File No: LTD/1980

February 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

3-Cyclopentene-1-butanal, α,2,2,3-tetramethyl-

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

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

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Director
NICNAS

TABLE OF CONTENTS

SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1980	International Flavours and Fragrances (Australia) Pty Ltd	3-Cyclopentene-1-butanal, α,2,2,3-tetramethyl-	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*
Skin corrosion/irritation (Category 2)	H315 – Causes skin irritation

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

Hazard classification	*Hazard statement*
Acute Category 1	H400 – Very toxic to aquatic life

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:
  – Skin corrosion/irritation (Category 2): H315 – Causes skin irritation

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

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 process:
  - Enclosed, automated processes, where possible
  - Ventilation system including local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during reformulation process:
  - Avoid skin and eye contact

- 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 process:
  - Coveralls
  - Impervious gloves
  - Protective goggles

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

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

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

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.

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;

- the concentration of the notified chemical exceeds or is intended to exceed 0.07% in deodorant and hand cream, 0.1% in hairstyling (non-spray) products, 0.15% in fine fragrances, 0.2% in body lotion and face cream, or 1% in other leave-on and rinse-off cosmetic products, household cleaning products and air care products;

or

(2) Under Section 64(2) of the Act; if

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

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

Safety Data Sheet

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

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

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for all physico-chemical endpoints except density and flash point.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
Low Volume Chemical Permit (NICNAS)

NOTIFICATION IN OTHER COUNTRIES
China, USA and Philippines

2. IDENTITY OF CHEMICAL

MARKETING NAME
Santafleur

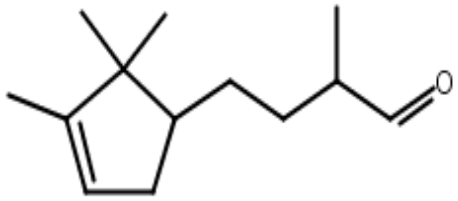
CAS NUMBER
65114-03-6

CHEMICAL NAME
3-Cyclopentene-1-butanal, α,2,2,3-tetramethyl-

OTHER NAME
α,2,2,3-Tetramethylcyclopent-3-ene-1-butyraldehyde

MOLECULAR FORMULA
$C_{13}H_{22}O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
194.32 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
~ 90%

The notified chemical is comprised of the following isomers in ~3:1 ratio:

3-Cyclopentene-1-butanal, α,2,2,3-tetramethyl-, (αR,1R)-*rel*- (major)
3-Cyclopentene-1-butanal, α,2,2,3-tetramethyl-, (α*R*,1*S*)-*rel*- (minor)

IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)

Chemical Name	Cyclopentanebutanal, α,2,2,3-tetramethyl-		
CAS No.	94201-30-6	*Weight %*	1.06
Chemical Name	Cyclopentanebutanol, β,2,2,3-tetramethyl-		
CAS No.	116965-41-4	*Weight %*	1.98

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Liquid

Property	Value	Data Source/Justification
Melting Point	33.79 °C	Calculated (EpiSuite v4.11)*
Boiling Point	252.25 °C at 101.3 kPa	Calculated (EpiSuite v4.11)*
Density	892 - 902 kg/m^3 at 20 °C	Measured. Test report not provided
Vapour Pressure	2.64×10^{-3} kPa at 25 °C	Calculated (EpiSuite v4.11)*
Water Solubility	0.0045 g/L at 25 °C	Calculated (EpiSuite v4.11)*. The notified chemical can be considered slightly soluble in water
Hydrolysis as a Function of pH	Not determined	The notified chemical does not contain any readily hydrolysable functionality
Partition Coefficient (n-octanol/water)	log Pow = 4.71 at 25 °C	Calculated (EpiSuite v4.11)*. The notified chemical has the potential to bioaccumulate
Adsorption/Desorption	log K_{oc} = 2.4 (MCI method) and 3.3 (Kow method) at 25 °C	Calculated (EpiSuite v4.11)*. The notified chemical is expected to have low to medium mobility in soil
Dissociation Constant	Not determined	The notified chemical does not contain any functionality that is expected to dissociate.
Flash Point	103 °C (closed cup)	Measured. Test report not provided
Flammability	Not determined	Not expected to be flammable based on flash point
Autoignition Temperature	Not determined	Not expected to autoignite
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties

*US EPA (2012)

DISCUSSION OF PROPERTIES

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 cannot be classified according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)*, as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia as a component of finished fragrance oil at ≤ 10% concentration for local reformulation into cosmetic and household products.

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

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

PORT OF ENTRY
Melbourne

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of fragrance oil in 208 L polypropylene-lined steel drums by sea. Within Australia the drums will be transported by road to the warehouse for storage and later distributed to the industrial customers by road. Finished consumer products containing the notified chemical will be transported primarily by road to retail stores in packages suitable for retail sale.

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

Finished Consumer Product	*Final Concentration of the Notified Chemical (%)*
Deodorant and hand cream products	≤ 0.07
Hair styling products	≤ 0.1
Fine fragrances	≤ 0.15
Face cream and body lotion products	≤ 0.2
Other leave-on and rinse-off cosmetic products, air-care products (candles and air freshener) and household cleaning products	≤ 1.0

OPERATION DESCRIPTION

Reformulation
The procedures for reformulating fragrance oils containing the notified chemical will vary and will depend on the nature of the cosmetic and household products, and may involve both automated and manual transfer steps. However, in general, it is expected that the reformulation process will be highly automated and occur in an enclosed system with adequate ventilation. This will be followed by automatic filling of the finished products into containers of various sizes which will be distributed to retail outlets. During the reformulation process, samples will be taken for quality control testing.

End-use
Household cleaning products
Finished household cleaning products containing the notified chemical (at ≤ 1% concentration) will be used by the general public and professional cleaners. The products may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines or open processes, and manually applied by sponge, mop, spray or brush followed by wiping or rinsing.

Cosmetics
The finished cosmetic products containing the notified chemical (at ≤ 1% 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 and warehouse workers	Unknown	Incidental exposure only
Plant operators-mixing/compounding	4	250
Plant operators-drum handling	1	250
Plant operators-drum cleaning/washing	2	250
Plant operators-equipment cleaning/washing	2	250
Plant operators-quality control	1	250
Professional users- (e.g. hairdressers, cleaners, etc.)	8	250

EXPOSURE DETAILS

Transport and distribution
Transport and storage workers may come into contact with the notified chemical at ≤ 10% concentration in fragrance oils and end-use products, only in the event of an unlikely accidental rupture of containers. If such an event occurs, workers may be exposed through dermal, ocular or perhaps inhalation exposure. Exposure should be minimised through the use of personal protective equipment (PPE) including protective coveralls, impervious gloves and eye protection as stated by the notifier.

Reformulation
Reformulation is expected to be highly automated and occur in an enclosed system with adequate ventilation, therefore limited exposure is expected. However, workers may be exposed to the notified chemical at ≤ 10% concentration via dermal, ocular and inhalation routes during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Exposure will be minimised through the use of PPE including protective clothing, eye protection, impervious gloves and respiratory protection (as appropriate) as stated by the notifier.

End-use
Exposure to the notified chemical in end-use products (at ≤ 1.0% concentration) may occur in professions where the services provided involve the application of cosmetics to clients (e.g. hair dressers and workers in beauty salons), or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure are also possible. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using the products containing the notified chemical.

6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the notified chemical (at ≤ 1% concentration) 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 (e.g. through the use of spray products) are also possible.

Data on typical use patterns of product categories in which the notified chemical may be used are shown in the following tables provided in various literatures (SCCS, 2016; Cadby et al., 2002; ACI, 2010; Loretz *et al.*, 2006). For the purposes of the exposure assessment, Australian use patterns for the various product categories are assumed to be similar to those in Europe. A dermal absorption (DA) of 100% was assumed for the notified chemical for calculation purposes. For the inhalation exposure assessment, a 2-zone approach was used (Steiling *et al.*, 2014; Rothe *et al.*, 2011; Earnest, Jr, 2009). An adult inhalation rate of 20 m^3/day (enHealth, 2012) was used and it was conservatively assumed that the fraction of the notified chemical inhaled is 50%. A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used for calculation purposes.

Cosmetic products (Dermal exposure):

Product type	Amount (mg/day)	C (%)	RF (unitless)	Daily systemic exposure (mg/kg bw/day)
Body lotion	7820	0.200	1	0.2444
Face cream	1540	0.200	1	0.0481
Hand cream	2160	0.070	1	0.0236
Fine fragrances	750	0.150	1	0.0176
Deodorant spray	1430	0.070	1	0.0164
Shampoo	10460	1.000	0.01	0.0163
Conditioner	3920	1.000	0.01	0.0061
Shower gel	18670	1.000	0.01	0.0292
Hand soap	20000	1.000	0.01	0.0313
Hair styling products	4000	0.100	0.1	0.0063
Total				**0.4393**

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

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

Product type	Amount (g/use)	C (%)	Product Retained (PR) (%)	Percent Transfer (PT) (%)	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	230	1.0	0.95	10	0.0341
Fabric softener	90	1.0	0.95	10	0.0134
Total					**0.0475**

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

Household products (Direct dermal exposure):

Product type	Frequency (use/day)	C (%)	Contact Area (cm^2)	Product Use C (g/cm^3)	Film Thickness (cm)	Time Scale Factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	1.0	1980	0.01	0.01	0.007	0.0003
Dishwashing liquid	3	1.0	1980	0.0093	0.01	0.03	0.0025
All-purpose cleaner	1	1.0	1980	1	0.01	0.007	0.0217
Total							**0.0245**

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

Hairspray (Inhalation exposure):

Product type	Amount (g/use)	C (%)	Inhalation rate (m^3/day)	Exposure duration zone 1 (min)	Exposure duration zone 2 (min)	Fraction inhaled (%)	Volume zone 1 (m^3)	Volume zone 2 (m^3)	Daily systemic exposure (mg/kg bw/day)
Hairspray	9.89	1.0	20	1	20	50	1	10	**0.0322**

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

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical at the maximum intended concentrations as specified by the notifier in various product types. This would result in a combined internal dose of 0.5435 mg/kg bw/day for the notified chemical. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household cleaning products (in addition to hair spray) may occur. However, it is considered that the combination of the conservative hair spray inhalation exposure assessment parameters, and the aggregate exposure from use of the dermally applied products, which assumes a conservative 100% absorption rate, is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray cosmetic and household products with low exposures (e.g. air fresheners and deodorants).

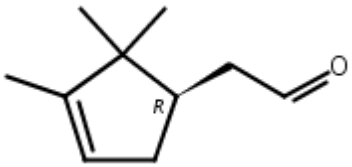
6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and analogue chemicals are summarised in the following table. For full details of the studies, refer to Appendix B.

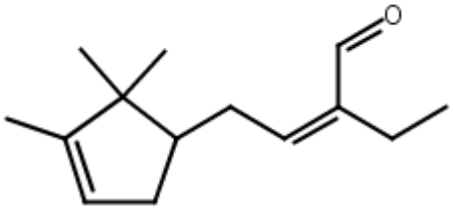
Endpoint	*Test substance*	*Result and Assessment Conclusion*
Rat, acute oral toxicity*	Analogue chemical 1	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, acute dermal toxicity*	Analogue chemical 1	LD50 > 5,000 mg/kg bw; low toxicity
Rabbit, skin irritation*	Analogue chemical 1	irritating
Mouse, skin sensitisation – Local lymph node assay	Analogue chemical 1	no evidence of sensitisation up to 50%
Human, skin sensitisation – RIPT	Notified chemical (1%)	no evidence of sensitisation
Rat, repeat dose gavage toxicity – 28 days	Analogue chemical 2	NOAEL = 100 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	Notified chemical	non mutagenic
Genotoxicity – *in vitro* mammalian cell micronucleus test	Notified chemical	non genotoxic

*Non-OECD guideline study

Analogue chemicals



Analogue chemical 1 (CAS No: 4501-58-0)



Analogue chemical 2 (CAS No: 65114-02-5)

Analogue chemical 1 (3-cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-, (1R)-) is similar in structure to the notified chemical. Both are aldehyde type compounds with a trimethylcyclopentene ring. The main difference is the length of the carbon chain between the aldehyde group and cyclopentene ring i.e. 3 carbons for the notified chemical and 1 carbon for the analogue. Given the slightly lower molecular weight of the analogue chemical (152 g/mol) and partition coefficient (log Pow = 3.31), the absorption potential is expected to be greater for analogue chemical 1 than the notified chemical. Therefore analogue chemical 1 is considered acceptable to estimate the toxicity of the notified chemical.

Analogue chemical 2 (2-butenal, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-,) is also similar in structure to the notified chemical containing an aldehyde group and a trimethylcyclopentene ring. The carbon chain between the aldehyde group and ring is the same i.e. 3 carbons, however the analogue chemical differs in that the aldehyde group is conjugated with a double bond. This is likely to increase the reactivity of the analogue chemical resulting in a greater potential for toxicity. The molecular weight (206 g/mol) and partition coefficient (log Pow = 5.11) of analogue chemical 2 are also similar to the notified chemical. Therefore analogue chemical 2 is considered acceptable to estimate the repeated dose toxicity of the notified chemical.

Toxicokinetics

Given the low molecular weight (194.32 g/mol), the notified chemical may be absorbed across the respiratory or gastrointestinal tract. However, based on the slight water solubility (0.0045 g/L at 25 °C) and high partition

coefficient (log Pow = 4.71), the notified chemical has a reasonably high lipophilicity, and hence percutaneous absorption is expected to be limited.

Acute toxicity
No studies were submitted for acute oral and dermal toxicity of the notified chemical.
Analogue chemical 1 was found to be of low acute oral and dermal toxicity in studies conducted in rats and rabbits, respectively.

No studies were submitted for acute inhalation toxicity of the notified chemical or analogue chemicals.

Irritation and sensitisation
No studies were submitted for skin irritation of the notified chemical.

In a skin irritation study conducted in rabbits with analogue chemical 1, all treated animals showed well defined erythema (grade 2) and very slight (grade 1) to very slight/slight oedema (grade 1.5) up to the 72 hour observation. One animal showed well-defined to moderate erythema (grade 2.5) at the 72 hour observation. At the day 7 observation, irritation effects were still present in all animals but showed signs of moderating. Desquamation and slight desquamation were also noted in two animals at the day 7 observation. The study was not continued after day 7 and therefore, the reversibility of the effects cannot be confirmed. NICNAS notes that there is another skin irritation study (conducted after the study provided by the notifier) in the REACH dossier for analogue chemical 1 warranting hazard classification as a Category 2 skin irritant under the GHS. Based on the available evidence, the notified chemical is expected to be a skin irritant warranting hazard classification.

No studies were submitted for eye irritation of the notified chemical or for the analogue chemical 1.

The notified chemical was not a skin sensitiser when tested at 1% concentration in a human repeat insult patch test (HRIPT) with 109 subjects completing the study. In addition, analogue chemical 1 was found to be non-sensitising in a mouse local lymph node assay (LLNA) at up to 50% concentration. Based on the data available, the notified chemical is not expected to be a skin sensitiser up to 50% concentration.

Repeated dose toxicity
No repeated dose toxicity studies were submitted of the notified chemical.

In a 28-day repeated dose oral toxicity study, rats received analogue chemical 2 daily by oral gavage at doses of 100, 350 and 1,000 mg/kg bw/day. Treatment related effects were observed on the liver, kidney, urinary bladder and spleen. The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 100 mg/kg bw/day in this study based on an increase in liver weight (13.6% and 16.5% increase in males and females respectively treated at 350 mg/kg bw/day and 22.0% and 34.0% increase in males and females respectively treated at 1,000 mg/kg bw/day) compared with the control groups and corresponding histopathology (diffuse hypertrophy of the hepatocytes) observed at 1,000 mg/kg bw/day. For risk assessment purposes a NOAEL of 100 mg/kg bw/day is used.

Mutagenicity/Genotoxicity
The notified chemical was negative in a bacterial reverse mutation assay and in an *in vitro* micronucleus assay in human lymphocytes.

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***
Skin corrosion/irritation (Category 2)	H315 - Causes skin irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the toxicological information available, the notified chemical is expected to be a skin irritant. The eye irritation potential of the notified chemical is not known.

Reformulation
During reformulation workers may be handling the notified chemical at ≤ 10% concentration. It is anticipated that engineering controls such as enclosed and automated processes and local ventilation will be implemented where possible, and appropriate PPE (coveralls, imperious gloves, eye protection) will be used to limit worker exposure to concentrations that could be irritating (i.e. 10%). Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use
Workers involved in professions where the services provided involve the application of cosmetic and household products containing the notified chemical to clients (*e.g.*, hairdressers, beauty salon workers and cleaners) or the use of household products in the cleaning industry may be exposed to the notified chemical at ≤ 1.0% concentration. Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the risk to such workers is expected to be of a similar or lesser extent than that experienced by consumers using the various products containing the notified chemical.

Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health
Cosmetic and household products containing the notified chemical will be available to the public. The main route of exposure is expected to be dermal, with some potential for accidental ocular or inhalation exposure.

Local Effects
The notified chemical is irritating to skin. The eye irritation potential of the notified chemical is not known. At the proposed low use concentrations (≤ 1%) of the notified chemical in cosmetic and household cleaning products, irritation effects are not expected.

Systemic Effects
The repeated dose toxicity potential of the notified chemical was estimated by calculation of the margin of exposure (MOE) using the worst case exposure scenario from use of multiple products of 0.5435 mg/kg bw/day (see Section 6.1.2) and the NOAEL of 100 mg/kg bw/day, which was established in a 28 day repeated dose oral toxicity study performed on an analogue chemical. The margin of exposure (MOE) was estimated to be 184 for a person using daily all types of products containing the notified chemical. A MOE greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences.

Therefore, based on the available information, the risk to the public associated with the use of the notified chemical at ≤ 0.07% in deodorant and hand cream products, ≤ 0.1% in hairstyling (non-spray) products; ≤ 0.15% in fine fragrances, ≤ 0.2% in face cream and body lotion products or ≤ 1% in other leave-on and rinse-off cosmetic products, air-care products (candles and air freshener) and household cleaning 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 as a component in fragrance oil or as a component in finished cosmetic and household products. Significant release of the notified chemical to the environment is not expected from transport and storage, except in the case of accidental spills and leaks. In the event of a spill, wastes containing the notified chemical are expected to be contained and collected with an inert absorbent material and disposed of to landfill.

At the customers' facilities, the fragrance oil containing the notified chemical will be blended with other ingredients for the manufacture of cosmetic and household products. The blending operations are expected to be automated and occur in closed systems with adequate ventilation. Therefore, it is not expected that there will be significant release of the notified chemical from this process. Any wastes containing the notified chemical residues during reformulation or repacking processes are expected to be discharged to an on-site wastewater

treatment plant or local municipal treatment plant according to the local government regulation. Empty import containers containing the notified chemical will be either recycled or be disposed of through an approved waste management facility. There may be minor air fugitive emissions at the consumer product manufacturing sites due to product sampling and consumer product compounding operations.

RELEASE OF CHEMICAL FROM USE
The notified chemical is expected to be released to the aquatic compartment through sewers during its use in cosmetic and household products.

While it was not possible to quantify, the notified chemical is expected to be released to the atmospheric compartment based on its vapour pressure. Therefore, its fate in air is also considered below.

RELEASE OF CHEMICAL FROM DISPOSAL
Wastes and residues of the notified chemical in empty containers are likely to either, share the fate of the containers and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling in accordance with current regulations.

7.1.2. Environmental Fate

Following its use in cosmetic formulations and household products in Australia, the majority of the notified chemical is expected to enter the sewer system, before potential release to surface waters nationwide. Based on the result of a biodegradability study, the notified chemical is readily biodegradable (63% in 28 days). For details of the study, please refer to Appendix C.

During the wastewater treatment process, the majority of the notified chemical is expected to partition to sludge, based on its low water solubility. In water the notified chemical is expected to be readily biodegradable, and is highly volatile (Henrys Law constant = 114 Pa m^3/mol) and hence is not expected to be persistent.

It is anticipated that the notified chemical in sewage sludge will be disposed of to landfill or applied to land when sludge is used for soil remediation. The notified chemical may have low to medium mobility in soil considering its estimated log Koc and the classification of McCall et al. (1981). However, in soil the notified chemical is not expected to persist, based on its readily degradable classification.

The notified chemical is volatile (vapour pressure = 2.37×10^{-3} kPa at 25 °C), and hence may be present in air as a result of blending into consumer products, use and disposal. However, the half-lives of the notified chemical in air are short, 1.1 and 3.7 hours based on reactions with hydroxyl radicals and ozone, respectively (calculated using EpiSuite v4.11; US EPA, 2012). Therefore, the notified chemical is not expected to persist in air.

The estimated log Pow (4.71 at 25 °C) indicates that the notified chemical has the potential to bioaccumulate. However, the notified chemical is readily biodegradable and not expected to persist in the environment.

The notified chemical is expected to degrade via biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the predicted environmental concentration (PEC) is summarised in the table below. As it will be used in cosmetics and household cleaning products, it was assumed that up to 100% of the total import volume of the notified chemical is released to the sewage treatment plants (STPs). Release is expected to occur over all days of the year. A worst case release scenario considering no removal in the STP. The actual concentration released from the STP is likely to be lower due to losses in the STP (volatilisation, biodegradation and partitioning to sludge) as described by Struijs (1996).

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:	2.7	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	24.4	million
Removal within STP	0%	

Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10	
PEC - River:	0.067	µg/L
PEC - Ocean:	0.56	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 3.7 x 10^{-3} mg/kg. The notified chemical is not expected to accumulate in soils after repeated applications.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on acceptable analogue of the notified chemical (analogue 2; see Section 6.2) are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	Analogue 2: 96 h LC50 = 0.775 mg/L	Very toxic to fish
Daphnia Toxicity	Analogue 2: 48 h EC50 = 0.22 mg/L	Very toxic to invertebrates
Algal Toxicity	Analogue 2: ErC50 = 2.69 mg/L	Toxic to algae

The empirical aquatic toxicity data for analogue chemical 2 presented in the table above indicates that it is very toxic to aquatic organisms. On this basis, the notified chemical is also expected to be very toxic to fish, aquatic invertebrates and toxic to algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is formally classified as "Acute Category 1: Very toxic to aquatic life". On the basis of acute toxicity data, NOEC value and ready biodegradability criteria, the notified chemical is not subject to GHS chronic classification for substances hazardous to the aquatic environment.

7.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive endpoint for Daphnia. A safety factor of 100 was used given acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 Daphnia (Analogue 2)	0.22	mg/L
Assessment Factor	100	
Mitigation Factor	1.0	
PNEC:	2.2	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.56	2.2	0.26
Q - Ocean	0.056	2.2	0.026

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach eco-toxicologically significant concentrations in surface waters, with consideration of its maximum annual importation quantity and use pattern. The notified chemical has bioaccumulation potential but is not likely to persist in the environment. Therefore, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Analogue chemical 1 (purity not recorded)
METHOD	Not specified
Species/Strain	Rat/strain not specified
Vehicle	Not specified
Remarks - Method	Only limited information (results and necropsy) has been provided.

RESULTS

Group	*Number of Animals**	*Dose (mg/kg bw)*	*Mortality*
1	10	2,470	0/10
2	10	3,510	4/10
3	10	5,000	8/10
4	10	7,120	7/10

*Sex of animals not specified

LD50	4,100 mg/kg bw/day
Signs of Toxicity	Signs of toxicity were observed in all exposure groups. At 2,470 mg/kg animals showed ptosis, lethargy, piloerection, chromodacryorrhea, ataxia, chromorhinorrhea, diarrhoea and bulging dyspnea. At 3,510 mg/kg animals showed diarrhoea, lethargy, ataxia, flaccid muscle, chromodacryorrhea, negative righting reflex, emaciation and piloerection. At 5,000 mg/kg animals showed lethargy, ataxia, chromodacryorrhea, diarrhoea, coma, emaciation and piloerection. At 7,120 mg/kg animals showed diarrhoea, chromorhinorrhea, ataxia, lethargy, prostration, chromodacryorrhea, piloerection, ptosis, coma, emaciation and bulging eyes.
Effects in Organs	At 2,470 mg/kg bw dark lungs were observed. At 3,510 mg/kg bw and above, dark and/or mottled liver, dark lungs, mottled kidneys, and dark, large and/or mottled spleens were observed.
CONCLUSION	Analogue chemical 1 is of low acute oral toxicity
TEST FACILITY	MBRL (1978)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Analogue chemical 1 (purity not recorded)
METHOD	Not specified
Species/Strain	Rabbit/strain not specified
Vehicle	Not specified
Type of dressing	Not specified
Remarks - Method	Only limited information (results and necropsy) has been provided.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	10 (sex not specified)	5,000	0/10

LD50	> 5,000 mg/kg bw
Signs of Toxicity - Local	On day 1, 7 animals showed severe redness and all animals showed moderate oedema. At necropsy, 6 animals showed oedema, 6 animals showed redness and 2 animals showed hard/thick skin.
Signs of Toxicity - Systemic	Prostration, yellow exudate from nose, tachypnea, ataxia, ptosis, respiratory distress, lethargy, diarrhoea, bloated abdomen, emaciated, mucous stool and alopecia were observed (number of animals affected not provided) at necropsy.
Effects in Organs	Bloated intestines were observed in 4 animals. Six animals showed discoloured (dark or bright red or bright orange) lungs.
CONCLUSION	Analogue chemical 1 is of low acute dermal toxicity
TEST FACILITY	MBRL (1978)

B.3. Irritation – skin

TEST SUBSTANCE	Analogue chemical 1 (purity not recorded)
METHOD	Similar to OECD TG 404 Acute Dermal Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White
Number of Animals	4 F
Vehicle	Nil
Observation Period	7 days
Type of Dressing	Semi-occlusive
Remarks - Method	0.5 mL of the test substance was applied on the dorsal shaved skin over an area of ~ 6 cm^2 surgical lint. The lint patches were held by elastic adhesive bandage. After 4 hours, the patches were removed and the treated sites were cleaned by gentle swabbing with cotton wool soaked in warm water.

RESULTS

Lesion	*Mean Score**				*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4			
Erythema/Eschar	2.0	1.5	2.0	2.17	2.5	> 7 days	1.5
Oedema	0.5	0.0	1.33	1.33	1.0	> 7 days	0.5

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

Remarks - Results

At the 24, 48 and 72 hour observations two animals showed well defined erythema (grade 2.0) and one animal showed very slight to well-defined erythema (grade 1.5). The remaining animal showed well defined erythema (grade 2.0) at the 24 and 48 hour observations increasing to well-defined to moderate erythema (grade 2.5) at the 72 hour observation. At the day 7 observation two animals showed very slight to well-defined erythema (grade 1.5) and one animal showed very slight erythema (grade 1.0).

Very slight (grade 1.0) to very slight/slight oedema (grade 1.5) was observed in two animals and grade 0.5 oedema observed in one animal at the 24, 48 and 72 hour observations. At the 7 day observation only grade 0.5 oedema was observed in two animals.

At the day 7 observation, desquamation and slight desquamation was observed in two animals.

As the study was not continued after day 7, the reversibility of the effects cannot be confirmed. Thus classification of the test substance as a Category 2 or Category 3 skin irritant cannot be determined.

CONCLUSION Analogue chemical 1 is at least mildly irritating to the skin.

TEST FACILITY Toxicol (1987)

B.4. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Analogue chemical 1 (purity not recorded)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/CBA/J
Vehicle Diethyl phthalate:ethanol (3:1)
Preliminary study Not conducted
Positive control Hexyl cinnamic aldehyde. Positive control was not conducted in parallel with the main study.
Remarks - Method A preliminary study was not conducted and dose selection for the main study was based on the reported use pattern of the chemical.

A concurrent positive control was not conducted. The results of a positive control study conducted by the laboratory prior to the main study, was provided. The EC3 for the positive control was 8.9%.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5F	354	-
2.5	5F	669	1.9
5.0	5F	375	1.1
10	5F	493	1.4
25	5F	698	2.0
50	5F	655	1.9
Positive Control			
0 (vehicle control)	Not stated	280	-
5	Not stated	540	1.9
15	Not stated	1308	4.7
35	Not stated	4310	15.4

Remarks - Results A stimulation index of ≥ 3 was not attained at any test dose concentration.

No signs of systemic toxicity were noted in either test animals or control animals during the test.

No mortality and no clinical signs of toxicity were observed in the test animals, with all animals gaining weight during the study.

The positive control confirmed the sensitivity of the test system.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the analogue chemical 1 up to 50% concentration.

TEST FACILITY Calvert (2012)

B.5. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical (1%)
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: Patches containing 0.2 mL of the test substance (1%) were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications during the induction period. Patches were removed by the subjects after 24 hours and graded by technicians after an additional 24 hours (or 48 hours for patches applied on Friday). Rest Period: 14 days Challenge Procedure: Patches were applied to a naïve site. The sites were scored 24, 48 and 72 hours after application.
Study Group	115 (98 F and 17 M); age range 18 - 69 years
Vehicle	Ethanol:diethyl phthalate (3:1)
Remarks - Method	Occluded. The test substance was applied on Park-Davis Readi-Bandage®. Negative control [1% distilled water in alcohol:diethyl phthalate (75:25)] was conducted in parallel with the test substance.
RESULTS	
Remarks - Results	109/115 subjects completed the study. Six subjects discontinued with the study for reasons unrelated to the test substance. No adverse responses were noted at induction and challenge.
CONCLUSION	The notified chemical at 1% concentration was non-sensitising under the conditions of the test.
TEST FACILITY	Essex (2001)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Analogue chemical 2 (95.5% purity)
METHOD	Notification 0402, No. 1, MOE (Japan) 28-Day Repeated Dose Toxicity Study in Mammals. Similar to OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Rats/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 days
Vehicle	Corn oil
Remarks - Method	In a dose range finding study, 3M and 3F rats were administered orally (gavage) with the analogue chemical 2, dissolved in corn oil, at 0, 30, 100, 300 and 1,000 mg/kg bw/day for 7 days. Increased relative liver weights were observed in females treated at 300 and 1,000 mg/kg bw/day. Females treated at 1,000 mg/kg bw/day also showed enlargement of liver. Based on these results 100, 350 and 1,000 mg/kg bw/day was chosen for the main study.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	5M/5F	0	0/10
low dose	5M/5F	100	0/10
mid dose	5M/5F	350	0/10
high dose	5M/5F	1,000	0/10

control recovery	5M/5F	0	0/10
high dose recovery	5M/5F	1,000	0/10

Mortality and Time to Death
No unscheduled mortalities were observed during the study.

Clinical Observations
Slight reduction in mobility was observed in four males and three females of the high dose group.

No treatment related effects were noted on body weights and food consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
In the high dose group, chloride level was decreased in males and females, and ALP and total cholesterol levels were increased in females.

Urine volume was increased in females of the high dose group and light violet substances, considered to be the test substance or metabolite, were observed in the urine sediments in females of the mid and high dose groups.

No significant changes were noted in the recovery group.

Effects in Organs
Absolute weight of the liver was increased in females of the mid (16.5% increase compared to control group) and high (34.0% increase compared to control group) dose groups and males of the high (22.0% increase compared to control group) dose group. Relative weight of the liver was increased in males in all dose groups (9.4%, 13.3% and 23.2% increase compared to control group in low, mid and high doses respectively) and females of the high dose (27.0% increase compared to control group) group. Relative weights of the kidneys were increased in males (11.8% increase compared to control group) and females (18.7% increase compared to control group) of the high dose group and absolute weights of the kidneys were increased in females (26.4% increase compared to control group) of the high dose group.

Enlargement of the cecum was observed in males and females of the high dose group.

In the high dose recovery group, the relative weights of the liver and kidneys, and absolute weights of the kidneys were increased in males and females. However, the effects in the liver and kidneys showed reversibility.

Histopathology
One female of the mid dose group, and all females and three males of the high dose group showed diffuse hypertrophy in the hepatocytes.

In females of the high dose group, dilatation of the tubules in two animals, regeneration of the collecting ducts in one animal and vacuolation of the proximal tubules in four animals were observed in the kidney. Hyperplasia of the urothelium of the urinary bladder in two animals and congestion of the spleen in four animals were observed.

In the high dose recovery group, dilatation and regeneration of the tubules of the kidney were observed in one female.

Remarks – Results
The test substance affected the liver, kidney, urinary bladder and spleen.

Although relative liver weights of males treated at all doses were increased, no corresponding histopathological changes were observed in males treated at 100 mg/kg bw/day and 350 mg/kg bw/day.

CONCLUSION
The No Observed Effect Adverse Effect Level (NOAEL) for the test substance in rats was established by the study authors as 100 mg/kg bw/day in this study, based on an increase in liver weight and corresponding histopathology (diffuse hypertrophy of the hepatocytes) observed at 350 mg/kg bw/day and 1,000 mg/kg bw/day.

TEST FACILITY CERI Hita (2014)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (95.2% purity)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation method
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98, TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix from Arcolor 1254 induced rat liver
Concentration Range in Main Test	Test 1 a) With metabolic activation: 5.0 – 5,000 µg/plate b) Without metabolic activation: 5.0 – 5,000 µg/plate Test 2 a) With metabolic activation: 5.0 – 1,600 µg/plate (for TA98, TA100, TA1535 and TA1537) and 16.0 – 5,000 µg/plate (for WP2uvrA) b) Without metabolic activation: 1.60 – 500 (for TA98, TA100, TA1535 and TA1537) and 5.0 – 1,600 µg/plate (for WP2*uvr*A).
Vehicle	Dimethylsulfoxide (DMSO)
Remarks - Method	Vehicle and positive control studies were conducted in parallel with the main study. Negative control: DMSO Positive control: With metabolic activation: 2-aminoanthracene (TA100, TA1535, TA1537 and WP2*uvr*A) and benzo[a]pyrene (TA98) Without metabolic activation: sodium azide (TA1535 and TA100), 2-nitrofluoroene (TA98), ICR-191 (TA1537) and 4-nitroquinoline-N-oxide (WP2*uvr*A). No protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 160	-	≥ 1,600	Negative
Test 2		≥ 160	1,600	Negative
Present				
Test 1	≥ 500	-	≥ 1,600	Negative
Test 2		1,600	≥ 1,600	Negative

Remarks - Results	In Test 1, toxicity was observed in all strains at ≥ 160 µg/plate without metabolic activation, except for WP2*uvr*A where toxicity was observed at ≥ 500 µg/plate. In the presence of metabolic activation, toxicity was observed at ≥ 500 µg/plate in TA1537 and in all other strains at ≥ 1,600 µg/plate. In Test 2, toxicity was observed in all strains at ≥ 160 µg/plate in the absence of metabolic activation. In the presence of metabolic activation toxicity was observed in all strains at 1,600 µg/plate except for WP2*uvr*A where toxicity was observed at ≥ 5,000 µg/plate. No biologically relevant increases in revertant colony numbers of any of the tester strains were observed during the test in either the presence or absence of metabolic activation.

The positive controls gave satisfactory responses, confirming the validity of the test system.

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

TEST FACILITY Covance (2015a)

B.8. Genotoxicity – *in vitro*

TEST SUBSTANCE Notified chemical (95.2% purity)

METHOD OECD TG 487 *In vitro* Mammalian Cell Micronucleus Test

Species/Strain	Human
Cell Type/Cell Line	Peripheral blood lymphocytes
Metabolic Activation System	S9 mix from Arcolor 1254 induced rat liver
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks - Method	Negative control: DMSO Positive control: without metabolic activation – mitomycin C with metabolic activation - cyclophosphamide In a range finding study, human peripheral blood lymphocytes were treated with the test substance at 19.6 to 1,943 µg/mL for 3 hours with or without metabolic activation and for approximately 24 hours without metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	9.72, 12.1, 15.2, 19.0*, 23.7, 29.6, 32.9*, 36.6, 40.7*, 45.2, 50.2 and 55.8	24 h	24 h
Test 2	23.7, 32.9, 40.7, 50.2, 55.8*, 62.0*, 69.9*, 76.5, 85.0 and 94.5	3 h	24 h
Present			
Test 1	62.0, 76.5, 94.5, 118.0*, 131.0*, 146.0*, 162.0, 180.0, 200.0 and 250.0	3 h	24 h

*Cultures selected for micronucleus assay.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity (> 50%) in Preliminary Test	*Cytotoxicity (> 50%) in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 54.4	≥ 40.7	> 55.8	Negative
Test 2	≥ 90.7	≥ 69.9	> 94.5	Negative
Present				
Test 1	≥ 151	≥ 146.0	> 250.0	Negative

Remarks - Results Precipitation was observed in the range finding study at ≥ 252 µg/mL.

A statistically significant increase in the frequency (1.25%) of binucleated cells with micronuclei was observed at 55.8 µg/mL in Test 2 (in the absence of metabolic activation). The study authors stated that the frequency was within the 95% reference vehicle control historical range of 0.20 to 1.41% therefore this was not considered as biologically relevant. No statistically significant increase in the frequency of binucleated cells was observed in any other concentration tested.

	The study authors stated that binucleated cells with micronuclei frequency for positive control in Test 2 (in the absence of metabolic activation) were lower (6.45%) than the 95% reference historical control range of 7.99 to 15.22%. This value, however, is statistically significant compared to the concurrent vehicle control therefore the value is considered acceptable.
CONCLUSION	The notified chemical was not clastogenic to human peripheral blood lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Covance (2015b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (96.0% purity)
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test
Inoculum	Secondary activated sludge obtained from a STP treating predominantly domestic wastewater.
Exposure Period	42 days
Auxiliary Solvent	None
Analytical Monitoring	Dissolved oxygen concentrations were determined electrochemically using an oxygen electrode and meter (WTW).
Remarks - Method	There were no reported deviations from the test protocols listed above.

RESULTS

Test substance		*Acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
7	8	7	74
14	23	14	78
21	57	-	-
28	63	-	-
35	73	-	-
42	75	-	-

Remarks - Results	All validity criteria for the TG were met. There was a mean oxygen depletion in the control was 1.0 mg/L at day 28 in the control bottles without silica gel. The concentration of oxygen in the test bottles was > 2.5 mg/L over the test period. The DT_{50} value was calculated, as approximately 20 days, based on the degradation between days 7 and 21.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	AkzoNobel (2018)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Analogue chemical 2 (95.5% purity)
METHOD	"Fish, Acute Toxicity Test" stipulated in the "Testing Methods for New Chemical Substances" of Japan OECD TG 203 Fish, Acute Toxicity Test - Semi-static regime OECD TG 23 Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures
Species	*Oryzias latipes*
Exposure Period	96 hours
Auxiliary Solvent	Acetone
Water Hardness	43 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography-mass spectrometry (GC-MS)
Remarks – Method	There were no reported deviations from the test protocols listed above. The test conditions (pH, dissolve oxygen and temperature) were suitable for the test species. The test was conducted in a closed system as the compound is volatile. Renewal of the test solutions occurred every 24

hours. The test solutions were colourless and clear at the start and before the renewal or at the time that mortality of all test organisms was confirmed. Acute toxicity test with a reference substance (Copper (II) sulfate pentahydrate) was periodically conducted.

RESULTS

Concentration mg/L		*Number of Fish*	*Cumulative Mortality (%)*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	7	0	0	0	0	0
5	0.229	7	0	0	0	0	0
10	0.469	7	0	0	0	0	0
20	0.911	7	14	14	29	71	71
40	2.30	7	43	100	100	100	100
80	4.94	7	100	100	100	100	100

LC50 0.775 mg/L at 96 hours (using an unspecified method).

Remarks – Results The validity criterion of the test guideline OECD 203 were met. The test concentrations were the geometric mean of the measured concentrations, and these were used to determine the LC50. A clear dose-response relationship was observed. Toxic effects were observed at measured levels of the notified chemical in the test solutions less than the water solubility limit. The 96-hour LC50 for the reference substance was 0.64 mg/L.

CONCLUSION The analogue chemical 2 is very toxic to fish.

TEST FACILITY CERI Kurume (2015a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Analogue chemical 2 (95.5% purity)

METHOD "Daphnia sp., Acute Immobilization Test" stipulated in the "Testing Methods for New Chemical Substances"
OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Semi-static regime
OECD TG 23 Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures

Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent Acetone
Water Hardness 43 mg $CaCO_3$/L
Analytical Monitoring GC-MS
Remarks - Method There were no apparent deviations from the test guideline that would have significantly affected the reliability of the results. 20 daphnids were used for each test level with 5 daphnids/test vessel. Six test concentrations of 100, 45.5, 20.7, 9.39, 4.27and 1.94% of stock solution content and a control were included in the test. A 48-hour acute immobilization test of a reference substance – potassium dichromate – was periodically conducted. Renewal of the test solutions occurred every 24 hours. The test was conducted in a closed vessel with no headspace as the compound is volatile.

RESULTS

Concentration mg/L		*Number of D. magna*	*Percent immobilisation*	
Nominal	*Actual*		*24 h*	*48 h*
Control	0	20	0	0
1.94	0.0668	20	0	0
4.27	0.161	20	0	0

9.39	0.365	20	0	5
20.7	0.818	20	100	100
45.5	1.92	20	100	100
100	4.06	20	100	100

LC50	0.518 mg/L at 48 hours (using an unspecified method)
Remarks - Results	The validity criterion of the OECD test guideline 202 were met. The 48 h EC50 for the reference substance was 0.22 mg/L. Immobilisation of all test species was observed was the three highest test concentrations. Toxic effects were observed at measured levels of the notified chemical in the test solutions less than the water solubility limit.

CONCLUSION The analogue chemical 2 is very toxic to aquatic invertebrates.

TEST FACILITY CERI Kurume (2015a)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Analogue chemical 2 (95.5% purity)

METHOD "Algal Growth Inhibition Test" stipulated in the "Testing Methods for New Chemical Substances"
OECD TG 201 Alga, Growth Inhibition Test
OECD TG 23 Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures

Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1 – 100 mg/L (% stock solution content 1.0, 3.16, 10, 31.6, 100 mg/L) Actual: 0.045 – 5.7 mg/L (geometric mean: 0.0451, 0.0160, 0.492, 1.65, 4.32 mg/L)
Auxiliary Solvent	Acetone
Water Hardness	Not reported
Analytical Monitoring	GC-MS
Remarks - Method	There were no reported deviations from the protocol that would have affected the reliability of the test. The test item concentration in test solution was reduced during the exposure period, which was expected to result from volatilisation to the headspace of the test vessel. The conditions of the test were maintained within recommended ranges with the exception of pH in the control and most of the test treatments. There was a geometric mean measured pH increase of >1.5 in all but the highest test dose. The increase in pH was attributed to the closed system test design, with no exchange of CO_2. This pH increase was not expected to have significantly affected test outcomes – see comments on growth rates in control below. Algae growth inhibition tests with a reference substance (potassium dichromate) are periodically conducted at the test facility.

RESULTS

Biomass		*Growth*	
EbC50 mg/L at 72 h (95% confidence intervals)	*NOEbC mg/L*	*ErC50 mg/L at 72 h (95% confidence intervals)*	*NOErC mg/L*
Not reported	Not reported	2.69 (2.66 – 2.71)	0.16

Remarks - Results	Cell growth in the controls was within the required parameters for the TG. The cell in the control grew exponentially during the exposure, and increased to ~54 times the number of initial cells. The mean coefficient of

	variation for section-by-section specific growth rate in the controls was 28%. The coefficient of variation of specific growth rate in replicate controls was 1.5%. There was a clear dose-response relationship for percent inhibition and cell concentration over the 72 exposure period. The EC50 was determined using a semi-qualitative method – visual inspection of % inhibition versus the logarithm of the measured concentration. The 3-day ErC50 for the reference compound was 1.1 mg/L was in the normal range 0.96 ± 0.17 mg/L (Mean ± Standard deviation). Toxic effects were observed at measured levels of the notified chemical in the test solutions less than the water solubility limit.
CONCLUSION	The analogue chemical 2 is toxic to algae.
TEST FACILITY	CERI Kurume (2015c)

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