File No.: LTD/2081

September 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Benzenepentanol, α,γ-dimethyl-

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/2081	Firmenich Pty Ltd	Benzenepentanol, α,γ-dimethyl-	Yes	≤ 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Skin irritant (Category 2)	H315 – Causes skin irritation
Specific target organ toxicity (single exposure; narcotic effects) (Category 3)	H336 – May cause drowsiness or dizziness

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*
Aquatic toxicity (Category 2)	H411 – Toxic to aquatic life with long lasting effects

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

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

Environmental Risk Assessment
On the basis of the PEC/PNEC ratio, 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 Irritation Category 2: H315 – Causes skin irritation
  - Specific Target Organ Toxicity Category 3: H336 – May cause drowsiness or dizziness

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

CONTROL MEASURES

Occupational Health and Safety

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

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

- 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
  - Safety glasses
  - Impervious gloves
  - Respiratory protection if inhalation exposure may occur

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

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

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

Storage

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

Emergency procedures

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

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory

obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the final use concentration of the notified chemical exceeds 0.2% in cosmetic/personal care/household products, 2% in fine fragrances, and 10% in air fresheners;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a fragrance ingredient, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

Safety Data Sheet
The SDS of the notified chemical provided by the notifier was 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)
Firmenich Pty Ltd (ABN: 86 002 964 794)
73 Kenneth Road
BALGOWLAH NSW 2093

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for adsorption/desorption, dissociation constant, flammability, explosive properties and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
ECHA (2019)

2. IDENTITY OF CHEMICAL

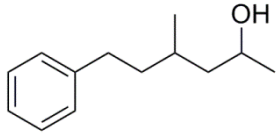
MARKETING NAME(S)
Yodanol

CAS NUMBER
72681-01-7

CHEMICAL NAME
Benzenepentanol, α,γ-dimethyl-

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

STRUCTURAL FORMULA



MOLECULAR WEIGHT
192.29 g/mol

ANALYTICAL DATA
Reference NMR, IR, GLC, GPC, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: clear colourless liquid

Property	*Value*	*Data Source/Justification*
Freezing Point	< -21 °C	Measured
Boiling Point	276 °C at 98.2 kPa	Measured
Density	942 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.68 × 10^{-4} kPa at 25 °C	Measured
Water Solubility	0.303 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Stable at pH 2-12 and 40°C over 28 days	Measured
Partition Coefficient (n-octanol/water)	log P_{ow} = 3.37	Measured
Surface tension	53.1 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.21	Measured
Dissociation Constant	Not determined	Not expected to dissociate in the environmental pH range of 4 - 9
Flash Point	136 °C	Measured
Flammability	Not determined	
Autoignition Temperature	394 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties

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

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

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

The notified chemical has a flash point of 136 ºC which is greater than 93 °C. Based on *Australian Standard AS1940* definitions for combustible liquid, the notified chemical may be considered as a Class C2 combustible liquid.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia either in the neat form, or as a component of fragrance formulations or finished 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
Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Pty Ltd

TRANSPORTATION AND PACKAGING

The imported notified chemical or products containing it will be transported by road via truck to the notifier's warehouse or customers' facilities for storage or reformulation. Fragrance formulations containing the notified chemical will be imported and distributed in lacquered drums of varying sizes from 5 – 180 kg. End-use products will be packaged in containers suitable for retail sale.

USE

The notified chemical will be used as a fragrance component in a variety of cosmetic and household products at typical final use concentrations of ≤ 0.2% in cosmetics products, ≤ 2% in fine fragrances, ≤ 0.2% in household cleaning products and ≤ 10% in air fresheners.

OPERATION DESCRIPTION

Reformulation

The reformulation processes for incorporating the notified chemical into end-use products will likely vary depending on the specific type of cosmetic and household products formulated. This may involve both automated and manual processes including transferring and blending the notified chemical with other formulations. According to the notifier, a typical blending operation will be highly automated and occur in a fully enclosed/contained environment, followed by automated filling using sealed delivery systems into containers of various sizes.

End-use

Household products

Finished household cleaning products containing the notified chemical will be used by consumers and professional cleaners. The products may be used in either closed systems with episodes of controlled exposure, for example automatic washing machines or open processes, and manually applied by sponge, mop, spray or brush followed by wiping or rinsing.

Cosmetics

Finished cosmetic products containing the notified chemical will be used by consumers and professionals (such as hairdressers and workers in beauty salons). Depending on the nature of the product, application of products may be done by hand, sprayed or through the use of an applicator.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and warehouse workers	unknown	unknown
Mixing	4	2
Drum handling	4	2
Drum cleaning/washing	4	2
Maintenance	4	2
Quality control	0.5	1
Packaging	4	2

EXPOSURE DETAILS

Transport and storage workers

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

Reformulation workers

During reformulation, dermal, ocular and possible inhalation exposure of workers to the notified chemical (at up to 100% concentration) may occur during weighing, transfer, blending, quality control analysis and

cleaning/maintenance of equipment. Exposure is expected to be minimised through the use of local exhaust ventilation and enclosed and automated systems, and through the use of personal protective equipment (PPE) such as impervious gloves, safety glasses, protective clothing and respiratory protection.

Professional end users
Exposure to the notified chemical in end-use products (at ≤ 10 % concentration) may occur in professions where the services provided involve the application of cosmetic products to clients or the use of cleaning products in the cleaning industry. The principal route of exposure is expected to be dermal, while ocular and inhalation exposures are also possible. Such professionals may use PPE to minimise repeated or prolonged exposure and ensure that good hygiene practices are in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of a variety of cosmetic and household products. The principal route of exposure will be dermal, while ocular and inhalation exposures are also possible, particularly if products are applied by spray.

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

Cosmetic products (Dermal exposure)

Product type	**Amount** (mg/day)	**C** (%)	**RF**	**Daily systemic exposure** (mg/kg bw/day)
Body lotion	7,820	0.2	1	0.2444
Face cream	1,540	0.2	1	0.0481
Hand cream	2,160	0.2	1	0.0675
Fragrances	750	2	1	0.2344
Deodorant (non-spray)	1,500	0.2	1	0.0469
Shampoo	10,460	0.2	0.01	0.0033
Hair conditioner	3,920	0.2	0.01	0.0012
Shower gel	18,670	0.2	0.01	0.0058
Hand wash soap	20,000	0.2	0.01	0.0063
Hair styling products	4,000	0.2	0.1	0.0125
Total				**0.6703**

C = concentration (%); RF = Retention Factor Daily Systemic Exposure = (Amount × C × RF × DA) / BW

Hair spray (inhalation exposure)

Product type	**Amount** (g/day)	**C** (%)	**Inhalation Rate** (m^3/day)	**Exposure Duration (Zone 1)** (min)	**Exposure Duration (Zone 2)** (min)	**Fraction Inhaled** (%)	**Volume (Zone 1)** (m^3)	**Volume (Zone 2)** (m^3)	**Daily systemic exposure** (mg/kg bw/day)
Hairspray	9.89	0.2	20	1	20	50	1	10	**0.0064**

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

Household products (Indirect dermal exposure – from wearing clothes)

Product type	**Amount** (g/use)	**C** (%)	**Product Retained (PR)** (%)	**Percent Transfer (PT)** (%)	**Daily systemic exposure** (mg/kg bw/day)
Laundry liquid	230	0.2	0.95	10	0.0068
Fabric softener	90	0.2	0.95	10	0.0027
Total					**0.0095**

Daily Systemic Exposure = (Amount × C × PR × PT)/body weight

Household products (Direct dermal exposure – from wearing clothes)

Product type	Frequency (use/day)	C (%)	Contact area (cm^2)	Product use C (g/cm^3)	Film thickness (cm)	Time scale factor	Daily systemic exposure (mg/kg bw/day)
Laundry liquid	1.43	0.2	1,980	0.01	0.01	0.007	0.0001
Dishwashing liquid	3	0.2	1,980	0.009	0.01	0.03	0.0005
All-purpose cleaner	1	0.2	1,980	1	0.01	0.007	0.0043
Total							**0.0049**

Daily Systemic Exposure = (Frequency × C × Contact area × Product Use Concentration × Film Thickness on skin × Time Scale factor × dermal absorption)/body weight

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical. This would result in a combined internal dose of 0.6914 mg/kg bw/day. It is acknowledged that inhalation exposure to the notified chemical from use of other cosmetic and household products (in addition to hair spray) may occur. However, it is considered that the combination of 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% absorption rate, is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of other spray cosmetic and household products with lower exposure factors (e.g. air fresheners).

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Acute oral toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute inhalation toxicity – rat (nose only)	LC50 > 5.14 mg/L/4 hour; narcotic effects
Skin irritation – *in vitro* reconstructed human epidermis test	Irritating
Eye irritation – *in vitro* isolated chicken eye test	No prediction can be made
Eye irritation – rabbit	Slightly irritating
Skin sensitisation – guinea pig maximisation test	No evidence of sensitisation
Repeat dose oral toxicity – rat, 28 days, with a mammalian erythrocyte micronucleus test	NOAEL = 1,000 mg/kg bw/day*
	Non genotoxic
Mutagenicity – bacterial reverse mutation	Non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration	Non genotoxic

* Established by the study authors

Toxicokinetics, Metabolism and Distribution

No data on toxicokinetics for the notified chemical was provided. For dermal absorption, molecular weights below 100 g/mol are favourable for absorption and molecular weights above 500 g/mol do not favour absorption (ECHA, 2017). Dermal uptake is likely to be moderate to high if the water solubility is between 100-10,000 mg/L and the partition coefficient (log P) values between 1 and 4 (ECHA, 2017). Based on the low molecular weight (192.29 g/mol), water solubility (303 mg/L) and partition coefficient (log Pow = 3.37 at 23.2 °C) of the notified chemical, absorption across biological membranes may occur.

Acute Toxicity

The notified chemical was found to have low acute oral and dermal toxicity in rats.

In the acute inhalation toxicity study, no animals died when exposed (nose-only) to the notified chemical at a concentration of 5.14 mg/L, for 4 hours. However, ataxia, lethargy, decreased respiration and difficult respiration were observed in all animals after exposure. The following macroscopic abnormalities were noted at necropsy: dark and pale patches on the lungs and pale lungs. Although abnormalities on the lungs were observed, no abnormalities were observed in the upper respiratory tract during necropsy. The notified chemical is therefore not expected to be a respiratory irritant. Based on the observations in this study, the notifiers have classified the notified chemical for single target organ toxicity with narcotic effects.

Irritation
According to the results of an *in vitro* assay using reconstructed human epidermis models, the notified chemical is considered to be irritating to skin, requiring hazard classification (GHS Category 2).

In an isolated chicken eye (ICE) test conducted on the notified chemical, no prediction can be made for eye irritation based on the results of this study. Based on an eye irritation study conducted in two rabbits, the notified chemical was a slight eye irritant, but does not require classification.

Sensitisation
The notified chemical was not a skin sensitiser in guinea pigs when tested in a maximisation test (induction and challenge by topical administration at 100% and 50% concentrations, respectively).

Repeated Dose Toxicity
In a repeated dose oral (gavage) toxicity study, the notified chemical was administered to rats at 100, 350 and 1,000 mg/kg bw/day for 28 days with a 14 day recovery period. All animals in the high dose group and males in the mid dose group showed a statistically significant increase in mean liver weight compared to the control groups (mostly > 10% increases), with a dose dependent response observed in males. The No Observed Adverse Effect Level (NOAEL) was established as 1,000 mg/kg bw/day in this study. However, based on the increased mean liver weights in treated animals and lack of recovery in the high dose females, a NOAEL of 350 mg/kg bw/day is used for the quantative risk assessment below (see Section 6.3.2).

Mutagenicity/Genotoxicity
The notified chemical showed negative results in a bacterial reverse mutation assay, an *in vitro* chromosomal aberration test using human lymphocytes and a mammalian erythrocyte micronucleus test in conjunction with the repeated dose toxicity study in rats.

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

Hazard Classification	***Hazard Statement***
Skin irritant (Category 2)	H315 – Causes skin irritation
Specific target organ toxicity (single exposure; narcotic effects) (Category 3)	H336 – May cause drowsiness or dizziness

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Reformulation
During reformulation, worker exposure will be limited through the use of engineering controls (such as enclosed, automated systems and local exhaust ventilation) and use of appropriate PPE by workers (eye protection and respiratory protection if inhalation exposure may occur), as stated by the notifier.

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.

End-Use
Workers involved in professions where the services provided involve the application of cosmetic and household products containing the notified chemical to clients (*e.g.* hairdressers, beauty salon workers and cleaners) or the use of household products in the cleaning industry may be exposed to the notified chemical at ≤ 0.1% concentration. PPE may be used by workers to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the risk to workers is expected to be of a similar or lesser extent than that for consumers using various products containing the notified chemical.

6.3.2. Public Health

Members of the public will experience widespread and frequent exposure to the notified chemical at ≤ 0.2% concentration through daily use of cosmetic and household products (≤ 2% in fine fragrances). The main route of exposure is expected to be dermal and inhalation, with some potential for accidental ocular or oral exposure.

Acute toxicity and irritation

The notified chemical may cause narcotic effects by inhalation and it is a skin irritant. However, these effects are not expected from the use of products containing the notified chemical at the proposed use concentrations in cosmetic and household products (≤ 0.2% and ≤ 2% in fine fragrances). The GHS cut-off for narcotic effects is ≥ 20% and therefore, use of the chemical at ≤ 10% concentration in air fresheners is not expected to pose an unreasonable risk to the public from inhalation exposure.

Repeated dose toxicity

The repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) to the notified chemical using the worst case exposure scenario from use of multiple products (at concentrations of ≤ 0.2% in cosmetics products, ≤ 2% in fine fragrances, ≤ 0.2% in household cleaning products) calculated as 0.6914 mg/kg bw/day (see Section 6.1.2). Using the NOAEL of 350 mg/kg bw/day derived from a 28-day repeated dose oral toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 506. A MOE value ≥ 100 is generally considered to be acceptable for taking into account intra- and inter-species differences.

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

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia as a component of end-use cosmetic and household products, or in either the pure form or as a component of fragrance solutions for reformulation into the end-use products. In general, the reformulation processes are expected to involve blending operations that will normally be automated and occur in an enclosed system, followed by automated filling of the finished products into end-use containers. Liquid waste from cleaning of the reformulation equipment will be reused. As estimated by the notifier, up to 0.1% of the import volume of the notified chemical may remain as residues in empty import containers which will either be recycled or disposed of through an approved waste management facility. Release of the notified chemical to the environment in the event of accidental spills or leaks during reformulation, storage and transport will be collected for disposal in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

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

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical in empty end-use containers are likely to either share the fate of the containers and be disposed of to landfill, or be released to the sewer system when the containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate

A biodegradability study conducted on the notified chemical indicates that it is not readily biodegradable but shows inherent biodegradability (66% degraded over 28 days in OECD 301F test; 10-day-window was not passed). For details of the biodegradability study, refer to Appendix C.

Based on its use as a component of cosmetic and household products, the majority of the notified chemical is expected to be released to sewers, and then to sewage treatment plants (STPs) before potential release to surface waters. Based on its moderate water solubility (303 mg/L) and log P_{ow} (3.37), the majority of the notified chemical is expected to present in the water phase in STPs. Based on the biodegradability test results, the notified chemical is expected to be removed effectively by biodegradation at STPs, and only a small proportion

of the notified chemical will be released to surface waters after STPs. A very small proportion of the notified chemical may adsorb to sludge in STPs. The waste sludge containing the notified chemical will be sent to landfill for disposal or agricultural land for remediation. A minor amount of the notified chemical may also be disposed of to landfill as collected spills and empty container residues. The notified chemical is expected to have low mobility in soil based on its log K_{oc} of 3.21. The notified chemical is not expected to significantly bioaccumulate in biota based on its biodegradability and log P_{ow}. In landfill, sludge and water, the notified chemical is expected to undergo degradation by biotic and abiotic processes, eventually forming water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has been calculated to assume the worst case scenario with 100% release of the notified chemical into sewer systems nationwide over 365 days per annum. It is also assumed under the worst-case scenario that there is no removal of the notified chemical during sewage treatment processes. The resultant PEC in sewage effluent on a nationwide basis is estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	1,000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	1,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	2.74	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor – River	1	
Dilution Factor – Ocean	10	
PEC – River:	0.56	µg/L
PEC – Ocean:	0.06	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1,500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.56 µg/L may potentially result in a soil concentration of approximately 3.74 µg/kg. Due to the notified chemical's biodegradability, annual accumulation is not expected.

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. The result for bacterial respiration inhibition was based on nominal concentration while other endpoints were based on the analytically confirmed concentrations.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 7.13 mg/L	Toxic to fish
Daphnia Toxicity	48 h EC50 = 10.1 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	72 h EC50 = 10.4 mg/L	Toxic to algae
Inhibition of Bacterial Respiration	3 h IC50 = 52.5 mg/L NOEC = 0.75 mg/L	Inhibitory to microbial respiration at STPs at concentrations > 0.75 mg/L

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS), the notified chemical is expected to be toxic to aquatic organisms. Therefore, the notified chemical is formally classified as "Acute Category 2; Toxic to aquatic life" under the GHS. Based on the acute toxicity and lack of readily biodegradation, the notified chemical is formally classified as "Chronic Category 2; Toxic to aquatic life with long lasting effects" under the GHS (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

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

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
96 h LC50 for fish	7.13	mg/L
Assessment Factor	100	
Mitigation Factor	1	
PNEC	71.3	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q – River	0.56	71.3	**0.008**
Q – Ocean	0.06	71.3	**0.001**

The conservative risk quotients (Q = PEC/PNEC) have been calculated to be much less than 1 for both the riverine and marine compartments, indicating that the notified chemical is unlikely to reach ecotoxicologically significant concentration in the aquatic environment based on its maximum annual importation quantity and the assessed use pattern. Therefore, based on the calculated risk quotient, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Freezing Point < -21 °C

Method OECD TG 102 Melting Point/Melting Range
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature
Remarks A pour point procedure was used.
Test Facility Envigo (2016a)

Boiling Point 276 °C at 98.2 kPa

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

Density 942 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 A pycnometer method was used.
Test Facility Envigo (2016a)

Vapour Pressure 1.68×10^{-4} kPa at 25 °C

Method OECD TG 104 Vapour Pressure
EC Council Regulation No 440/2008 A.4 Vapour Pressure
Remarks The gas saturation method was used.
Test Facility Envigo (2017a)

Water Solubility 0.303 g/L at 20 °C

Method OECD TG 105 Water Solubility
EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks Flask Method
Test Facility Noack-Laboratorien (2016a)

Hydrolysis as a Function of pH

Method The notified chemical was dissolved in the pH buffer containing the surfactant and put into an oven at 40°C. Small aliquots of the test solution were extracted with an organic solvent (cyclohexane or ethyl acetate) containing a hydrocarbon standard (typically C12, C17 or C20) on a regular basis throughout the test (at time = 0, 0.25, 1, 2, 4, 7, 15, 21 and 28 days). The extracts were analysed by GC-FID and the results were plotted as (Area/Area Std) expressed in %.

pH	*T (°C)*	*Results*
2	40	Not hydrolysed
5	40	Not hydrolysed
7	40	Not hydrolysed
8.5	40	Not hydrolysed
12	40	Not hydrolysed

Remarks The notified chemical is stable at pH 2-12 and 40°C over 28 days.
Test Facility Firmenich (2015)

Partition Coefficient (n-octanol/water) log P_{ow} = 3.37

Method OECD TG 117 Partition Coefficient (n-octanol/water).

	EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method; the column temperature was 23.2 °C.
Test Facility	Noack-Laboratorien (2016b)

Surface Tension 53.1 mN/m at 20 °C

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

Adsorption/Desorption log K_{oc} = 3.21

Method	OECD TG 121 Estimation of Adsorption Coefficient Using HPLC Method
Remarks	The column temperature was 30 °C
Test Facility	Envigo (2016b)

Flash Point 136 ± 2 °C

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	The closed cup method was used.
Test Facility	Envigo (2017b)

Autoignition Temperature 394 ± 5 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Test Facility	Envigo (2017b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

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

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method (2001) EC Council Regulation No 440/2008 B.1 bis Acute Toxicity (Oral) Fixed Dose Method
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	Arachis oil BP
Remarks – Method	GLP Certificate No protocol deviations.

RESULTS

Main Study

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity	No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks – Results	The animals showed expected body weight gain over the observation period.

CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Envigo (2016c)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (1987) EC Council Regulation No 440/2008 B.3 Acute Toxicity (Dermal)
Species/Strain	Rat/Wistar (RccHan™:WIST)
Vehicle	None.
Type of dressing	Semi-occlusive
Remarks – Method	GLP Certificate No significant protocol deviations. A preliminary study (Group 1) was conducted in 1 male and 1 female animal at a dose of 2,000 mg/kg bw. The dose of 2,000 mg/kg bw was selected for the Group 2 study based on the results of the Group 1 study.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	1 F, 1 M	2,000	0/2
2	4 F, 4 M	2,000	0/8

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Very slight erythema was observed at the test site of the female in group 1 on day 1 after dosing.
Signs of Toxicity – Systemic	No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks – Results	The animals showed expected body weight gain over the observation

period.

CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Envigo (2016d)

B.3. Acute Inhalation Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity (2009) EC Council Regulation No 440/2008 B.2 Acute Toxicity (Inhalation)
Species/Strain	Rat/Wistar RccHan™:WIST
Vehicle	None
Method of Exposure	Nose-only exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	Mean mass aerodynamic diameter (MMAD): 1.69 μm
Remarks – Method	GLP Certificate No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 F, 5 M	15.25	5.14	0/10

LC50	> 5.14 mg/L/4 hours
Signs of Toxicity	All animals exhibited decreased respiratory rate during exposure. After being removed from the chamber, all animals showed decreased repiratory rate and ataxia. The animals appeared to be normal 1 hour after exposure. One day after exposure, all animals exhibited lethargy, decreased respiratory rate, noisy respiration, hunched posture and piloerection. On days 2 - 5, there were frequent instances of decreased respiratory rate, noisy respiration, sneezing, piloerection, staining around the eyes and snout and hunched postures. Animals recovered on days 8 - 11 post exposure.
Effects in Organs	Dark and/or pale patches on the lungs and/or pale lungs were noted in 8 animals at necropsy.
Remarks – Results	All animals showed weight losses one day after exposure. Three males and one female had body weight losses on days 2 and 3. Body weight gains were normal for all animals 4 days after exposure. Although abnormalities on the lungs were observed, no abnormalities were observed in the upper respiratory tract during necropsy.
CONCLUSION	The notified chemical is of low acute toxicity via inhalation.
TEST FACILITY	Envigo (2018)

B.4. Skin Irritation – *In Vitro* Reconstructed Human Epidermis Model

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method (2015) EPISKIN™ Reconstructed Human Epidermis Model
Vehicle	None

Remarks – Method	GLP Certificate No protocol deviations Negative control (Dulbecco's phosphate buffered saline) and positive control (5% sodium dodecyl sulfate in water) were run concurrently with the notified chemical. The MTT tetrazolium salt [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to determine cell viability.

RESULTS

Test Material	*Mean OD_{562} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.626 ± 0.022	100	3.5
Test substance	0.095 ± 0.020	15.2	3.3
Positive control	0.089 ± 0.014	14.2	2.2

OD = optical density; SD = standard deviation

Remarks – Results	The MTT solution containing the test substance did not turn blue, showing that it did not directly reduce MTT. The solution containing the test substance did not produce a coloured solution. It was therefore decided not to run colour correction tissues. The relative mean viability of the tissues treated with the test substance was ≤ 50% (predicted as irritant according to the criteria). The positive and negative controls gave satisfactory results, confirming the validities of the test systems. The mean concentration of inflammatory mediator IL-1α was markedly increased for the test substance than for the negative control. This showed that the test substance was able to induce significant IL-1α release (>60 pg/mL).
CONCLUSION	The notified chemical was considered irritating to the skin under the conditions of the test. Based on the mean tissue viability of ≤ 50%, the notified chemical should be classified for skin irritation (Category 2) according to the GHS criteria.
TEST FACILITY	Envigo (2016e)

B.5. Eye Irritation – *In Vitro* Isolated Chicken Eye Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 438 Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants (2013)
Vehicle	None.
Remarks – Method	GLP Certificate No significant protocol deviations Negative control (0.9% sodium chloride) and positive control (5% benzalkonium chloride in water) were run concurrently with the notified chemical.

RESULTS

Test Material	*Maximal mean score for corneal opacity (ICE Class)*	*Mean score of Fluorescein retention (ICE Class)*	*Maximal corneal swelling (ICE Class)*
Negative control	0	0.5	-1.45%
Test substance	2.0 (III)	1.3 (II)	17.65% (II)
Positive control	4.0	3.0	32.66%

Remarks – Results

Translucent corneal opacity was observed in all eyes treated with the test substance. Some degree of fluorescein staining was noted in test substance treated eyes. No morphological effects were noted in test substance treated eyes.

The combined scores (2 × Class II, 1 × Class III, according to the TG) were not sufficient to classify the test substance for eye irritation (Category 1) according to the GHS criteria.

The positive and negative controls gave satisfactory results, confirming the validities of the test systems.

CONCLUSION

No prediction for eye irritation can be made based on the results of this study.

TEST FACILITY

Envigo (2016f)

B.6. Eye Irritation – Rabbit

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion (2012)
EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation)

Species/Strain: Rabbit/New Zealand White
Number of Animals: 1 M, 1 F
Observation Period: 7 days
Remarks – Method: GLP Certificate
No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva – Redness	1.67	1.33	2	< 7 days	0
Conjunctiva – Chemosis	1.33	1	2	< 7 days	0
Conjunctiva – Discharge	0.67	0.33	2	< 48 hours	0
Corneal Opacity	0	0	0	-	0
Iridial Inflammation	0.33	0	1	< 48 hours	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 1 hour after treatment and persisted in one treated eye at the 24 hour observation. Moderate conjunctival irritation was noted in both treated eyes 1 and 24 hours after treatment, with minimal conjunctival irritation at the 48 hour and 72 hour observations.

All effects on treated eyes were fully reversible within 7 days.

CONCLUSION

The notified chemical is slightly irritating to the eye.

TEST FACILITY

Envigo (2016g)

B.7. Skin Sensitisation – Guinea Pig Maximisation Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation – Magnusson and Kligman (1992)
Species/Strain	Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY	Maximum non-irritating concentration: Topical:100%, 50%, 20% and 10%
MAIN STUDY	
Number of Animals	Test Group: 10 F Control Group: 5 F
Vehicle	Liquid paraffin (topical), olive oil (intradermal)
Positive Control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-hexylcinnamaldehyde.
INDUCTION PHASE	Induction concentration: Intradermal: 10% and 20% Topical: 100%
Signs of Irritation	Dryness of the skin was noted in 8 of 10 animals after topical induction, and scabs were noted in 2 of 10 animals.
CHALLENGE PHASE	
Challenge	Topical: 50% and 100%
Remarks – Method	GLP Certificate No significant protocol deviations

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after Challenge*	
		24 h	*48 h*
Test Group	50%, 100%	0/10, 0/10	0/10, 0/10
Vehicle Control	50%, 100%	0/5, 0/5	0/5, 0/5

Remarks – Results	There were no mortalities. The animals showed expected body weight gain over the observation period.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Phycher (2016)

B.8. Repeat Dose Oral Toxicity – Rats

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (2008) OECD TG 474 Mammalian Erythrocyte Micronucleus Test (2014)
Species/Strain	Rat/Sprague-Dawley Crl:CD® (SD) IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation (recovery) period: 14 days
Vehicle	Arachis oil BP
Remarks – Method	GLP Certificate No protocol deviations

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5 F, 5 M	0	0/10
Low Dose	5 F, 5 M	100	0/10
Mid Dose	5 F, 5 M	350	0/10
High Dose	5 F, 5 M	1,000	0/10

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

Mortality and Time to Death
There were no unscheduled deaths of animals in all dose groups.

Clinical Observations
All animals in the high dose group showed increased salivation from day 2 (females) and day 5 (males) onwards. All animals in the mid dose group showed similar increased salivation from day 9 onwards.

No significant clinical observations were detected during the recovery period.

Males in the high dose group showed a statistically significant reduction in body weight gain during the first week of treatment. These males continued to show a slight reduction in body weight gain during weeks 2 and 4 although it was not statistically significant. The body weight gain for these males was reduced by 24%.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Females in the high dose group showed a statistically significant reduction in activated partial thromboplastin time. This change was reversibile after the recovery period.

Males in the high dose group showed a statistically significant increase in alkaline phosphatase and a reduction in phosphorus. Females in the high dose group showed statistically significant reductions in albumin/globulin ratio, aspartate aminotransferase and alanine aminotransferase and increases in bilirubin, bile acids and triglyceride levels.

There were no toxicologically significant effects in urine parameters observed at the end of the treatment and recovery periods.

Effects in Organs
All animals in the high dose group and males in the mid dose group showed a statistically significant increase in liver weight (> 10%). Minimal cortical vacuolation was observed in two females in the high dose group, but these changes were not considered to be toxicologically significant by study authors.

Remarks – Results
Bone marrow samples were extracted from euthanised animals after the final dosing. There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes (PCEs) in any of the treated groups. The test substance was considered to be non-genotoxic under the conditions of the micronucleus test. The positive and vehicle controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1,000 mg/kg bw/day in this study, based on that some statistically significant metabolic changes observed in animals treated at 1,000 mg/kg bw/day were of no toxicological significance.

TEST FACILITY Envigo (2017c)

B.9. Genotoxicity – Bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1997)
EC Council Regulation No 440/2008 B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria
Plate incorporation procedure (test 1)/Pre incubation procedure (test 2)

Species/Strain *Salmonella typhimurium*: TA1535, TA1537, TA98, TA100
Escherichia coli: WP2uvrA

Metabolic Activation System A rat liver homogenate metabolising system (10% liver S9 in standard co-factors)

Concentration Range in Test 1 with and without metabolic activation: 0, 1.5, 5, 15, 50, 150, 500,

Main Test	1,500 µg/plate Test 2 with and withoutmetabolic activation: 0, 0.15, 0.5. 1.5, 5, 15, 50, 150, 500,- 1,500µg/plate
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks – Method	GLP Certificate No significant protocol deviations. There was no dose range-finding study. The dose range used for test 2 was determined by the results of test 1. Vehicle control and the following positive controls were run concurrently with the test substance: With metabolic activation: 2-aminoanthracene (WP2uvrA, TA100, TA1535, TA1537); benzo(a)pyrene (TA98) Without metabolic activation: *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine (WP2uvrA, TA100, TA1535); 9-aminoacridine (TA1537); 4-nitroquinoline-*N*-oxide (TA98)

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:* *Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 500	> 5,000	Negative
Test 2	≥ 500	> 5,000	Negative
Present			
Test 1	≥ 1,500	> 5,000	Negative
Test 2	≥ 500	> 5,000	Negative

Remarks – Results	No significant increases in the frequency of revertant colonies were observed for any of the bacterial strains, at any test concentration, either with or without metabolic activation. The positive and vehicle controls gave satisfactory responses confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Envigo (2017d)

B.10. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (2014)
Species/Strain	Human
Cell Type/Cell Line	Peripheral lymphocytes
Metabolic Activation System	A rat liver homogenate metabolizsing system (10-20% liver S9 in standard co-factors)
Vehicle	DMSO
Remarks – Method	GLP Certificate No significant protocol deviations. The dose selection for the main tests was based on toxicity and precipitation noted in the range finding study. Vehicle control and positive controls (mitomycin C and cyclophosphamide) were run concurrently with the test substance.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 7.5, 15*, 30*, 60*, 120*, 160, 200, 240	4 h	20 h
Test 2	0*, 7.5*, 15*, 30*, 60*, 120, 160	24 h	24 h
Present (2%)			
Test 1	0*, 7.5, 15, 30*, 60*, 120*, 160*, 200, 240	4 h	20 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 120.19			
Test 1		> 120	> 120	Negative
Test 2		> 60	> 60	Negative
Present	≥ 120.19			
Test 1		> 160	> 160	Negative

Remarks – Results

The toxicity curve of the test substance was extremely steep which made achieving optimum toxicity difficult for all three exposure conditions. The study authors considered that the test substance had been adequately tested since it was tested to cytotoxic dose levels. Toxicity was demonstrated by a reduction in cell numbers or by a reduction in mitotic index in all three exposure conditions.

No statistically significant increases in the frequency of cells with structural or numerical chromosome aberrations were observed in the presence or absence of metabolic activation. No indication of endoreduplication was obaserved. The positive and negative controls gave 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

Envigo (2017e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated sludge from a municipal STP
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen uptake by OxiTop® system
Remarks – Method	No major deviations from the test guidelines were reported. The test substance was directly added to the test medium. A toxicity control was run.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
4	0	4	71.3
6	10.5	6	75.9
18	58.1	18	91.8
28	66.2	28	93.8

Remarks – Results	All validity criteria for the test were satisfied. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days was 66%. The 10-d window was not passed.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Suzhou Research (2016a)

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	Zebra-fish (*Brachydanio rerio)*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	80 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	The definitive test was designed based on the preliminary test results. No major deviations from the test guidelines was reported. The test substance was directly added to the test solution. The test solution was renewed daily. The test solution was sampled at the start of the exposure, before and after renewal, and the end of the exposure for analysis of the test substance.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Initial Measured*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	7	0	0	0	0	0
4.0	4.18	7	0	0	0	0	0
4.8	4.93	7	0	0	0	0	0
5.8	5.92	7	0	0	0	1	1
7.0	7.20	7	0	0	2	3	3
8.5	8.82	7	0	0	3	6	6

LC50	7.128 mg/L (95% confidence limit of 6.346 – 8.295 mg/L) at 96 hours (calculated using Probit analysis programme).
Remarks – Results	All validity criteria for the test were satisfied. The dissolved oxygen (DO) was > 72% during the test. The analysed test substance concentration during the test was within ± 20% of the nominal concentration so the results are based on the analytically confirmed concentrations.

CONCLUSION The test substance is toxic to fish.

TEST FACILITY Suzhou Research (2016b)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Semi-static
EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia – Semi-static

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	258 mg $CaCO_3$/L
Analytical Monitoring	HPLC – Diode Array Detector (DAD)
Remarks – Method	The definitive test was designed based on the preliminary test results. No major deviations from the test guidelines was reported. A stock solution of the test substance (40 mg/L) was prepared in test water and stirred under light exclusion and without headspace for 24 hours before being used at the highest test concentration. Lower test concentrations were achieved by diluting the stock solution. The test solutions were renewed daily. The test solutions were sampled at the start (0 and 24 hours) and the end of the exposure intervals (24 and 48 hours) for analysis of the test substance. A reference test with potassium dichromate was run.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*% Immobilised*	
Nominal	*Initial Measured*		*24 h*	*48 h*
Control	Control	20	0	0
2.5	2.57	20	0	5
5	5.45	20	0	0
10	10.7	20	30	40
20	21.6	20	100	100
40	43.6	20	100	100

EC50	10.1 mg/L (95% confidence limit of 5.42 – 18.9) at 48 hours (calculated using sigmoidal dose – response regression)
Remarks – Results	All validity criteria for the test were satisfied. The dissolved oxygen was >

6.86 mg/L during the test. The analysed test substance concentration during the test was within ± 20% of the nominal concentration so the results were based on the analytically confirmed concentrations. The 48 h EC50 for *D.magna* exposed to potassium dichromate was 1.89 mg/L which was within the range of expected responses.

CONCLUSION The test substance is toxic to aquatic invertebrates.

TEST FACILITY Noack-Laboratorien (2016c)

C.2.3. 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: Control, 1.25, 2.50, 5.0, 10, 20 mg/L Innitial measured: Control, 1.26, 2.64, 4.96, 9.92, 19.9 mg/L
Auxiliary Solvent	None
Water Hardness	0.24 mmol Ca+Mg/L
Analytical Monitoring	HPLC – DAD
Remarks – Method	The definitive test was designed based on the preliminary test results. No major deviations from the test guidelines was reported. The test substance was directly added to test water and stirred for 24 hours before testing. The test solutions were sampled at the start, after 24 hours and the end of the exposure for analysis of the test substance. A reference test with potassium dichromate was run.

RESULTS

Biomass		*Growth*	
EyC50 (mg/L at 72 h)	*NOEC (mg/L)*	*ErC50 (mg/L at 72 h)*	*NOEC (mg/L)*
3.72 (95% CL of 3.37 – 4.13)	1.25	10.4 (95% CL of 9.21 – 11.9)	2.50

Remarks – Results All validity criteria for the test were satisfied. The mean cell density in the control increased 82 times after 72 hours. The analysed test substance concentration during the test was within ± 20% of the nominal concentration so the results were based on the analytically confirmed concentrations. The 48 h ErC50 for algae exposed to potassium dichromate was 1.15 mg/L which was within the historical range.

CONCLUSION The test substance is toxic to algae.

TEST FACILITY Noack-Laboratorien (2016d)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test

Inoculum	Activated sludge from a municipal STP
Exposure Period	3 hours
Nominal Concentration	0.75, 5.6, 32, 180 and 1,000 mg/L
Remarks – Method	The definitive test was designed based on the preliminary test results. No major deviations from the test guidelines was reported. The test substance was directly added to test water. A reference test with copper sulfate

	pentahydrate was run.
RESULTS	
IC50	52.5 mg/L (95% CL of 47.2 – 59.7 mg/L) at 3 hours (calculated using sigmoidal dose – response regression)
NOEC	0.75 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The 3 h IC50 for microorganisms exposed to copper sulfate pentahydrate was 96 mg/L which was within the historical ranges.
CONCLUSION	The test substance can inhibit microbial respiration at STPs at concentrations > 0.75 mg/L
TEST FACILITY	Noack-Laboratorien (2016e)

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