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April 2018

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (3a*R*,7a*R*)-*rel*-

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2022	International Flavours and Fragrances (Australia) Pty Ltd	Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (3a*R*,7a*R*)-*rel*-	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 sensitiser (Category 1B)	H317 – May cause an allergic skin reaction

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

Hazard classification	*Hazard statement*
Acute (Category 2)	H401 – Toxic to aquatic life

Human health risk assessment
Provided that the recommended controls are being adhered to, 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 sensitiser (Category 1B): H317 – May cause an allergic skin reaction

  The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation processes:
  - Enclosed, automated processes, where possible
  - Good general ventilation, including local exhaust ventilation 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 processes:
  - 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

  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.

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 containment, physical 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 use concentration of the notified chemical exceeds or is intended to exceed 1% in fine fragrances and body lotion, 0.5% in deodorants, and 1.5% 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.

Safety Data Sheet
The SDS of 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(S)
International Flavours and Fragrances (Australia) Pty Ltd (ABN: 77 004 269 658)
301 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 dissociation constant, particle size, reactivity and genotoxic damage *in vivo.*

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
China (2017)
EU (2016)
Japan (2017)
Philippines (2018)

2. IDENTITY OF CHEMICAL

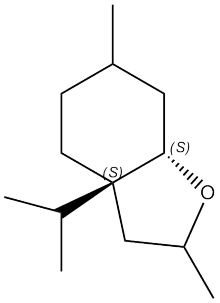
MARKETING NAME(S)
Floriane (≥ 90% concentration of the notified chemical)

CAS NUMBER
2222985-46-6

CHEMICAL NAME
Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (3a*R*,7a*R*)-*rel*-
(The notified chemical is a multi-constituent substance containing inseparable isomers)

MOLECULAR FORMULA
$C_{13}H_{24}O$

STRUCTURAL FORMULA
The relative stereochemistry is shown below.



MOLECULAR WEIGHT
196.33 g/mol

ANALYTICAL DATA

Method	NMR
Remarks	Consistent with structure
Test Facility	International Flavours and Fragrances

Method	IR
Remarks	Consistent with structure
Test Facility	International Flavours and Fragrances

Method	UV
Remarks	Maximum absorbance peaks at wavelength of 199 and 243 nm were observed at pH 7
Test Facility	International Flavours and Fragrances

Method	GC-MS
Remarks	Eight isomers were identified
Test Facility	International Flavours and Fragrances

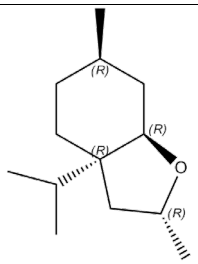
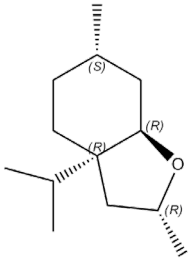
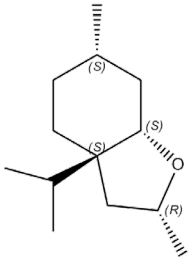
3. COMPOSITION

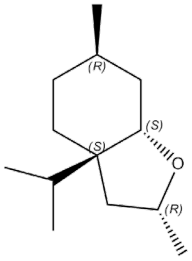
DEGREE OF PURITY
≥ 90% (isomer mixture)

CHEMICAL CONSTITUENTS
The chemical name (see Section 2) includes 4 isomers.

Based on the analytical report submitted, there are 6 inseparable isomers identified using GC-MS for the notified chemical. Four of the isomers are the major constituents, displaying trans-fusion and are represented by the relative stereochemistry (3a*R*,7a*R*). The stereochemistry has been specified in the chemical name (see Section 2).

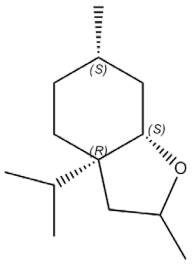
Major constituents

Isomer Name	*Structure*	*Weight %*
Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (2*R*,3a*R*,6*R*,7a*R*)-*rel*-		55.82
Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (2*R*,3a*R*,6*S*,7a*R*)-*rel*-		17.30
Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (2*R*,3a*S*,6*S*,7a*S*)-*rel*-		7.54

Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (2*R*,3a*S*,6*R*,7a*S*)-*rel*-		4.11

Two minor isomers identified by GC-MS are not covered by the chemical name but are considered in this assessment as inseparable constituents of the notified chemical.

Minor constituents

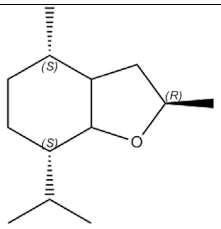
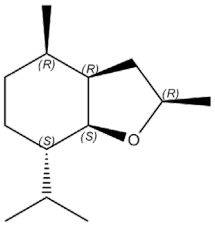
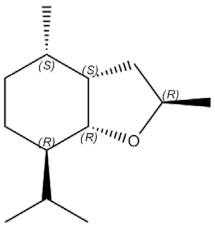
Isomer Name	*Structure*	*Weight %*
Benzofuran, octahydro-2,6-dimethyl-3a-(1-methylethyl)-, (3a*R*,6*S*,7a*S*)-*rel*-*		2.67

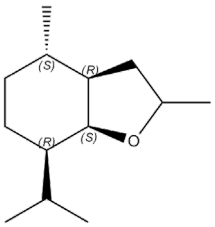
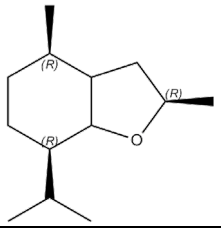
* Refers to two isomers as stereo positions for Carbon 2 have not been separately specified.

IMPURITIES (> 1% BY WEIGHT)

Chemical Name	2,4-dimethyl-7-(propan-2-yl)octahydro-1-benzofuran (mixture of 5 isomers, see below structures)		
CAS No.	Not assigned	*Weight %*	≤ 10
Hazardous Properties	Not determined		

Other components in the reaction mixture were also identified. These components are considered as impurities and are not included in this assessment.

Impurities Isomer Name	*Structure*	*Weight %*
Benzofuran, octahydro-2,4-dimethyl-7-(1-methylethyl)-, (2*R*,4*S*,7*S*)-*rel*-		3.96
Benzofuran, octahydro-2,4-dimethyl-7-(1-methylethyl)-, (2*R*,3a*R*,4*R*,7*S*,7a*S*)-*rel*-		1.89
Benzofuran, octahydro-2,4-dimethyl-7-(1-methylethyl)-, (2*R*,3a*S*,4*S*,7*R*,7a*R*)-*rel*-		1.50

Benzofuran, octahydro-2,4-dimethyl-7-(1-methylethyl)-, (3a*R*,4*S*,7*R*,7a*S*)-*rel*-		1.41
Benzofuran, octahydro-2,4-dimethyl-7-(1-methylethyl)-, (2*R*,4*R*,7*R*)-*rel*-		0.70

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Clear liquid

Property	Value	Data Source/Justification
Freezing Point	< -20 °C	Measured
Boiling Point	241 °C at 98.2 kPa	Measured
Density	922 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.015 kPa at 25 °C	Measured
Water Solubility	4.93 × 10^{-2} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	t½ > 1 year at 25 °C and pH 4, 7, 9	Measured
Partition Coefficient (n-octanol/water)	log P_{ow} = 4.32 at 25 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.59 – 3.76 at 30 °C	Measured
Dissociation Constant	Not determined	No dissociable functionalities
Flash Point	99 °C at 101.3 kPa	Measured
Flammability	Combustible liquid*	Based on measured flash point
Autoignition Temperature	240 °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

* Based on *Australian Standard AS1940* definitions for combustible liquids.

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.

The notified chemical has a flash point of 99 °C. Based on *Australian Standard AS1940* definitions for combustible liquids, a liquid that has a flash point of 150 °C or less is a Class C1 combustible liquid.

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 and will not be imported into Australia in neat form. It will be imported into Australia as a component of finished fragrance oil products. The fragrance oil products will contain the notified chemical at ≤ 10% concentration and will be reformulated locally to produce household and cosmetic consumer 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

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

TRANSPORTATION AND PACKAGING
The finished fragrance oil products containing the notified chemical (at ≤ 10% concentration) will be imported in 205 L polypropylene-lined steel drums. The imported products containing the notified chemical will be transported by road to the International Flavours and Fragrances (IFF) facility in Victoria and then distributed to reformulation sites. The end-use products will be packaged in consumer containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient and incorporated into a variety of cosmetic and household products in Australia. In finished consumer products the maximum proposed use concentrations of the notified chemical are ≤ 1% in fine fragrances and body lotion, ≤ 0.5% in deodorants, and ≤ 1.5% in other cosmetic and household products.

OPERATION DESCRIPTION
The notified chemical will not be manufactured in Australia. No reformulation or repackaging of products containing the notified chemical will occur at the IFF facility. The imported finished fragrance oils containing the notified chemical (at ≤ 10% concentration) will be stored at the IFF facility until they are sold and distributed to customer facilities for reformulation into end-use cosmetic and household products.

Reformulation
At the customer reformulation sites, procedures for incorporating fragrance oil products containing the notified chemical into end-use products will likely vary depending on the nature of the formulated products and may involve both automated and manual transfer steps. In general, it is expected that the products containing the notified chemical will be weighed and added to the mixing tank where mixing with additional additives will occur to form finished cosmetic and household products. Subsequently, automated filling of the reformulated products into containers of various sizes will occur. The blending and filling operations are expected to be typically automated with enclosed systems and adequate ventilation. During the reformation process, samples of products containing the notified chemical will be taken for quality control purposes.

End use

Cosmetic products
The finished cosmetic products containing the notified chemical at ≤ 1% concentration in fine fragrances and ≤ 0.5% concentration in deodorants will be used by consumers and professionals such as beauticians and hairdressers. Depending on the nature of the products, applications may be by hand, spray or through the use of applicators.

Household products
Household products containing the notified chemical at ≤ 1.5% concentration may be used by consumers and professional workers such as cleaners. The products may be used in either closed systems with episodes of controlled procedures, for instance automatic washing machine cycles, or open manual processes including spraying, brushing, dipping, wiping and rinsing.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	Incidental	Incidental
Compounding	4	250
Drum handling	1	250
Drum cleaning	2	200
Maintenance	2	250
Quality control	1	250

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical as a component of finished fragrance oils (at ≤ 10% concentration), only in the unlikely event of an accidental breach of import containers.

Reformulation

During reformulation, dermal, ocular and inhalation exposure of workers to the notified chemical (at ≤ 10% concentration) may occur during weighing, transfer, blending, quality control analysis, cleaning and maintenance. The use of engineering controls including local exhaust ventilation and enclosed systems, and the use of personal protective equipment (PPE) such as coveralls, goggles, impervious gloves and appropriate respiratory protection by workers are expected to minimise exposure to the notified chemical.

End-use

Exposure to the notified chemical in end-use products (at concentrations of ≤ 1% in fine fragrances, ≤ 0.5% in deodorants, and ≤ 1.5% in other cosmetic and household products) may occur in professions where the services provided involve the application of cosmetic products to clients (i.e., hair and beauty salons) or where the cleaning products are used in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposures are also possible. Such professionals may use PPE to minimise repeated exposure and good hygiene practices are expected to be in place. If appropriate PPE is used, exposure of such workers to the notified chemical is expected to be similar or to a lesser extent of that experienced by consumers using the same products.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical (at concentrations of ≤ 1% in fine fragrances, ≤ 0.5% in deodorants, and ≤ 1.5% in other cosmetic and household products) through the use of a wide range of cosmetic and household products. The main route of exposure will be dermal, while ocular and inhalation exposures (e.g. through the use of spray products) are also possible.

Data on typical use patterns of various types of consumer products (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 exposure assessment via the dermal route, Australian use patterns for various products are assumed to be similar to the consumer use patterns in Europe. In the absence of dermal absorption data and based on the low molecular weight of the notified chemical (196.33 g/mol), a dermal absorption (DA) value of 100% was assumed (ECHA, 2017; SCCS, 2012). For inhalation exposure estimation of spray products, a 2-zone approach was used (Steiling *et al*., 2014; Rothe *et al*., 2011; Earnest, Jr, 2009) with an adult inhalation rate of 20 m^3/day (enHealth, 2012). It was conservatively assumed that the fraction of the notified chemical inhaled will be 50%, with the remainder ending up on the targets as intended. A lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was applied in the calculations.

Cosmetic products (dermal exposure)

Product type	*Amount (mg/day)*	*Chemical concentration (%)*	*Retention Factor (RF)*	*Daily systemic exposure (mg/kg bw/day)*
Body lotion	7,820	1.5	1.000	1.8328
Face cream	1,540	1.5	1.000	0.3609

Product type	*Amount (mg/day)*	*Chemical concentration (%)*	*Retention Factor (RF)*	*Daily systemic exposure (mg/kg bw/day)*
Hand cream	2,160	1.5	1.000	0.5063
Fine fragrance	750	1.0	1.000	0.1172
Deodorant (spray)	1,430	0.5	1.000	0.1117
Deodorant (non-spray)	1,500	0.5	1.000	0.1172
Shampoo	10,460	1.5	0.010	0.0245
Conditioner	3,920	1.5	0.010	0.0092
Shower gel	18,670	1.5	0.010	0.0438
Hand wash soap	20,000	1.5	0.010	0.0469
Hair styling products	4,000	1.5	0.100	0.0938
Facial cleanser	800	1.5	0.010	0.0019
Total				**3.2642**

Daily systemic exposure = (Amount × Chemical concentration × RF × DA) / BW
RF = retention factor; DA = dermal absorption; BW = body weight

Household Products (Indirect dermal exposure – from wearing clothes)

Product type	*Amount (g/use)*	*C (%)*	*Product Retained (%)*	*Product Transferred (%)*	*Daily systemic exposure (mg/kg bw/day)*
Laundry liquid	230	1.5	0.95	10	0.0512
Fabric softener	90	1.5	0.95	10	0.0200
Total					**0.0713**

Daily systemic exposure = (Amount × C × PR × PT × DA) / BW
C = chemical concentration; PR = product retained; PT = product transferred; DA = dermal absorption; BW = body weight

Household products (Direct dermal exposure)

Product type	*Frequency (use/day)*	*C (%)*	*Contact Area (cm^2)*	*Product Usage (g/cm^3)*	*Film Thickness (cm)*	*Time Scale Factor*	*Daily systemic exposure (mg/kg bw/day)*
Laundry liquid	1.43	1.5	1,980	0.01	0.01	0.007	0.0005
Dishwashing liquid	3	1.5	1,980	0.009	0.01	0.03	0.0038
All-purpose cleaner	1	1.5	1,980	1	0.01	0.007	0.0325
Total							**0.0367**

Daily systemic exposure = Frequency × C × Contact Area × Product Usage × Film Thickness × Time Scale Factor × DA / BW
C = chemical concentration; DA = dermal absorption; BW = body weight

Aerosol products (Inhalation exposure)

Product type	*Amount (g/day)*	*C (%)*	*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	1.5	1	20	50	1	10	**0.0483**

Daily systemic exposure = [(Amount × C × 20 m^3/day Inhalation Rate × 50% Fraction Inhaled × 0.1) / BW × 1440)] × [(Exposure Duration Zone 1 / Volume Zone 1) + (Exposure Duration Zone 2 / Volume Zone 2)]
C = chemical concentration; BW = body weight

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical. This would result in a combined internal dose of 3.42 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 products (in addition to hair spray) may occur. However, it is considered that the combination of the conservative (screening level) hair spray inhalation exposure assessment parameters, and the aggregate exposure from use of the dermally applied products, which assumes a conservative 100% dermal 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 and deodorants).

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	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 > 5.33 mg/L/4 hour; low toxicity
Skin Corrosion – *in vitro* Human Skin Model Test (EpiDerm™)	non-corrosive
Skin Irritation – *in vitro* Reconstructed Human Epidermis Model Test (EpiSkin™)	non-irritating
Rabbit, acute dermal irritation	mildly irritating
Eye irritation – *in vitro* Reconstructed Human EpiOcular Model Test (EpiOcular™)	non-irritating
Eye irritation – *in vitro* Bovine Corneal Opacity and Permeability (BCOP) Test	no prediction can be made
Rabbit, acute eye irritation	mildly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation, EC3 = 56.6%
Human, skin sensitisation – HRIPT	no evidence of sensitisation at 6% concentration
Rat, repeat dose oral toxicity – 28 days	NOAEL = 744 mg/kg bw/day in females and 670 mg/kg bw/day in males (highest dose tested)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration test in human lymphocytes	non genotoxic

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted. Given the low molecular weight (196.33 g/mol) of the notified chemical and a log P_{ow} of 4.32, absorption across biological membranes may occur.

Acute toxicity

The notified chemical is of low acute oral and dermal toxicity based on studies conducted in rats.

In an acute inhalation study conducted in rats, 1 animal died as a result of 4 hour exposure to a mean achieved atmosphere concentration of 5.33 mg/L of the notified chemical. Dark patches on the lungs were noted and macroscopic abnormalities detected. The study authors concluded that the death of the animal was attributable to systemic toxicity. Dark patches on the lungs were noted at necropsy in 2 of 9 animals that survived until the end of the recovery period. No macroscopic abnormalities were detected amongst the other surviving animals. It was therefore concluded by the study authors that the acute inhalation median lethal concentration (4 hour LC50) of the notified chemical in rats was > 5.33 mg/L.

Irritation

The notified chemical was non-corrosive and non-irritating to skin based on *in vitro* skin corrosion (EpiDerm™) and *in vitro* skin irritation (EpiSkin™) studies.

In a dermal irritation study in rabbits the notified chemical was found to be mildly irritating.

In an *in vitro* eye irritation (EpiOcular™) study the notified chemical was non-irritating. Based on a bovine corneal opacity and permeability (BCOP) test, the *in vitro* irritancy score (IVIS) was between > 3 and ≤ 55, indicating no prediction could be made under the conditions of the test.

The notified chemical was mildly-irritating based on an eye irritation study conducted in rabbits.

Sensitisation

In a mouse Local Lymph Node Assay (LLNA), there was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical and the EC3 value was calculated to be 56.6%. The notified chemical is considered to be a skin sensitiser.

In a repeated insult patch test (RIPT) conducted in human volunteers, 101 subjects completed the study and the notified chemical at 6% concentration did not demonstrate a potential for eliciting dermal irritation or sensitisation under the test conditions.

Repeated dose toxicity
In a 28-day repeated dose oral toxicity study, the notified chemical was administered to rats for a period of 28 days consecutively at dietary concentrations of 1,000, 3,500 and 10,000 ppm, with the actual mean achieved dose levels in the animals being 70, 246 and 670 mg/kg bw/day in males, and 77, 526 and 744 mg/kg bw/day in females, respectively. In animals of either sex treated at the high dose resulted in treatment related effects including centrilobular hepatocyte hypertrophy in liver and follicular cell hypertrophy in thyroid. These effects were also observed in males treated at the mid and low doses. In addition, increased liver and kidney weights were noted in groups treated at the high and mid doses. Females treated at the high dose also showed a statistically significant increase in total protein, cholesterol and bilirubin.

The above microscopic liver and thyroid changes and associated blood chemistry changes were considered by the study authors to be likely adaptive and not adverse. A No Observed Adverse Effect Level (NOAEL) for systemic effects was established by the study authors as 10,000 ppm (equivalent to 744 mg/kg bw/day in females and 670 mg/kg bw/day in males) for the notified chemical based on the highest doses tested with no effects deemed as adverse.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation assay and not considered to be genotoxic in an *in vitro* chromosome aberration test using 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 sensitiser (Category 1B)	H317 – May cause an allergic skin reaction

6.3. Human Health Risk Characterisation
The notified chemical is a skin sensitiser that may cause an allergic skin reaction. At high concentrations, the notified chemical may also cause irritation effects to skin and eye.

6.3.1. Occupational Health and Safety

Reformulation
Reformulation workers may come into contact with the notified chemical at ≤ 10% concentration. Main routes are expected to be dermal and accidental ocular exposure is also possible. Safe work practices, engineering controls and use of PPE, including impervious gloves, coveralls and eye protection would reduce the risk of adverse health effects.

End-use
Cleaners and beauty care professionals will handle end use products containing the notified chemical at ≤ 1.5% concentration. As certain protective measures including PPE may be used by these professionals, the risk to the workers is expected to be of a similar or lesser extent than that experience by members of the public who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

Overall, provided that the recommended controls are being adhered to, 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
Members of the public may experience repeated exposure to the notified chemical through the use of cosmetic and household products (containing the notified chemical at concentrations of ≤ 1% in fine fragrances, ≤ 0.5% in deodorants, and ≤ 1.5% in other cosmetic and household products). The main route of exposure is expected to be dermal with some potential for inhalation and for accidental ocular or oral exposure.

Local effects
The notified chemical is mildly irritating to skin and eye. However, given the relatively low proposed use concentration (≤ 1.5%), significant irritation effects are not expected from the use of finished consumer products containing the notified chemical.

Skin sensitisation
Proposed methods for the quantitative risk assessment of the dermal sensitisation have been the subject of significant discussion (i.e., Api *et al.,* 2008 and RIVM, 2010). Using face cream as an example product that may contain the notified chemical at 1.5% concentration, as a worst case scenario, the Consumer Exposure Level (CEL) for the notified chemical is estimated to be 40.9 μg/cm^2/day (Cadby *et al.,* 2002). When tested in an LLNA study, the notified chemical was a skin sensitiser with an EC3 value of 56.6%. Consideration of the study details and application of appropriate safety factors allowed the derivation of an Acceptable Exposure Level (AEL) of 43.5 μg/cm^2/day. In this instance, the factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (3.16), use/time factor (3.16) and database factor (1), giving an overall safety factor of > 300 (300 used for calculation).

As the CEL is estimated to be less than the AEL, the risk to the public of induction of sensitisation that is associated with the use of face cream (a worst case example of a leave-on cosmetic product) is not considered to be unreasonable. Based on the lower expected exposure level from other cosmetic and household products, by inference, the risk of induction of sensitisation associated with the use of the products containing the notified chemical is also not considered to be unreasonable. However, it is acknowledged that consumers may be exposed to multiple products containing the notified chemical, and a quantitative assessment based on aggregate exposure has not been conducted.

Systemic effects
The potential systemic exposure to a typical user of the public from the use of the notified chemical in cosmetics and household products was estimated to be 3.42 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 670 mg/kg bw/day for males and 744 mg/kg bw/day for females, which was derived from an oral (diet) repeated dose toxicity study in rats, the margin of exposure (MOE) was estimated to be 196 for males and 218 for females, respectively. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and interspecies differences.

Therefore, based on the information available, the risk to the public associated with the maximum proposed use concentrations of the notified chemical at ≤ 1% in fine fragrances, ≤ 0.5% in deodorants, and ≤ 1.5% in other cosmetic and 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
No significant release of the notified chemical is anticipated during storage or transport. Any spills or leaks are expected to be contained by use of sand or inert absorbent and disposed of according to local regulations. Release of the notified chemical at the consumer product manufacturing site, is also anticipated to be very low given the low use concentration in products. Any wash waters resulting from the blending and cleaning operations are likely to be discharged to an on-site wastewater treatment plant or a local municipal treatment plant. Fugitive emissions to air are expected during product sampling and compounding operations due to the volatility of the notified chemical.

RELEASE OF CHEMICAL FROM USE
The notified chemical will be in consumer products in the form of formulated fragrance oils in cosmetics, personal care products and cleaning/household products. Therefore, the majority of the notified chemical is expected to be released into sewers all over Australia. In addition, there is a potential for the notified chemical to be released to the air during use given the volatility of the chemical.

RELEASE OF CHEMICAL FROM DISPOSAL
All spilled material(s) and clean-up absorbent are expected to be collected and placed in sealed containers and disposed of to landfill. Empty containers are anticipated to have very low residues of the notified chemical given

the expected concentration in finished consumer products, and will be disposed of to landfill. Any recycled containers containing residues of the notified chemical are expected to be rinsed, with the rinsate disposed of into the sewer.

7.1.2. Environmental Fate

For the details of the environmental fate studies, refer to Appendix C. The notified chemical is not readily biodegradable and is resistant to hydrolysis; hence it is expected to persist in the environment. The measured bioaccumulation factor (BCF) for the notified chemical ranged between 200 – 530 L/kg indicating it is not likely to bioaccumulate. In soils and sludge the notified chemical is not expected to be mobile, based on its soil organic carbon-water partitioning coefficient (K_{oc}). The notified chemical may end up in the air due to its volatility (i.e., high vapour pressure), but it is not expected to persist in the air, based on an estimated half-life of ~4 hours for the chemical (AopWin v1.92, US EPA, 2012).

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. Based on the worst case scenario it was assumed that there would be no removal of the notified chemical during sewage treatment. 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)	24.4	million
Removal within STP	0%	
Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10	
PEC - River:	0.056	µg/L
PEC - Ocean:	0.56	µg/L

It is expected that there will be some volatilisation and partitioning to sludge during treatment, and hence the PEC are likely to be less than those calculated under the worst case scenario above. Based on the estimated log P_{ow} of the notified chemical, in addition to the fact that the notified chemical is not readily biodegradable, it was estimated that there may be up to 70% removal of the notified chemical during sewage treatment processes, mainly due to volatilisation (~50%), but also partitioning to sludge (~20%).

Based on 20% of the notified chemical partitioning to sludge, soil PECs resulting from biosolids application was determined. Partitioning to biosolids in sewage treatment plants (STPs) Australia-wide may result in an average biosolids concentration of 1.0 mg/kg (dry weight). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1,500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 7.0×10^{-3} mg/kg in applied soil. As the notified chemical is not readily biodegradable it was assumed that it can accumulate in soil. The concentration of the notified chemical in soil under repeated biosolids application may approximate 3.5×10^{-2} and 7.0×10^{-2} mg/kg after 5 and 10 years, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 2.7 mg/L 96 h LC50 = 1.1 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 9.4 mg/L	Toxic to aquatic organisms
Algal Toxicity	72 h ErC50 = 16 mg/L	Harmful to algae

Inhibition of Bacterial Respiration	3 h IC50 > 1,000 mg/L	Not inhibitory to bacterial respiration.
Earthworm Toxicity	14 d LC50 = 73 mg/kg dry weight	Moderately toxic to earthworms.

The empirical aquatic toxicity data for the notified chemical presented in the table above indicates that it is toxic to aquatic organisms. 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 2: Toxic to aquatic life".

7.2.1. Predicted No-Effect Concentration

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

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 Fish	1.1	mg/L
Assessment Factor	100	
Mitigation Factor	1.0	
PNEC:	11	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	11	0.051
Q - Ocean	0.056	11	0.0051

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 their maximum annual importation quantity and use pattern. The notified chemical is likely to persist in the environment, but is not expected to bioaccumulate. Therefore, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -20 °C

Method OECD TG 102 Melting Point/Melting Range
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks The test substance showed no change in appearance during cooling from 20 to -21 °C
Test Facility Harlan (2014a)

Boiling Point 241 °C at 98.2 kPa

Method OECD TG 103 Boiling Point
EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks Differential scanning calorimetry method was used
Test Facility Envigo (2016a)

Density 922 kg/m^3 at 20 ± 0.5 °C

Method OECD TG 109 Density of Liquids and Solids
EC Council Regulation No 440/2008 A.3 Relative Density
Remarks The pycnometer method was used
Test Facility Envigo (2016a)

Vapour Pressure 0.015 kPa at 25 °C

Method OECD TG 104 Vapour Pressure
EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks Vapour pressure balance method was used
Test Facility Envigo (2016b)

Water Solubility 4.93×10^{-2} g/L at 20 ± 0.5 °C

Method OECD TG 105 Water Solubility
EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks Slow stir adaptation of the standard test method
Test Facility Harlan (2014a)

Hydrolysis as a Function of pH t½ > 1 year at 25 °C and pH 4, 7, 9

Method OECD TG 111 Hydrolysis as a Function of pH
EC Council Regulation No 440/2008 C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH

pH	*T (°C)*	*t½ years*
4	50.0 ± 0.5 °C	> 1
7	50.0 ± 0.5 °C	> 1
9	50.0 ± 0.5 °C	> 1

Remarks At pH 4 and 7 less than 90% of the initial concentration of the test substance was recovered for an incubation time of 120 hours due to suspected volatility. Therefore, the testing duration was increased to 356 hours for testing at pH 4 and 7 (as opposed to 120 hours for testing at pH 9) to evaluate temporal trends. The concentration of the test substance decreased slightly over time indicating some loss of the notified chemical from the test system, probably due to volatilisation. Replicate analysis was also increased from two to four replicate samples in testing at pH 4 and 7 for 356 hours. The relative distribution of individual components/peaks did not show significant shifts during incubation of sample solutions under the test conditions.

It was extrapolated that < 10% hydrolysis after 5 days at 50 °C was equivalent to a half-life greater than 1 year at 25 °C. Therefore, the test substance is considered to be resistant to

hydrolysis.
Test Facility Envigio (2016a)

Partition Coefficient (n-octanol/water) $\log P_{ow} = 4.32$ at 25.0 ± 0.5 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks Slow-stirring method, which was designed to be compatible with the above methods, involving slow stirring of a stock solution of the test substance with a volume of n-octanol saturated water until an equilibrium concentration was reached. There was no statistically significant temporal increase or decrease in test substance concentration, confirming the equilibrium between water and n-octanol phases.

The log P_{ow} is calculated based on a weighted average of series tests derived from three different vessels, with a variance weighted standard deviation of 3.37×10^{-3}.
Test Facility Envigio (2016a)

Surface Tension 64.5 mN/m at 21.5 ± 0.5 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions
EC Council Regulation No 440/2008 A.5 Surface Tension
Remarks Concentration: at 90% saturation
Test Facility Envigo (2016a)

Adsorption/Desorption – screening test $\log K_{oc} = 3.59 – 3.76$ at 30 °C

Method OECD TG 106 Adsorption - Desorption Using a Batch Equilibrium Method
Remarks HPLC method determined based on two peaks
Test Facility Envigo (2016c)

Flash Point 99 ± 2 °C at 100.4 kPa

Method EC Council Regulation No 440/2008 A.9 Flash Point
Remarks A closed cup flash point tester was used.
Test Facility Envigo (2016c)

Flammability Non-pyrophoric

Method EC Council Regulation No 440/2008 A.13 Pyrophoric properties
Remarks Three filter papers were observed for ignition or charring
Test Facility Envigo (2016d)

Autoignition Temperature 240 ± 5 °C at 100.2 – 101.5 kPa

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Remarks A flask heater was used
Test Facility Envigo (2016c)

Explosive Properties Predicted negative

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks The prediction was based on the chemical structure
Test Facility Envigo (2016c)

Oxidizing Properties Predicted negative

Method EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids)
Remarks The prediction was based on the chemical structure

Test Facility Envigo (2016c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 420 Acute Oral Toxicity - Fixed Dose Method
EC Council Regulation No 440/2008 B.1 bis Acute toxicity (oral) fixed dose method

Species/Strain: Rat/Wistar (RccHan™:WIST)
Vehicle: Arachis oil BP
Remarks - Method: No analysis was conducted to determine the homogeneity, concentration or stability of the test substance formulation.

No other major deviations from the test guideline were reported.

For the purpose of the 2,000 mg/kg bw dose level the test substance was used as supplied.

RESULTS

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

LD50: > 2,000 mg/kg bw
Signs of Toxicity: No signs of systemic toxicity were noted in the animal treated at 300 mg/kg bw or at 2,000 mg/kg bw.

Hunched posture and ataxia were observed in all animals of group 3. Other signs of systemic toxicity noted in these animals were noisy respiration, increased salivation, pilo-erection, tiptoes gait, sneezing and red/brown staining around the eyes. These 4 animals appeared normal 2 days after dosing.
Effects in Organs: No abnormities were observed at necropsy
Remarks - Results: All animals showed expected body weight gain during the observation period.

CONCLUSION: The notified chemical is of low acute toxicity via the oral route.

TEST FACILITY: Envigo (2016e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test
EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal) – Limit Test

Species/Strain: Rat/Wistar (RccHan™:WIST)
Vehicle: None
Type of dressing: Semi-occlusive
Remarks - Method: The test substance was used as supplied.

Absorption of the test substance was not determined.

No major deviations from the test guideline were reported.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	No signs of dermal irritation were noted
Signs of Toxicity - Systemic	No signs of systemic toxicity were observed
Effects in Organs	No abnormities were observed at necropsy
Remarks - Results	All animals had expected body weight gain during the observation period

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

TEST FACILITY Envigo (2016f)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 403 Acute Inhalation Toxicity
EC Council Regulation No 440/2008, 93/21/EEC B.2 Acute Toxicity (Inhalation)

Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	None
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	Mass median aerodynamic diameter = 3.40 μm with an average geometric standard deviation of 2.50
Remarks - Method	No major deviations from the test guideline were reported.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	10 (5 M/5 F)	31.7	5.33 ± 0.14	1/10

LC50	> 5.33 mg/L/4 hours
Signs of Toxicity	During exposure, all animals exhibited decreased respiratory rate, ataxia and isolated instances of lethargy. One day after exposure, 1 male animal exhibited decreased respiratory rate, laboured respiration, dehydration, hypothermia and prostration. This animal was euthanised on Day 1. All remaining animals exhibited decreased respiratory rate, hunched posture and pilo-erection. All female animals also exhibited sneezing. The surviving animals (9/10) recovered and appeared normal from Day 5 – 6 post exposure.
Effects in Organs	Dark patches on the lungs were noted at necropsy in the animal that was euthanised. Dark patches on the lung were also noted in 2 female animals that survived until the end of the observation period. No macroscopic abnormalities were detected in the other 7/10 animals.
Remarks - Results	A slight loss of body weight was noted in all male animals and 4 of 5 female animals on the day after exposure. All animals recovered from the initial body weight loss within a week and exhibited normal growth during the second week of the observation period. Due to the clinical observations noted and macroscopic abnormalities detected, the study authors concluded that the contributing factor to the death of 1 animal may have been systemic toxicity.

CONCLUSION The notified chemical is of low acute toxicity via inhalation.

TEST FACILITY Envigo (2016g)

B.4. Irritation – skin (*in vitro* EpiSkin™ Reconstituted Human Epidermis Model)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method
EC Council Regulation No 440/2008 B.46. *In vitro* Skin Irritation – Reconstructed Human Epidermis Model Test (2009)

Vehicle None

Remarks - Method The EpiSkin™ test system was used. The positive control was sodium dodecyl sulfate (SDS) at a concentration of 5% and the negative control was Dulbecco's Phosphate Buffered Saline (DPBS) with Ca^{2+} and Mg^{2+}. Test substance was used as supplied.

No major deviations from the test guideline were reported.

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*
Negative control	0.900 ± 0.030	100* ± 3.4
Test substance	0.878 ± 0.119	97.6 ± 13.2
Positive control	0.122 ± 0.052	13.6 ± 5.8

OD = optical density; SD = standard deviation
* The mean % viability of the negative control tissue is set at 100%

Remarks - Results The solution containing the test substance was colourless. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) solution containing the test substance did not turn blue, indicating that the test substance did not interfere with MTT reaction.

The criteria for acceptance of both the negative and positive controls were satisfied, as were the requirements for standard deviation between the replicates.

As the relative mean viability of tissues treated with the test substance was > 50%, the test substance does not meet the criteria for classification according to the GHS.

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

TEST FACILITY Envigo (2016h)

B.5. Corrosion – skin (*in vitro* EpiDerm™ Reconstructed Human Epidermis Model)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 431 *In vitro* Skin Corrosion - Human Skin Model Test
EC Council Regulation No 440/2008 B.40 BIS. *In vitro* Skin Corrosion - Human Skin Model Test

Vehicle None

Remarks - Method The EpiDerm™ test system was used. Test substance was used as supplied.

The test substance was able to directly reduce MTT. Therefore, an additional procedure using freeze-killed tissues was performed. However, the results obtained showed that negligible interference due to direct reduction of MTT occurred. It was concluded by the study authors that it

was unnecessary to use the results of the freeze-killed tissues for quantitative correction of results or for reporting purposes.

The positive control used was potassium hydroxide (8 N) and the negative control was sterile distilled water.

RESULTS

Exposure of 3 minutes

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*
Negative control	1.657 ± 0.055	100*
Test substance	1.739 ± 0.182	104.9
Positive control	0.150 ± 0.035	9.0

Exposure of 60 minutes

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*
Negative control	1.784 ± 0.049	100*
Test substance	1.671 ± 0.078	93.6
Positive control	0.106 ± 0.019	5.9

OD = optical density
* The mean % viability of the negative control tissue is set at 100%

Remarks - Results

Results for the test substance were similar to the results of the negative control at both exposure durations.

The solution containing the test substance did not become coloured, indicating that the test substance did not have the potential to cause colour interference.

The acceptance criteria for both the negative and positive controls were satisfied, and the variation between replicates was satisfactory.

As the relative mean viability of tissues exposed to the test substance was > 50% after both 3 minute and 60 minute exposure, the test substance did not meet the criteria for classification according to the GHS.

CONCLUSION

The notified chemical was not corrosive to the skin under the conditions of the test.

TEST FACILITY

Envigo (2016i)

B.6. Irritation – skin

TEST SUBSTANCE

Notified chemical

METHOD

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	2
Vehicle	None
Observation Period	14 days
Type of Dressing	Semi-occlusive
Remarks - Method	Test substance was used as supplied.

No major deviations from the test guideline were reported.

RESULTS

Lesion	*Mean Score* Animal No.*	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*

	1	2			
Erythema/Eschar	2	2	2	< 14 d	0
Oedema	2	2	2	< 14 d	0

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

Remarks - Results

Very slight erythema was noted at both treated skin sites immediately after patch removal. One hour after patch removal very slight erythema and very slight oedema were detected. Well-defined erythema and slight oedema were noted at both treated skin sites at the 24, 48 and 72 hour observations.

Severe desquamation, preventing accurate evaluation of erythema and oedema was observed at one treated skin site with moderate desquamation noted at the other treated skin site at the Day 7 observation. Both treated skin sites appeared normal at the Day 14 observation.

The test substance produced a primary irritation index of 4.0 and was classified by the study authors as a moderate irritant to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

CONCLUSION

The notified chemical is a mild irritant to the skin.

TEST FACILITY

Envigo (2016j)

B.7. Irritation – eye (*in vitro* Bovine Corneal Opacity and Permeability (BCOP) Assay)

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

Vehicle

None

Remarks - Method

The BCOP test system was used. The positive control was ethanol and the negative control was sodium chloride solution at 0.9% concentration. Test substance was used as supplied.

No major deviations from the test guideline were reported.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0.7	0.010	0.8
*Test substance**	2.7	0.075	3.8
*Positive control**	30.7	1.210	48.8

IVIS = *in vitro* irritancy score * Corrected for background values

Remarks - Results

The acceptance criteria for both the negative and positive controls were satisfied, and the variation between replicates was satisfactory.

The corneas treated with the test substance were clear post-treatment and incubation. The corneas treated with the negative control were clear, and the corneas treated with the positive control were cloudy.

As the *in vitro* irritancy score (IVIS) for the test substance was 3.8, the test substance does not meet the criteria for classification as category 1 irritant or no irritation. According to the prediction model no prediction of eye irritation can be made for the test substance as the IVIS score was > 3 and ≤ 55.

CONCLUSION	No prediction could be made for the notified chemical under the conditions of the test.
TEST FACILITY	Envigo (2016l)

B.8. Irritation – eye (*in vitro* EpiOcular™ Reconstructed Human Model)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 492 Reconstructed Human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage
Vehicle	None
Remarks - Method	The EpiOcular™ test system was used. The test substance was used as supplied. The positive control was methyl acetate and the negative control was sterile distilled water. The test substance was able to directly reduce MTT. Therefore, an additional procedure using freeze-killed tissues was performed. However, the results obtained showed that negligible interference due to direct reduction of MTT occurred. It was concluded by the study authors that it was unnecessary to use the results of the freeze-killed tissues for quantitative correction of results or for reporting purposes. The mean OD_{562} of the negative control tissues was 2.503, which was marginal compared to the assay acceptance criterion of 2.500. This was considered borderline and deemed unnecessary to repeat the test by the study authors. The deviations were considered by the study authors not to affect the purpose or integrity of the study.

RESULTS

Test material	*Mean OD_{562} of duplicate tissues*	*Relative mean viability (%)*
Negative control	2.503	100*
Test substance	1.645	65.7
Positive control	0.853	34.1

OD = optical density
* The mean % viability of the negative control tissue is set at 100%

Remarks - Results	The acceptance criteria for both the negative and positive controls were satisfied, and the variation between replicates was satisfactory. As the relative mean viability of tissues exposed to the test substance was > 60% after 30 minutes exposure period followed by the 2 hour post-exposure incubation period, the test substance does not meet the criteria for classification according to the GHS.
CONCLUSION	The notified chemical was considered to be non-irritating to the eye under the conditions of the test.
TEST FACILITY	Envigo (2016k)

B.9. Irritation – eye

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

Species/Strain	Rabbit/New Zealand White
Number of Animals	2
Observation Period	7 days
Remarks - Method	Test substance was used as supplied. No major deviations from the test guideline were reported.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: redness	1.33	1.33	2	< 7 d	0
Conjunctiva: chemosis	0.67	1	2	< 7 d	0
Conjunctiva: discharge	0.67	0.33	2	< 7 d	0
Corneal opacity	0	0	0	–	0
Iridial inflammation	0	0.33	1	< 48 h	0

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

Remarks - Results	No corneal effects were noted during the study. Iridial inflammation was noted in the treated eye of the first animal 1 hour after the treatment, and in the treated eye of the second animal at the 24 hour observation. Moderate conjunctival irritation was noted in both treated eyes 1 and 24 hours after treatment with minimal conjunctival irritation observed at the 48 and 72 hour observations. All ocular effects had resolved in both animals at the 7 day observation. Both animals showed expected gain in body weight during the study.
CONCLUSION	The notified chemical is a mild irritant to the eye.
TEST FACILITY	Envigo (2016m)

B.10. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/CaCrl
Vehicle	Acetone/olive oil 4:1
Preliminary study	Yes
Positive control	α-Hexylcinnamaldehyde
Remarks - Method	No major deviations from the test guideline were reported.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5 F	2,130.21 ± 427.75	–
25	5 F	3,523.92 ± 614.61	1.65
50	5 F	6,102.91 ± 1,318.10	2.86
100	5 F	8,356.83 ± 1,657.03	3.92
Positive Control			

25	5 F	11,020.69 ± 5,176.54	5.17

EC3	56.60%
Remarks - Results	There were no deaths. No signs of systemic toxicity were observed in the test or control animals. Body weight change of test animals between Day 1 and 6 were comparable to the corresponding control animals over the same period. The test substance meets the criteria for classification as a skin sensitiser (sub-category 1B) according to the GHS.
CONCLUSION	There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Envigo (2016n)

B.11. Skin sensitisation – human volunteers

TEST SUBSTANCE	Notified chemical (at 6% concentration)
METHOD	Repeated insult patch test with challenge
Study Design	Induction Procedure: Patches containing 0.2 mL test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications. Patches were removed by the applicants after 24 hours and graded after additional 24 hours (or 48 hours for patches applied on Friday). Sites were graded at 24 and 48 hour post-patch removal. Rest Period: 10 – 15 days Challenge Procedure: A patch was applied to previously untreated test sites. Patches were removed by the test facility technician after 24 hours. Sites were re-evaluated at 48 and 72 hour.
Study Group	90 F, 22 M; age range 18 – 70 years
Vehicle	Alcohol
Remarks - Method	The test substance was spread on a 3.63 cm^2 occlusive patch and applied to the patch site.
RESULTS	
Remarks - Results	101/112 subjects completed the study. Eleven subjects discontinued participation in the study for reasons unrelated to the test material. No adverse events were reported during the study.
CONCLUSION	The notified chemical at 6% concentration was non-sensitising under the test conditions.
TEST FACILITY	CRL (2015)

B.12. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Rat/Wistar (RccHan™:WIST)
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Basal laboratory diet
Remarks - Method	No major deviations from the test guideline were reported.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration*			*Mortality*
		Nominal dietary concentration (ppm)	*Actual mean dose level (mg/kg bw/day)*		
			Male	Female	
control	10 (5 M/5 F)	0	0	0	0/10
low dose	10 (5 M/5 F)	1,000	70.2	77.2	0/10
mid dose	10 (5 M/5 F)	3,500	246.2	252.8	0/10
high dose	10 (5 M/5 F)	10,000	669.7	744.3	0/10
control recovery	10 (5 M/5 F)	0	0	0	0/10
high dose recovery	10 (5 M/5 F)	10,000	669.7	744.3	0/10

Mortality and Time to Death
All animals survived until scheduled necropsy.

Clinical Observations
No abnormality observed during the study and 14 day post exposure observation period.

One recovery female had fur loss between Days 41 and 43. This was considered by the study authors as incidental and unrelated to treatment.

Animals of either sex treated with high dose showed a reduction in body weight gain during the first week of treatment. Females continued to show a reduction in body weight gain during the second week of treatment and males showed a slight reduction during the final two weeks of treatment. No toxicological significant effect on body weight gain was evident in animals of either sex treated with mid or low doses.

Animals of either sex treated with high dose showed a reduction in food consumption and food conversion efficiency during the first week of treatment. No such effects were detected in animals of either sex treated at mid and low doses.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
There were no treatment related adverse effects on clinical chemistry, haematology or urinalysis reported.

Females treated with high dose, showed a statistically significant increase in total protein, cholesterol and bilirubin. Although all of the individual values were within the historical control ranges, the study authors concluded that a potential association with the histopathological changes observed in the liver as a result of the test substance cannot be excluded.

No toxicologically significant effects were detected in males treated with any dose level or in females treated with mid or low doses. Recovery males at the high dose showed a statistically significant increase in albumin/globulin ratio and a statistically significant reduction in phosphorus. All of the individual values were within the historical control ranges for these parameters. In the absence of an observed similar effect at the end of the treatment period, the inter-group difference was considered by the study authors to be of no toxicological importance.

Effects in Organs
No toxicologically significant macroscopic abnormalities were detected.

The following microscopic abnormalities were detected but not considered by the study authors to be toxicologically significant:

Liver: minimal centrilobular hepatocyte hypertrophy was evident in animals of either sex treated with high dose and in males treated with mid and low doses. The microscopic changes in the liver correlated with a significant increase in liver weight in treated males and females. The study authors considered the hepatic response to be consistent with enzyme induction leading to hepatocellular hypertrophy. Minimal liver hypertrophy in the absence of associated degenerated-necrotic changes and the lack of significant changes of the serum liver enzymes activity was considered by the study authors to be adaptive and not adverse. Partial recovery was evident in animals of either sex following 14 days without treatment and minimal centrilobular hypertrophy was

still present in a few animals following the recovery period.

Thyroid: follicular cell hypertrophy was evident in animals of either sex treated with high dose and in males treated with mid and low dose. The morphological changes seen in the thyroid were considered by the study authors to be an adaptive physiological response of the thyroid gland to hepatic enzyme induction and stimulation of the hypothalamic-pituitary-thyroid axis. Full recovery was evident in affected animals of either sex following 14 days without treatment.

Kidneys: accumulation of hyaline droplets in the tubular epithelium of the proximal tubules consistent with the accumulation of alpha-2u-globulin was evident in males from all treatment groups. The increase in hyaline droplets in the kidney was not associated with any damage to the cells or any sign of compromised function. Hyaline droplet accumulation is known to be specific to the male rats and is not considered to be relevant for humans. Full recovery was evident in affected males following 14 days without treatment.

Remarks – Results

The oral administration of the test substance to rats for a period of 28 consecutive days at dietary concentrations of 1,000, 3,500 and 10,000 ppm resulted in some treatment related effects. The study authors concluded that the microscopic liver and thyroid changes, and associated blood chemistry changes identified in animals of either sex treated at high dose and males at low and mid doses, likely to represent adaptive changes. The study authors concluded that 10,000 ppm dietary exposure, the highest level tested, may be regarded as a No Observed Adverse Effect Level (NOAEL) for animals of either sex.

CONCLUSION

The NOAEL was established by the study authors for the notified chemical as the highest dose tested equivalent to 744.3 mg/kg bw/day in females and 669.7 mg/kg bw/day in males.

TEST FACILITY Envigo (2016o)

B.13. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test Plate incorporation and pre incubation procedure
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98 and TA100 *Escherichia coli*: WP2*uvr*A
Metabolic Activation System	Phenobarbitone (PB) and β-Naphthoflavone (βNF) induced rat liver S9 mix
Concentration Range in Main Test	a) With metabolic activation: 50 – 5,000 µg/plate b) Without metabolic activation: 50 – 5,000 µg/plate
Vehicle	Dimethyl sulphoxide (DMSO)
Remarks - Method	Concentrations for main test were chosen based on the plate incorporation method conducted on TA100, TA1535 and WP2*uvr*A (base-pair substitution type) and on TA98 and TA1537 (frameshift type) results. Tests with vehicle control and positive controls were run concurrently. Positive controls were: - With metabolic activation: 2-aminoanthracene (TA1537, TA1535, TA100 and WP2*uvr*A) and benzo[*a*]pyrene (TA98) - Without metabolic activation: *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (TA1535, TA100 and WP2*uvr*A); 9-aminoacridine (TA1537), 4-nitroquinoline-1-oxide (TA98). No major deviations from the test guideline were reported.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*

Absent				
Test 1 (plate incorporation)*	> 5,000	–	> 5,000	negative
Test 2 (pre incubation)	–	> 5,000	> 5,000	negative
Present				
Test 1 (plate incorporation)*	> 5,000	–	> 5,000	negative
Test 2 (pre incubation)	–	> 5,000	> 5,000	negative

* Preliminary cytotoxicity test only

Remarks - Results

The test substance did not result in an increase of more than twice the number of revertant colonies in comparison to the negative control. In addition, no dose-related response was observed in any strains of base-pair substitution type or frame-shift type, with or without metabolic activation.

The positive and negative controls provided a satisfactory response confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY

Harlan laboratories (2014b)

B.14. Genotoxicity – *in vitro* mammalian chromosome aberration test

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test
EC Directive 2000/32/EC B.10 Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test

Species: Human
Cell Type: Peripheral blood lymphocytes
Metabolic Activation System: Phenobarbitone (PB) and β-Naphthoflavone (βNF) induced rat liver S9 mix
Vehicle: Acetone
Remarks - Method: A preliminary toxicity test was conducted with a dose range of 7.66 – 1,962 µg/mL.

Mitomycin C (MMC) and cyclophosphamide (CP) were used as positive controls.

No major deviations from the test guideline were reported.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1 (main)	0*, 7.5*, 15*, 30*, 60, 80, 100, 120	4 h	20 h
Test 2 (main)	0*, 3.75, 7.5, 15*, 30*, 60*, 80 120	24 h	24 h
Present			
Test 1 (main)	0*, 7.5*, 15*, 30*, 60, 80, 100, 120	4 h	20 h
Test 2 (main)	0*, 3.75, 7.5, 15*, 30*, 45*, 60*, 80	4 h	20 h

* Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1 (main)	≥ 7.5	> 120	negative
Test 2 (main)	≥ 30	> 120	negative
Present			
Test 1 (main)	≥ 7.5	> 120	negative

Test 2 (main)	≥ 15	> 80	negative

Remarks - Results

In the preliminary toxicity test precipitation of the test substance was observed at the end of the exposure at ≥ 245.25 µg/mL in the 4(20)-hour exposure group in the absence of S9-mix, 490.5 µg/mL in the 4(20)-hour exposure group in the presence of S9-mix and at ≥ 122.63 µg/mL in the 24 hour continuous exposure group.

Haemolysis was also observed following exposure to the test item in the preliminary test at ≥ 30.66 µg/mL in the 4(20)-hour exposure group in the absence of S9-mix, ≥ 15.33 µg/mL in the 4(20)-hour exposure group in the presence of S9-mix and at ≥ 61.31 µg/mL in the 24 hour continuous exposure group.

In main test 1 and 2, the test substance did not induce any statistically significant increases in the frequency of cells with chromosome aberrations either in the absence or presence of metabolic activation.

The positive and negative controls provided a satisfactory response confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY

Harlan (2014c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability – Closed bottle test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test
Inoculum	Secondary treated effluent from a domestic wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Theoretical Oxygen Demand (ThOD)
Remarks - Method	The purity of the test substance was 94%. Sodium benzoate was used as a reference substance (procedural control). An inoculum blank, a toxicity control and a procedural control were also included in the test design. No information was provided about the characteristics of the stock solution. It was assumed that the test substance was well mixed. The test substance concentration in the test suspension was less than the water solubility of the test substance (~ 49 mg/L). No major deviations from the test guideline were reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
7	10.93	7	63.47
14	4.86	14	71.56
28	0.55	28	not reported

Remarks - Results	All validity criteria were met. Oxygen depletion in the inoculum blank was 0 mg/L. The residual oxygen concentration in the test bottles was > 5.8 mg/L in all treatments and the inoculum control. The pH of the test solution was in the range of 6.99 – 7.67 over the testing period. The percentage degradation of the reference compound surpassed the threshold level of 60% by Day 7 (63.47%) and reached 71.56% degradation by Day 14. Therefore, the test indicates the suitability of the inoculums. The percentage degradation of the toxicity control surpassed the threshold level of 25% by Day 14 (35.64%; 14 days), showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The mean percentage degradation of the test substance was 4.86% on Day 14 and 0.55% on Day 28, respectively. The 10 day window requirement was not considered as the test substance is a mixture of isomers.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Suzhou (2015a)

C.1.2. Ready biodegradability – CO_2 evolution test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test
Inoculum	Sewage sludge micro-organisms were obtained from the secondary treatment stage of a sewage treatment plant, which treats predominantly domestic sewage.
Exposure Period	28 days
Auxiliary Solvent	Acetone

Analytical Monitoring	CO_2 production was determined by measuring inorganic carbon (IC), using TOC analyser.
Remarks - Method	The purity of the test substance was > 87.4%. Sodium benzoate was used as a reference substance. The test substance concentration in the test suspension was less than the water solubility (~ 49 mg/L). Preliminary water solubility tests showed an oily film being formed on the surface in the mineral medium. The most accurate final dispersion, involving the use of an auxiliary solvent was used to prepare the test substance. There is no description of the test substance used in the test, but it was assumed there was a clear colourless media column with an oily slick on the surface and filter paper visible on the bottom of the vessel, based on the results of preliminary testing. No major deviations from the test guideline were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
8	1	8	86
14	0	14	87
28	1	28	89

Remarks - Results	All validity criteria of the test guideline were met. The percentage degradation of the reference compound surpassed the threshold level of 60% by Day 8 (86% degradation) and reached 87% degradation by Day 14. Therefore, the test indicates the suitability of the inoculums. The percentage degradation of the toxicity control surpassed the threshold level of 25% by Day 14 (40% degradation), showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The mean percentage degradation of the test substance was 1% on Day 28. The 10 day window requirement was not considered as the test substance is a mixture of isomers.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Harlan (2014d)

C.1.3. Ready biodegradability – Modified MITI test (I)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)
Inoculum	Activated sludge originated from 10 locations in Japan, including surface waters, sediments and return sludge from sewage treatment plants.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical oxygen demand (BOD)
Remarks - Method	The purity of the test substance was 98%. Aniline was used as a reference substance. The test substance concentration in the test suspension was greater than the water solubility of the test substance (~ 49 mg/L). At the start of incubation the test substance was not dissolved in the test solutions. The pH of the test solutions was not measured at the end of incubation because of the volatility of the test substance. The percentage biodegradation of each of the peaks corresponding to the test substance components was calculated in order to confirm if there was any change over the testing period. No major deviations from the test guideline were reported.

RESULTS

Test substance in vessels 2, 3 and 4		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	1, 0 and 0	7	83
14	0, -4 and -5	14	90
21	0, -5 and -6	21	89
28	-1, -6 and -7	28	91

Remarks - Results: All validity criteria of the test guideline were met. The oxygen uptake of the control blank (≈ inoculum blank) was 22 mg/L. The pH at the start of the test was 7. The difference between the extremes of replicate values for the percentage biodegradation of the test substance was 3%. The percentage degradation of aniline calculated from the oxygen consumption was 83% after 7 days and 90% after 14 days. The mean percentage degradation of the test substance was < 1% on Day 14 and 28, respectively. The 10 day window requirement was not considered as the test substance is a mixture of isomers.

CONCLUSION: The notified chemical is not readily biodegradable.

TEST FACILITY: CERI (2016a)

C.1.4. Bioaccumulation

TEST SUBSTANCE: Notified chemical

METHOD: Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body

Species: Common carp (*Cyprinus carpio*)

Exposure Period: Exposure: 28 days; Depuration: depuration period not included in the study

Auxiliary Solvent: None

Concentration Range: Nominal: 0.005 – 0.055 mg/L

Analytical Monitoring: Gas chromatography-mass spectrometry (GC-MS)

Remarks - Method: High exposure level (level 1) and low exposure level (level 2) of 50 and 5 µg/L were included in the test design. The concentration of the test substance was below its limit of solubility in the test water. The bioconcentration test was carried out in a flow-through system, where the flow rate for 1,152 L/day of test water was 0.04 mL/min for stock solution and 800 mL/min for dilution water.

The test water before and during the uptake phase was maintained at a temperature range of 24 to 25 °C, dissolved oxygen range of 7.3 to 8 mg/L and pH range of 7.8 to 8.0. Total organic carbon before and after the uptake phase ranged from 21 to 26 mg C/L. Total hardness of the level 1 test solution and the control was 19 and 25 mg $CaCO_3$/L, respectively.

Hydrogenated castor oil (HCO-40) and *N,N*-dimethylformamide were used as solubilising agents to prepare the stock solution for the test.

No major deviations from the test guideline were reported.

RESULTS

Bioconcentration Factor: Steady state BCF (BCF_{ss}) ranged between 200 – 530 L/kg and 170 – 380 L/kg for level 1 and 2 exposure concentrations, respectively.

CT50

Remarks - Results: The validity criteria of test method were met. The water temperature variation was less than ± 2 °C of set water temperature 25 °C. The

concentration of dissolved oxygen did not fall below 60% of the saturated concentration of 8.1 mg/L at 25 °C. The concentration of the test substance in the test tank was maintained within ± 20% of the mean of the measured values during the uptake phase. The mortality or other adverse effects/disease in both control and test groups was < 10% at the end of the test. Eight peaks were detected with GC-MS analysis, and no interfering peak was observed at the peak positions of the test substance for the control water before or after the uptake phase. The measured concentrations of the test substance test water were maintained at ≥ 80% of nominal concentrations and the variations were within ± 20% of the average measured concentrations.

The average test fish concentration from samples taken at Day 15, 22, 26 and 28 were within ± 20% of each other for all peaks. Therefore, it was confirmed that steady-state had been reached in the uptake phase.

CONCLUSION The notified chemical is not expected to bioaccumulate.

TEST FACILITY CERI (2016b)

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

Species	Zebrafish (*Brachydanio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	Acetone
Water Hardness	50 mg $CaCO_3$/L
Analytical Monitoring	GC-flame ionisation detector (FID)
Remarks – Method	The test conditions (pH, dissolved oxygen and temperature) were suitable for the test species. The body lengths of test fish were within the range of 2.4 – 3.0 cm, which met the requirement of the test guideline. Test solutions were renewed every 24 hours. Acute toxicity test with the reference substance potassium dichromate was conducted to test the sensitivity of the test species. US EPA Probit analysis program (version 1.5) was used for calculating LC values. No major deviations from the test guideline were reported.

RESULTS

Concentration mg/L		*Number of Fish*	*% Cumulative mortality*				
Nominal	*Actual (geometric mean 0 – 96 h)*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	–	7	0	0	0	0	0
Solvent control	–	7	0	0	0	0	0
0.65	0.55	7	0	0	0	0	0
0.98	0.80	7	0	0	0	0	0
1.47	1.23	7	0	0	0	0	14
2.21	1.75	7	0	0	0	0	14
3.32	2.66	7	0	0	14	14	43
4.98	4.48	7	0	14	14	14	86

LC50	2.7 mg/L at 96 hours (95% confidence limits of 2.0 – 4.3)
Remarks – Results	The validity criteria of the test guideline OECD 203 were met. The measured test substance concentrations were in the range of 60 – 102% of the nominal concentrations throughout the test. Therefore, geometric average measured concentrations were used to calculate results where the

deviation from the nominal concentration was > 20%. 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 test substance was 2.7 mg/L. The 24 hour LC50 of potassium dichromate was 300 mg/L.

CONCLUSION The notified chemical is toxic to fish.

TEST FACILITY Suzhou (2015b)

C.2.2. Acute toxicity to fish – Study 2

TEST SUBSTANCE Notified chemical

METHOD Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body

Species	Ricefish (*Oryziru latipes*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	18.9 and 25.1 mg $CaCO_3$/L in the test substance (50 µg/L concentration) and control, respectively
Analytical Monitoring	GC-MS
Remarks – Method	Hydrogenated castor oil (HCO-40) and *N,N*-dimethylformamide were used as solubilising agents to prepare the stock solution for the test. No major deviations from the test guideline were reported.

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*			
Nominal		*24 h*	*48 h*	*72 h*	*96 h*
Control	10	0/10	0/10	0/10	0/10
1.25	10	0/10	0/10	0/10	0/10
2.5	10	0/10	0/10	0/10	0/10
5	10	0/10	0/10	0/10	0/10
10	10	0/10	0/10	0/10	0/10

LC50	> 10 mg/L at 96 hours
Remarks – Results	The validity criteria of test method were met. The water temperature variation was less than ± 2 °C of the set water temperature at 25 °C. The concentration of dissolved oxygen did not fall below 60% of saturated concentration 8.1 mg/L at 25 °C. The concentration of the test substance in the test tank was maintained within ± 20% of the mean of the measured values during the uptake phase. The mortality or other adverse effects/disease in both control and test group was < 10% at the end of the test. Eight peaks were detected with GC-MS analysis, and no interfering peak was observed at the peak positions of the test substance for the control water before or after uptake phase.

CONCLUSION The notified chemical is not toxic to fish under the test conditions.

TEST FACILITY CERI (2016b)

C.2.3. Acute toxicity to fish – Fish embryo acute toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 236 Fish Embryo Acute Toxicity (FET) Test – Semi-static

Species	Zebra fish (*Danio rerio*)
Exposure Period	4 days

Auxiliary Solvent	None
Water Hardness	125 mg $CaCO_3$/L
Analytical Monitoring	GC-FID
Remarks – Method	Precautions were made during preparation of test solutions and the testing period so as to avoid possible losses of the test substance by evaporation. The test media were prepared prior to the start of exposure and each test medium renewal. The medium was renewed every 24 hours. The NOEC for development of larvae, hatching success, and mortality, and the LOEC for hatching rate of larvae and mortality of embryos/larvae was determined by a Fisher's exact binomial test (one-sided greater, $\alpha = 0.05$). The reference substance was 3,4-dichloroaniline (positive control) at a concentration of 4.0 mg/L. No major deviations from the test guideline were reported.

RESULTS

Concentration mg/L		*Larvae hatched/viable egg*				*Hatching rate*	*% of*
Nominal	*Actual*	*1*	*2*	*3*	*4*	*(%)*	*control*
Control	–	0/20	0/20	10/10	18/1	90	–
Filtrate 1:460	0.065	0/20	0/20	14/5	18/1	90	100
Filtrate 1:220	0.13	0/20	0/20	8/12	18/1	90	100
Filtrate 1:100	0.29	0/19	0/18	11/7	17/1	85	94
Filtrate 1:46	0.63	0/19	0/16	4/9	11/2	55	61
Filtrate 1:22	1.35	0/15	0/12	1/7	8/0	40	44
Reference substance	4	0/14	0/6	0/0	0/0	0	0

LC50 (embryo and larvae)	1.1 mg/L at 96 hours (95th percentile confidence interval: 0.85 – 1.6 mg/L)
Remarks – Results	The validity criteria for the test guideline were met. The water temperature during the test was between 26.2 – 26.7 °C. Overall survival of embryos in the control was 95% until the end of the 96 hour exposure. Exposure to the positive control resulted in a minimum mortality of 100% at the end of the 96 hour exposure. The hatching rate in the control was 90% at the end of 96 hour exposure. At the end of the 96 hour exposure, the dissolved oxygen concentration in the control and highest test concentration was 8.2 mg/L (note 7.9 mg/L ≈ 97% saturation. A clear dose response relationship was observed. Two peaks were detected with GC-FID analysis. The NOEC and LOEC for hatching success were 0.29 and 0.63 mg/L at 96 hours, respectively.

CONCLUSION The notified chemical is toxic to fish with long lasting effects

TEST FACILITY Harlan (2015a)

C.2.4. 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

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Hexane
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	GC-FID
Remarks - Method	Test substance was > 87.4% purity. The test concentrations used in the definitive test (nominal: 0.10, 1.0, 10, and 100% v/v saturated solution) were determined by a preliminary range finding test. Given the low solubility of the test substance, the method was modified in the

preparation of the test media. A saturated solution was prepared by stirring an excess (100 mg/L) of test substance in test water for a period of 24 hours prior to removing any undissolved test substance present by filtration to give a saturated solution. Throughout the duration of the test, the test preparations were observed to be clear colourless solutions. The test involved daily renewal of the test preparations.

Temperature (20 °C) and dissolved oxygen levels (8.9 – 9.3 mg O_2/L) were kept relatively stable throughout the test.

The reference substance was potassium dichromate (positive control) at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L. Exposure conditions for the positive control were similar to those in the definitive test.

EC values were determined using the maximum-likelihood Probit method and by the trimmed Spearman-Karber method at 24 and 48 hours, respectively.

No major deviations from the test guideline were reported.

RESULTS

Concentration mg/L		*Number of D.*	*Cumulative Immobilized Daphnia*	
Nominal (%v/v saturated solution)	*Actual (mg/L)*	*magna*	*24 h*	*48 h*
Control	–	10	0	0
10	3.4	10	0	0
18	6.2	10	0	0
32	9.3	10	0	0
56	15	10	0	0
100	25	10	10	0

LC50 13 mg/L at 24 hours (95% confidence intervals: 11 – 14 mg/L)
9.4 mg/L at 48 hours (95% confidence intervals: 8.5 – 10 mg/L)

Remarks - Results All validity criteria for the test were satisfied. There was no immobilisation observed in the control, and the dissolved oxygen was ≥ 3 mg/L in the control and test vessels. The results were based on geometric mean measured concentrations. Immobilisation of all test species was observed at the highest test concentration. Toxic effects were observed at measured levels of the notified chemical in the test solutions less than the water solubility limit. The 24 and 48 hour EC50 (95% confidence intervals) for the reference substance were 0.87 (0.75 – 1.0) and 0.71 (0.65 – 0.78) mg/L.

CONCLUSION The notified chemical is harmful to aquatic invertebrates.

TEST FACILITY Harlan (2014e)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test
EC Council Regulation No 440/2008 C.3 Algal Inhibition Test

Species *Pseudokirchneriella subcapitata*
Exposure Period 72 hours
Concentration Range Nominal: 1:100 – undiluted filtrate (loading rate 100 mg/L)
Actual: 0.31 – 34 mg/L
Auxiliary Solvent None
Water Hardness 15 mg $CaCO_3$/L
Analytical Monitoring GC-FID

Remarks - Method	Test substance purity was > 87.4%. Intense stirring over 24 hours at room temperature in the dark, was used to dissolve a maximum amount of the test substance in the dispersion. No auxiliary solvent or emulsifier was used. The dispersion was then filtered prior to using as a stock solution for the test media. All test media were clear solutions throughout the test period. The pH of the control and the test media was 7.7 – 8.4 and 7.6 – 8.5, respectively. The temperature of the test was 23 – 24 °C. Potassium dichromate was used as a reference substance to evaluate the sensitivity of the test system. This positive control is tested twice a year. The EC values for the inhibition of average growth rate and yield and their 95% confidence intervals were calculated using Weibull analysis. No major deviations from the test guideline were reported.

RESULTS

Yield *EyC50 (95% confidence intervals)* *mg/L at 72 h*	*Growth* *ErC50 (95% confidence intervals)* *mg/L at 72 h*
7.1 (6.3 – 7.9)	16 (15 – 17)

Remarks - Results	The validity criteria of the test guideline were met. At the end of the test, 70 – 93% of the initial measured concentrations were found. Therefore, the results were based on the geometric means of the concentrations measured at the start and end of the test. In the control, the biomass increased by a factor of 362 over 72 hours. The mean coefficient of variation for section-by-section specific growth rate in the controls was 22%. The coefficient of variation of specific growth rate in replicate controls was 1.1%. There was a clear dose-response relationship for percent inhibition and cell concentration over the 72 hour exposure period. The 72 hour ErC50 for the reference compound was 1.1 mg/L was in the historical range (0.71 – 1.6 mg/L) for the test facility. Toxic effects were observed at measured levels of the notified chemical in the test solutions less than the water solubility limit.
CONCLUSION	The notified chemical is harmful to algae.
TEST FACILITY	Harlan (2015b)

C.2.6. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sewage from the aeration stage of a sewage treatment plant treating predominantly domestic sewage.
Exposure Period	3 hours
Concentration Range	Nominal: 100 – 320 mg/L
Remarks – Method	The purity of the test substance was 95%. The test was carried out based on results of a range-finding test. The test substance was not measured over the duration of the test. The reference substance used was 3,5-dichlorophenol. The test substance dispersed directly in water. No undissolved test substance was observed in the test solutions. The percentage inhibition values were plotted against concentration for the reference substance only; a line fitted using the Xlfit software package (IDBS) and the EC10, EC20, EC50 and EC80 values determined from the

equation for the fitted line. For the reference substance EC50 value using the method of Litchfield and Wilcoxon, 95% confidence limits were calculated. One way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure for comparing several treatments with a control was carried out on the oxygen consumption data after 3 hours for the control and all test concentrations to determine any statistically significant differences between the test and control groups. All statistical analyses were performed using the SAS computer software package.

No major deviations from the test guideline were reported.

RESULTS

IC50	> 1,000 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The 3 hour IC50 for 3,5-dichlorophenol was 9.5 mg/L (with 95% confidence intervals of 7.6 – 12 mg/L). The specific respiration rate of the blank controls was 27 mg oxygen/g dry weight of sludge per hour. The coefficient of variation of oxygen uptake rate in control replicates was 4.1%. In the definitive test no statistically significantly toxic effects were shown at the test concentrations of 100 and 180 mg/L, but statistically significantly toxic effects ($P < 0.05$) were shown at the test concentration of 320 mg/L. However, based on the results of the range finding test the effect of the test substance on the respiration of activated sewage sludge gave a 3 hour EC50 value of > 1,000 mg/L.

CONCLUSION The notified chemical is not inhibitory to microbial respiration.

TEST FACILITY Envigo (2016p)

C.2.7. Acute toxicity to earthworms

TEST SUBSTANCE Notified chemical

METHOD OECD TG 207 Earthworm, Acute Toxicity Tests

SPECIES *Eisenia foetida*

Remarks - Method

The test involved acute exposure of test species in artificial soil with 10% sphagnum peat. Based on the results of the preliminary range-finding test, nominal concentrations for the definitive test were 10, 18, 33, 59, 106 and 190 mg/kg (dry weight). The purity of the test substance was 94%. The reference substance used to determine the sensitivity of the test was chloracetamide.

A control group was run concurrently with the test substance. There were 4 replicates per test treatment and control, and 10 earthworms in each vessel. Mortality was recorded after 7 and 14 days of exposure. The earthworms were kept under continuous light and the test system was kept at a temperature of 19.2 – 20.8 °C, a soil moisture content of 34 – 40%, and a soil pH range of 5.8 – 6.0.

The LC50 and the 95% confidence limits were estimated using Probit analysis (SPSS 16.0).

No major deviations from the test guideline were reported.

RESULTS

Remarks - Results

The 14 day LC50 for the reference substance was 26 mg/kg dry weight and met the requirement for sensitivity of the test method. No mortalities were observed in the control. A clear dose response relationship was observed for the test substance. Weak peristalsis ability, yellow body fluid

	exudation, body disruption, and ulceration of body surface were observed in some surviving earthworms in treated groups. Earthworms in the 190 mg/kg (dry weight) treatment group all died. The 14 day LC50 (95% confidence limits) of the notified chemical was 73 (48 – 126) mg/kg dry weight.
CONCLUSION	The notified chemical is moderately toxic to earthworms.
TEST FACILITY	Suzhou (2015c)

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