates.
TEST FACILITY	Dr U Noack-Laboratorien (2015b)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Council Regulation No 761/2009 C.3 Algal Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.5, 1, 2, 4, 8 mg/L Measured: 0.341, 0.625, 1.32, 2.66, 5.42 mg/L
Auxiliary Solvent	None
Water Hardness	0.24 mmol Ca+Mg/L
Analytical Monitoring	Gas chromatography mass spectrometry (GC-MS)
Remarks - Method	No significant deviations from the test guidelines were reported. A stock solution of 8 mg/L of the test substance was prepared in dilution water, and the lower test concentrations were prepared by further diluting the stock solution. The test substance in the test water was measured at 0, 24, 48 and 72 h.

RESULTS

Biomass		*Growth*	
EC50	*NOEC*	*EC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
1.11 (95% CL: 0.929-1.38)	0.341	1.51 (95% CL: 1.34-2.19)	0.341

Remarks - Results	All validity criteria for the test were satisfied. The mean cell density in the control increased by 133 times The measured test substance concentration of all samples ranged from 59% to 77% of the nominal concentration so the test results were described based on geometric means of measured concentrations.

CONCLUSION	The test substance is toxic to alga.
TEST FACILITY	Dr U Noack-Laboratorien (2015c)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge from a local STP
Exposure Period	3 hours
Concentration Range	Nominal: 10, 32, 100, 320, 1,000 mg/L
Remarks – Method	No significant deviations from the test guidelines were reported. The test substance was directly added to the test vessels.
RESULTS	
IC50	> 1,000 mg/L
Remarks – Results	All validity criteria for the test were satisfied.
CONCLUSION	The test substance does not inhibit microbial activity in STPs
TEST FACILITY	Dr U Noack-Laboratorien (2015d)

BIBLIOGRAPHY

ACI (2010) Consumer Product Ingredient Safety, Exposure and risk screening methods for consumer product ingredients, 2nd Edition, American Cleaning Institute, Washington DC.

Cadby, P.A., Troy, W.R. and Vey, M.G.H. (2002) Consumer Exposure to Fragrance Ingredients: Providing Estimates for Safety Evaluation. Regulatory Toxicology and Pharmacology, **36**: 246-252.

CERI (2016) Biodegradation Study of [Notified Chemical] (Study No. 16216, March, 2016). Kurume, Japan, Chemicals Evaluation and Research Institute (Unpublished report submitted by the notifier).

CERI (2017) Bioconcentration Study of [Notified Chemical] in Common Carp (Study No. 46194, October, 2017). Kurume, Japan, Chemicals Evaluation and Research Institute (Unpublished report submitted by the notifier).

Dr U Noack-Laboratorien (2015a) [Notified Chemical]: Slow Stirring Water Solubility – Flask Method (Study ID. 141104FH/CWE16292, September, 2015). Sarstedt, Germany, Dr U Noack-Laboratorien (Unpublished report submitted by the notifier).

Dr U Noack-Laboratorien (2015b) [Notified Chemical]: Acute Immobilisation Test to *Daphnia magna*, Semi-static, 48 hours, in a Closed System (Study ID. 141104FH/DAI16292, December, 2015). Sarstedt, Germany, Dr U Noack-Laboratorien (Unpublished report submitted by the notifier).

Dr U Noack-Laboratorien (2015c) [Notified Chemical]: Alga Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 hours (Study ID. 141104FH/SPO16292, December, 2015). Sarstedt, Germany, Dr U Noack-Laboratorien (Unpublished report submitted by the notifier).

Dr U Noack-Laboratorien (2015d) [Notified Chemical]: Respiration Inhibition Test with Activated Sludge (Study ID. 141104FH/BBR16292, June, 2015). Sarstedt, Germany, Dr U Noack-Laboratorien (Unpublished report submitted by the notifier).

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

ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance, June 2017, version 3.0. European Chemicals Agency, https://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf.

enHealth (2012) Australian Exposure Factor Guide, companion document to: Environmental Health Risk Assessment: Guidelines for assessing human health risks from environmental hazards, EnHealth, Commonwealth of Australia.

Envigo (2015a) [Notified Chemical] Acute Dermal toxicity (Limit Test) in the Rat (Study No. 41403143, November, 2015). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier).

Envigo (2016) [Notified Chemical] Toxicity Study by Oral Administration to CD Rats for 4 Weeks Followed by a 2 Week Recovery Period (Study No. HIK0058, January, 2016). Suffolk, United Kingdom, Envigo CRS Limited (Unpublished report submitted by the notifier).

Envigo (2019) Historical macropathology Data HAN Wistar Rats. Selected Findings. Short Term Inhalation Studies (Study No. 190201 - 41403142, February, 2019). Derbyshire, United Kingdom, Envigo (Unpublished report submitted by the notifier).

Firmenich (2011) [Notified Chemical] Partition Coefficient Log P_{ow} (March, 2011). Geneva, Switzerland, Process Safety and Analytical Development Laboratory (Unpublished report submitted by the notifier).

Firmenich (2016a) [Notified Chemical] Stability Test (August, 2016). Geneva, Switzerland, Firmenich S.A Geneva laboratory (Unpublished report submitted by the notifier).

Firmenich (2016b) [Notified Chemical] Assessment of Ready Biodegradability Manometric Respirometry Test (October, 2016). Geneva, Switzerland, Firmenich Biodegradation laboratory (Unpublished report submitted by the notifier).

Guangdong Detection Center of Microbiology (2015) Acute Toxicity Test of [Notified Chemical] with Zebra Fish (Study No. 2015ESG0051, December, 2015). Guangzhou, China, Guangdong Detection Center of Microbiology (Unpublished report submitted by the notifier).

Harlan (2015a) [Notified Chemical]: Acute Inhalation Toxicity (Nose Only) Study in the Rat (Study No. 41403142, July, 2015). Derbyshire, United Kingdom, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2015b) [Notified Chemical]: Determination of Skin Irritation Potential Using the EPISKIN™ Reconstructed Human Epidermis Model (Study No. 41403137, August, 2015). Derbyshire, United Kingdom, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2015c) [Notified Chemical]: Isolated Chicken Eye Test for Identifying (i) Chemicals Inducing Serious Eye Damage and (ii) Chemicals Not requiring Classification for Eye Irritation or Serious Eye Damage (Study No. 41403138, April, 2015). Derbyshire, United Kingdom, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2015d) [Notified Chemical]: Reverse Mutation Assay 'Ames Test' Using *Salmonella typhimurium* and *Escherichia coli* (Study No. 41402646, March, 2015). Derbyshire, United Kingdom, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Harlan (2015e) [Notified Chemical]: Chromosome Aberration Test in Human Lymphocytes *in vitro*. (Study No. 41402647, June, 2015). Derbyshire, United Kingdom, Harlan Laboratories Ltd (Unpublished report submitted by the notifier).

Huntingdon (2015a) [Notified Chemical]: Acute Oral Toxicity to the Rat (Acute Toxic Class Method) (Study No. HIK0059, February, 2015). Cambridgeshire, United Kingdom, Huntingdon Life Sciences (Unpublished report submitted by the notifier).Loretz, L., Api, A.M., Barraj, L., Burdick, J. Davis, D.A., Dressler, W., Gilberti, E., Jarrett, G., Mann, S., Pan, Y.H.L., Re, T., Renskers, K., Scrafford, C., Vater, S. (2006) Exposure data for personal care products : Hairspray, spray perfume, liquid foundation, shampoo, body wash, and solid antiperspirant, Food and Chemical Toxicology **44**:2008-2018.

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

PBD (2016) [Notified Chemical] Assessment of Sensitising Properties on Albino Guinea Pigs. Maximisation Test According to Magnusson and Kligman (Study No. SMK-PH-15/0640, March, 2016). Martillac, France, Phycher Bio Développement (Unpublished report submitted by the notifier).

Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, Gronewold C (2011) Special aspects of cosmetic spray evaluations: Principles on inhalation risk assessment. Toxicology Letters, **205**:97-104.

SCCS (2012) Notes of Guidance for testing of Cosmetic Ingredients and Their Safety Evaluation (7th revision) European Commission - Scientific Committee on Consumer Safety.

Scognamiglio J, Letizia CS, Api AM (2013) Fragrance material review on 1-(5,5-dimethylcyclohexen-1-yl)pent-4-en-1-one. Food and Chemical Toxicology, **62**:S74-S82.

SGS (2018) Final Report – Repeated Insult Patch Test (RIPT) (Study No. 18-112, May, 2018). Union, New Jersey, United States of America, SGS Harrison Research Laboratories , Inc (Unpublished report submitted by notifier).

Steiling W, Bascompta M, Carthew P, Catalano G, Corea N, D'Haese A, Jackson P, Kromidas L, Meurice P, Rothe H, Singal M (2014) Principle considerations for the risk assessment of sprayed consumer products. Toxicology Letters, **227**:41-49.

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

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >.

US EPA (2012) Estimation Programs Interface (EPI) SuiteTM for Microsoft® Windows, v 4.10. United States Environmental Protection Agency. Washington DC, USA.

WIL (2015a) Determination of the Melting and Boiling Temperature, Density and the Flash Point of [Notified Chemical] (Study No. 507585, March, 2015). 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).

WIL (2015b) Determination of the Vapour Pressure of [Notified Chemical] (Study No. 507587, January, 2015). 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).

WIL (2015c) Determination of the Surface Tension of [Notified Chemical] (Study No. 507467, January, 2015). 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).

WIL (2015d) Determination of the Adsorption Coefficient of [Notified Chemical] (Project No. 507586, January, 2015). 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).

WIL (2015e) Determination of the Explosive properties, Auto-ignition Temperature and the Oxidizing Properties of [Notified Chemical] (Study No. 507588, January, 2015). 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).