File No.: LTD/2096

January 2020

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Carbonic acid, 2-hydroxyethyl (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl ester

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 Agriculture, Water and the Environment.

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

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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	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2096	Colgate-Palmolive Pty Ltd	Carbonic acid, 2-hydroxyethyl (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl ester	Yes	≤ 1 tonne per annum	Cosmetic ingredient (oral and lip products)

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*
Serious eye damage/eye irritation (Category 2A)	H318 – Causes serious eye damage

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 3)	H402 – Harmful to aquatic life

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

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  – Serious eye damage/eye irritation (Category 2A): H318 – Causes serious eye damage

  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:
  - Enclosed/automated processes
  - 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:
  - Avoid contact with skin and eyes
  - Avoid inhalation of aerosols/dusts

- 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:
  - Protective clothing
  - Impervious gloves
  - Eye protection
  - Respiratory protection if inhalation of aerosols/dust 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 physical containment, collection and subsequent safe 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 0.65% in lip and oral care cosmetic products;
- further information becomes available on the skin sensitisation potential of the notified chemical:

or

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

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

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Colgate-Palmolive Pty Ltd (ABN: 79 002 792 163)
Level 14, 345 George Street
SYDNEY NSW 2000

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: specific other names, analytical data, purity, impurities and identity of test facilities.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for hydrolysis as a function of pH, dissociation constant, flammability, explosive properties and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANTS
None

NOTIFICATION IN OTHER COUNTRIES
USA (2019)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Carbonic acid, 2-hydroxyethyl (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl ester

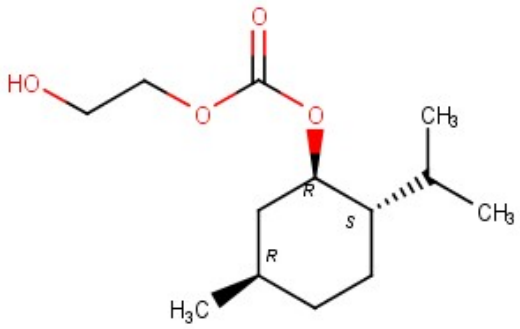
CAS NUMBER
156324-78-6

CHEMICAL NAME
Carbonic acid, 2-hydroxyethyl (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl ester

OTHER NAME
Carbonic acid, 2-hydroxyethyl 5-methyl-2-(1-methylethyl)cyclohexyl ester, [1*R*-(1α,2ß,5α)]
Menthyl Ethylene Glycol Carbonate

MOLECULAR FORMULA
$C_{13}H_{24}O_4$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
244.33 g/mol

ANALYTICAL DATA
Reference NMR, FTIR, GC-MS, UV spectra and optical activity were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: White solid

Property	*Value*	*Data Source/Justification*
Melting Point	52 °C	Measured
Boiling Point	Decomposes without boiling at 225 °C	Measured
Density	1,136 kg/m^3 at 21 °C	Measured
Vapour Pressure	1.024×10^{-5} kPa at 25 °C	Measured
Water Solubility	0.148 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	pH 4 < 10% after 5 days at 50 °C pH 7 $t_{1/2}$ 428 hours at 50 °C pH 7 $t_{1/2}$ (estimated): 7,420 hours at 25 °C pH 9 $t_{1/2}$: 133 hours at 25 °C	Measured
Partition Coefficient (n-octanol/water)	log Pow = 3.55 at 20 °C	Measured
Surface Tension	53.2 mN/m at 19.5 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.093	Calculated (KOCWIN v2.00 US EPA, 2012)
Dissociation Constant	Not determined	Does not contain dissociable functional groups
Flash Point	157 °C	Measured
Flammability	Not determined	Combustible liquid
Autoignition Temperature	345 °C	Measured
Explosive Properties	Not determined	Contain no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contain 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 157 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 if the chemical has a flash point below the boiling point.

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. It will be imported into Australia as a component of finished cosmetic products at ≤ 0.65% concentration. In the future the notified chemical may also be imported neat as a solid for reformulation into finished cosmetic 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 and Melbourne

TRANSPORTATION AND PACKAGING
The finished cosmetic products containing the notified chemical at ≤ 0.65% concentration will be imported in containers suitable for retail sale (i.e. ≤ 500 mL mainly plastic (HDPE) bottles or tubes), and transported by road or rail for distribution to customers and retailers.

In the future the notified chemical may also be imported neat in 180 kg steel drums for reformulation into finished cosmetic products.

USE
The notified chemical will be used as a component of lip and oral care cosmetic products (including toothpaste, mouthwash and lipstick) at ≤ 0.65% concentration.

OPERATION DESCRIPTION
Reformulation
The procedures for incorporating 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. However, in general, it is expected that for the reformulation process, the notified chemical will be weighed and added to the mixing tank where it will be blended with additional additives to form the finished cosmetic products. This will be followed by automated filling of the reformulated products into containers of various sizes. The blending operations are expected to be highly automated and use closed systems and/or adequate ventilation. During the reformulation process, samples of the notified chemical and the finished end-use products will be taken for quality control testing.

End-use
Finished cosmetic products containing the notified chemical at ≤ 0.65% concentration will be used by consumers and professionals such as dentists.

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	4	12
Compounder	8	12
Chemist	3	12
Packers	8	12
Store persons	4	12
Professional end users (dentists, etc.)	8	365

EXPOSURE DETAILS
Transport and storage
Transport, distribution and warehouse workers are not expected to be exposed to the notified chemical except in the unlikely event of an accident.

Reformulation
During reformulation, dermal and ocular exposure to the notified chemical at ≤ 100% concentration may occur during weighing and transfer stages, blending, quality control analysis, and cleaning and maintenance of

equipment. Given the low vapour pressure (1.024×10^{-5} kPa at 25 ºC) of the notified chemical, inhalation exposure to the notified chemical is not expected unless aerosols are formed.

The notifier states that exposure is expected to be minimised through the use of local exhaust ventilation and/or enclosed systems, and through workers using personal protective equipment (PPE) such as protective clothing, goggles, impervious gloves and respiratory protection (in cases where there is inadequate ventilation).

End-use
Exposure to the notified chemical in end-use products at ≤ 0.65% concentration may occur in professions where the services provided involve the application of oral care cosmetic products to clients (e.g. dentists). The principal route of exposure will be dermal. Such professionals are expected to use PPE, including gloves and overalls, to minimise repeated exposure, and good hygiene practices are expected to be in place.

6.1.2. Public Exposure
There will be widespread and repeated exposure of the public to the notified chemical at ≤ 0.65% concentration in lip and oral care cosmetic products. The principal route of exposure will be oral, while accidental dermal and ocular exposure are also possible.

Data on typical use patterns
Data on typical use patterns of cosmetic products in which the notified chemical is proposed to be used are shown in the following tables for young children (2-4 year olds) and adults, respectively. The use of toothpaste is separately estimated for young children, as they represent a more susceptible group. For the purposes of the exposure assessment, Australian use patterns for the product categories are assumed to be similar to those in Europe (SCCS, 2018). A child bodyweight of 12.5 kg (RIVM, 2006) and an adult bodyweight of 64 kg (enHealth, 2012) have been used for calculation purposes. In addition, 100% systemic exposure has been assumed. For adults it is assumed that the main exposure route is dermal absorption (i.e. mucous membranes), whereas in children it is assumed to be oral ingestion. Using these data, the total systemic exposure to the notified chemical is estimated to be 0.894 mg/kg bw/day for children and 0.2394 mg/kg bw/day for adults.

Children's exposure (2-4 year old)

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Toothpaste	1,720[1]	0.65	1	**0.894**

C = concentration (%); RF = retention factor; assumed brushing twice daily
Daily systemic exposure = (amount × C (%) × RF x oral absorption)/body weight (12.5 kg)

Adults' exposure

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Lipstick	57	0.65	1.0	0.0058
Toothpaste	2,750	0.65	0.05	0.0140
Mouthwash	21,620	0.65	0.1	0.2196
Total				**0.2394**

C = concentration (%); RF = retention factor; assumed brushing twice daily and using mouth rinse 4 times/day
Daily systemic exposure = (amount × C (%) × RF x dermal absorption)/body weight (64 kg)

[1]RIVM (2006)

6.2. Human Health Effects Assessment
The results from toxicological investigations conducted on the notified chemical is 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
Skin irritation – rabbit	slightly irritating
Eye irritation – rabbit	severely irritating
Skin sensitisation – guinea pig, maximisation test	no evidence of sensitisation

Endpoint	*Result and Assessment Conclusion*
Skin sensitisation – HRIPT (n = 92)	evidence of sensitisation (3% notified chemical in 91% water, 3% octyldodecanol and 2.4% sodium hydroxide)
Repeat dose oral toxicity – rat, 28 days	NOAEL = 150 mg/kg bw/day*
Mutagenicity – bacterial reverse mutation assay	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	non clastogenic
Genotoxicity – *in vitro* mammalian cell gene mutation test	non mutagenic

*established by the study authors

Toxicokinetics
Given the low molecular weight (244.33 g/mol) of the notified chemical, it may be absorbed across biological membranes.

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

No acute inhalational toxicity data were provided.

Irritation
The notified chemical was found to be slightly irritating to the skin in a study conducted in rabbits. Very slight erythema and oedema were observed in treated animals which resolved at the 48 hour observation.

The notified chemical is severely irritating to eyes based on a study conducted in rabbits. All four animals showed moderate conjunctival irritation, slight to moderate corneal opacity and slight iridial inflammation, which were fully resolved at the day 14 observation. One animal showed vascularisation of the cornea at the day 7 and day 14 observation. The study was not extended to determine if the vascularisation resolved. Based on the results of this study, the notified chemical warrants classification as a Category 2A eye irritant according to the GHS criteria.

Sensitisation
The notified chemical (at 50% topical induction concentration) was not a skin sensitiser in a guinea pig maximisation test (GPMT).

In a human repeated insult patch test (HRIPT), the notified chemical at 3% concentration in a cooling solution (contains 3% notified chemical, 91% water, 3% octyldodecanol and 2.4% sodium hydroxide) was found to be sensitising. In the study 3/92 subjects developed reactions greater than mild erythema at challenge. Following rechallenge with these three subjects, the reaction observed at 48 hours increased in severity for one subject at 96 hours confirming a sensitisation reaction; however, reaction appeared to be subsided in the other two subjects.

The notified chemical may also have the potential to cause skin sensitisation. However, the impact of other ingredients used in the HRIPT may have caused the positive skin reactions as the GPMT gave negative results.

An analogue of the notified chemical (differing in chemical structure by only one carbon) was negative in a GPMT and showed no evidence of sensitisation at 0.5% and 1% concentration in diethyl phthalate/ethanol (3:1). Therefore the notified chemical at concentrations up to 0.65% is not expected to cause skin sensitisation.

Repeated Dose Toxicity
A repeated dose oral (gavage) toxicity study on the notified chemical was conducted in rats, in which the test substance was administered at 15, 150 and 1,000 mg/kg bw/day for 28 consecutive days, with a 14-day recovery period. The No Observed Adverse Effect Level (NOAEL) was established by the study authors as 150 mg/kg bw/day based on effects observed in the kidney. Speckled kidneys were observed in all high dose males and slightly speckled kidneys in 3/5 mid dose males but was not evident in the recovery group animals. The study authors stated the toxicological significance of these findings were unclear because no associated histopathological lesions were observed in the kidney and no evidence of kidney dysfunction. However, high dose males showed statistically significant increased relative kidney weights compared to control animals, indicative of a treatment related renal effects at high dose. As no change in mean kidney weight in mid dose males (compared to the control mean), the speckled appearance in the kidneys in mid dose males was not considered to be toxicologically significant by the

study authors. These findings were no longer evident in recovery group males. Treated females did not show speckled kidneys or evidence of kidney dysfunction.

Mutagenicity/Genotoxicity
The notified chemical was not mutagenic in a bacterial reverse mutation assay and in an *in vitro* gene mutation test in Chinese hamster V79 cells, and was not clastogenic in an *in vitro* chromosome aberration test in human lymphocytes.

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 is presented in the following table.

Hazard Classification	*Hazard Statement*
Serious eye damage/eye irritation (Category 2A)	H318 – Causes serious eye damage

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the toxicological information provided, the notified chemical is a severe eye irritant and is slightly irritating to the skin. Systemic effects at high concentrations may occur.

Reformulation
Workers may experience dermal, ocular and perhaps inhalation exposure to the notified chemical at ≤ 100% concentration during reformulation. The use of local ventilation, enclosed/automated processes and PPE (i.e. protective clothing, goggles, impervious gloves and respiratory protection, if inhalation exposure may occur) are expected to minimise exposure.

Therefore, provided adequate control measures are in place to minimise worker exposure, including the use of automated processes and PPE, the risk to workers from use of the notified chemical is not considered to be unreasonable.

End-use
Workers involved in professions where the services provided involve the use of oral care cosmetic products, for example dentists, may be exposed dermally to the notified chemical. Such professionals are expected to use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the exposure of such workers is expected to be very low and 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 lip oral and dermal (on lip) care cosmetic products containing the notified chemical at ≤ 0.65% concentration. The principal route of exposure will be oral and dermal while accidental ocular exposure is also possible.

Irritation
The notified chemical is a severe eye irritant and slightly irritating to the skin. However, significant effects are not expected from the use of products containing the notified chemical at the proposed low use concentrations in lip and oral care cosmetic products.

Systemic Effects
The potential systemic exposure to young children (2-4 year olds) from the use of the notified chemical in toothpaste only was estimated to be 0.894 mg/kg bw/day, while the potential systemic exposure to adults from the use of the notified chemical in lip and oral care cosmetic products was estimated to be 0.2394 mg/kg bw/day. Using a NOAEL of 150 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 270 and 627 in children and adults, respectively. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences.

Overall, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 0.65% concentration in lip and oral care cosmetic products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia. Release of the notified chemical at sites is expected to be limited to accidental spills during transport, storage and product reformulation. Reformulation sites are expected to utilise engineering controls to limit release into the environment, but the notifier estimates that 1% of the notified chemical will remain as residues from import containers, which will be washed to sewer after on-site treatment. Accidental spills and equipment washings are to be collected using absorbent materials placed in sealed containers, and disposed of according to local government regulations.

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemical will primarily be rinsed into the sewer system as a part of its use in cosmetic products.

RELEASE OF CHEMICAL FROM DISPOSAL

A small proportion of the notified chemical may remain in the end use and bulk containers as residues, which are likely to be recycled or disposed of to landfill. The notifier expects this to account for 4% of the total import volume. During recycling of containers, residues containing the notified chemical are expected to be rinsed out with water and washed to sewer after on-site treatment.

7.1.2. Environmental Fate

The majority of the notified chemical will be washed into the sewer as a part of its use in cosmetic products, where it is expected to be partially removed by the STP. Approximately 3% of the notified chemical may remain in the end use containers which are either recycled or disposed of to landfill. The notified chemical is readily biodegradable (86% degradation after 28 days) and is not expected to bioaccumulate (QSAR BCF = 101.6). For the details of the biodegradability study refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

A predicted environmental concentration (PEC) for the worst case scenario has been calculated. It was assumed that 100% of the annual import quantity of the notified chemical is released to the sewer from oral care cosmetic uses over 365 days/year, with no removal of the notified chemical by sewage treatment plant (STP) processes. The extent to which the notified chemical is removed from the effluent in STP processes based on the properties of the notified chemical has not been considered for the worst-case scenario.

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	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.56	µg/L
PEC - Ocean:	0.06	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 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 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.56 µg/L may potentially result in a soil concentration of approximately 3.7 µg/kg. Since the notified chemical is readily biodegradable, no accumulation is 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.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 = 13 mg/L	Harmful to fish
Daphnia Toxicity	EC50 = 42 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	ErC50 = 39 mg/L NOEC = 2.4 mg/L	Harmful to algae
Inhibition of Bacterial Respiration	EC50 > 100 mg/L	Not harmful to microbial respiration

Based on the above ecotoxicological endpoints, the notified chemical is expected to be harmful to fish, aquatic invertebrates and algae. Therefore, the notified chemical is classified as 'Acute Category 3: Harmful to aquatic life' according to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). The notified chemical is readily biodegradable and is not bioaccumulative. Therefore, the notified chemical is not formally classified under the GHS for long-term hazard.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) was calculated using the most sensitive acute endpoint for the notified chemical (Fish LC50 = 13 mg/L). An assessment factor of 100 was used given acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50 (Fish).	13	mg/L
Assessment Factor	100	
Mitigation Factor	1	
PNEC:	130	µg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River:	0.56	130	< 0.01
Q - Ocean:	0.06	130	< 0.01

The risk quotient (Q=PEC/PNEC) has been calculated based on the worst case scenario. The conservative risk quotient has been calculated to be significantly less than 1 in both river and ocean compartments.

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.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 52 °C

Method Commission Directive 92/69/EEC A.1 Melting/Freezing Temperature
Remarks Determined using a melting point apparatus with a liquid bath
Test Facility Exempt Information (11)

Boiling Point Decomposes without boiling at 225 °C

Method Commission Directive 92/69/EEC A.2 Boiling Temperature
Remarks Determined by Siwoloboff method
Test Facility Exempt Information (11)

Density 1,136 kg/m^3 at 21 °C

Method Commission Directive 92/69/EEC A.3 Relative Density
Remarks Determined by pycnometer
Test Facility Exempt Information (11)

Vapour Pressure 1.024×10^{-5} kPa at 25 °C

Method Commission Directive 92/69/EEC A.4 Vapour Pressure
Remarks Determined using a vapour pressure balance
Test Facility Exempt Information (12)

Water Solubility 0.148 g/L at 20 °C

Method Commission Directive 92/69/EEC A6 Water Solubility
Remarks Shake flask Method
Test Facility Exempt Information (11)

Hydrolysis as a Function of pH

Method Commission Directive 92/69/EEC C7 Hydrolysis as a Function of pH

pH	*T (°C)*	*t½ (hours)*
4	50	< 10% after 5 days
7	50	428
7	25	7,420 (estimated)
9	25	133

Remarks The notified chemical is expected to hydrolyse under environmental conditions, increasing at higher pH levels.
Test Facility Exempt Information (11)

Partition Coefficient (n-octanol/water) log Pow = 3.55 at 20 °C

Method Commission Directive 92/69/EEC A8 Partition Coefficient
Remarks Flask Method
Test Facility Exempt Information (11)

Surface Tension 53.2 mN/m at 19.5 °C

Method Commission Directive 92/69/EEC A.5 Surface Tension
Remarks Concentration: 6.75×10^{-2} g/L
Test Facility Exempt Information (11)

Flash Point 157 °C

Method	Commission Directive 92/69/EEC A.9 Flash Point
Remarks	Closed cup method
Test Facility	Exempt Information (13)

Autoignition Temperature 345 °C

Method	Commission Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases)
Test Facility	Exempt Information (13)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity (1987)
Species/Strain	Rat/Sprague-Dawley
Vehicle	Arachis oil BP
Remarks – Method	No protocol deviations. A range finding study was conducted with 2 animals (1M/1F) dosed at 2,000 mg/kg bw.

RESULTS

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

*range finding study

LD50	> 2,000 mg/kg bw
Signs of Toxicity	In the range finding study, clinical signs of toxicity included lethargy and ataxia. Both animals appeared normal at the day 3 observation. In the main study, clinical signs of toxicity included lethargy, ataxia and in one animal hunched posture. All animals appeared normal at the day 1 observation.
Effects in Organs	No abnormalities were observed during necroscopy.
Remarks – Results	The animals showed expected body weight gain during the 14 day observation period
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Exempt Information (8)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (1981)
Species/Strain	Rat/Sprague Dawley
Vehicle	Arachis oil BP
Type of dressing	Semi-occlusive
Remarks – Method	The test material in powder form was directly applied to shorn skin which had previously been moistened with the vehicle. No protocol deviations.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Moderate erythema (grade not provided) was observed in all 5 females on observation days 2 and 3. A female showed desquamation on observation days 3 to 5. All symptoms were resolved at the day 6 observation.
Signs of Toxicity – Systemic	No signs of systemic toxicity were observed.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks – Results	All animals showed expected bodyweight gain during the study period.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.

TEST FACILITY Exempt Information (9)

B.3. Skin Irritation – Rabbit

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion (1981)
Species/Strain Rabbit/New Zealand White
Number of Animals 4 F
Vehicle Diethyl phthalate
Observation Period 14 days
Type of Dressing Semi-occlusive
Remarks – Method Animals were treated with 1%, 5%, 10%, 20% and 100% of the test substance.

RESULTS

Results for test substance at 100% concentration only:

Lesion	*Mean Score**				*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4			
Erythema/Eschar	0	0.33	0	0.33	1	< 48 h	0
Oedema	0	0	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 Very slight (grade 1) erythema was observed in an animal exposed to 20% concentration of the test substance and in 2 animals exposed to 100% concentration of the test substance at the 24 hour observation. The symptom was resolved at the 48 hour observation.

One animal exposed to 1% concentration of the test substance showed very slight oedema (grade 1) at the 4.5 hour observation and another animal exposed to 100% concentration of the test substance at the 24 hour observation. The symptom was resolved at the 24 hour and 48 hour observations, respectively.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY Exempt Information (6)

B.4. Eye Irritation – Rabbit

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion (1987)
Species/Strain Rabbit/New Zealand White
Number of Animals 3M/1F
Observation Period 14 days
Remarks – Method No protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*				*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4			
Conjunctiva – Redness	2	1.7	2	1.7	2	< 14 days	0
Conjunctiva – Chemosis	2	1.3	2	1.3	2	< 14 days	0
Conjunctiva – Discharge	1.7	0.3	0.3	1.7	3	< 14 days	0

Corneal Opacity	1.3	1	1	1	2	< 14 days	0
Iridial Inflammation	1	0.7	1	1	1	< 14 days	0

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

Remarks – Results

Residual test material around the eyes was observed in all four animals at the 1 hour observation.

Moderate (grade 2) redness was observed in all four animals at the 1 hour observation and it was reduced to slight in 2 animals at the 72 hour observation. The redness was resolved in 3 animals at the day 7 observation and in the fourth animal at the day 14 observation. One animal showed petechial haemorrhage of the nictitating membrane at the 1 hour observation which was resolved at the 24 hour observation.

Moderate (grade 2) chemosis was observed at the 1 hour (in all four animals), at the 24 hour (in 2 animals) and at the 72 hour (in 2 animals) observations. The symptom subsided to slight (grade 1) in 2 animals at the 48 hour observation and in 1 animal at the day 7 observation. The symptom was resolved in 3 animals at the day 7 observation and in one animal at the day 14 observation.

Severe (grade 3 in 2/4 animals) to moderate (grade 2 in 2/4 animals) discharge was observed at the 1 hour observation. Moderate (grade 2) discharge persisted in 2 animals at the 24 and 48 hour observations. The symptom became slight (grade 1) at the 72 hour observation. All animals appeared normal at the day 14 observation.

Dulling of the normal lustre of the corneal surface was observed at the 1 hour observation in all animals. The symptom progressed to slight corneal opacity (grade 1) in all animals at the 24, 48 and 72 hour observations, except for one animal where opacity progressed to moderate (grade 2) at the 72 hour observation. The symptom was resolved at the day 7 observation in 3 animals with slight corneal opacity persisting in one animal at the day 7 observation. This animal also showed vascularisation of the cornea at the day 7 and day 14 observations. No observations were made beyond day 14.

Slight iridial inflammation (grade 1) was observed in all four animals at the 1 hour observation which was resolved at the 72 hour observation in 1 animal, at the day 7 observation in 2 animals and at the day 14 observation in the fourth animal.

CONCLUSION The notified chemical is severely irritating to the eye.

TEST FACILITY Exempt Information (10)

B.5. Skin Sensitisation – Guinea Pig Maximisation Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 406 Skin Sensitisation – Maximisation Test (1981)

Species/Strain Guinea pig/Albino SPF

PRELIMINARY STUDY Maximum non-irritating concentration:
Intradermal: 5% (maximum tested)
Topical: 50% (maximum tested)

MAIN STUDY

Number of Animals Test Group: 20 (sex not stated) Control Group: 20 (sex not stated)

Vehicle Intradermal induction: groundnut oil
Dermal induction/challenge: diethyl phthalate

Positive Control Not conducted.

INDUCTION PHASE	Induction concentration: Intradermal: 5% Topical: 50%
Signs of Irritation	Intradermal injections of Freund's complete adjuvant with vehicle or test substance or sterile distilled water elicited irritation (no further information provided). No skin reactions were observed following topical induction.
CHALLENGE PHASE	
1st Challenge	Topical: 50%
Remarks – Method	In the preliminary intradermal irritancy study, no reactions were observed at 0.63% - 5% test concentrations. No skin reactions were observed in the one tested animal in the preliminary topical irritancy study conducted at 25% and 50% concentration of the test substance. All control and test group animals were pre-treated with 0.5 g of sodium lauryl sulphate (10% in petrolatum) one day prior to topical induction. 22 males and 18 females were used in the main study.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st Challenge		*2nd Challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	50%	2	1	-	-
Control Group	50%	1	0	-	-

Remarks – Results	No unscheduled deaths or test substance-related signs of toxicity were observed during the study. One control animal showed slight erythema (grade 1) 24 hours after challenge which was resolved at the 48 hour observation. Two test animals showed slight erythema 24 hours after challenge which persisted in one animal at the 48 hour observation. Normal body weight gains were recorded for all animals during the study.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical (up to 50% topical induction concentration) under the conditions of the test.
TEST FACILITY	Exempt Information (7)

B.6. Skin Sensitisation – Human Volunteers

TEST SUBSTANCE	Notified chemical (3% notified chemical in 91% water, 3% octyldodecanol and 2.4% sodium hydroxide))
METHOD	Repeated insult patch test with challenge
Study Design	Induction procedure: patches containing 0.3 mL of the test substance were applied 3 times per week (Monday, Wednesday and Friday) for a total of 9 applications during the induction period. Patches were removed by the subjects after 24 hours and graded by technicians after an additional 24 hours (or 48 hours for patches applied on Friday). Rest Period: 14 days Challenge procedure: Patches were applied to the induction site and to a naïve site for 24 hours. The sites were scored 48 and 96 hours after application.

	Rechallenge (timing of rechallenge not stated): If reactions were observed, these were re-challenged for 24 hours and the sites were scored 48 and 96 after re-challenge application. Three subjects that did not show significant reaction during challenge to the test substance were used as control.
Study Group	79 F, 18 M; age range 21-65 (and above) years
Vehicle	None
Remarks – Method	Occluded. The test substance was spread on a 2 cm × 2 cm patch
RESULTS	
Remarks – Results	92/97 subjects completed the study. Five subjects discontinued (during induction) from the study for reasons unrelated to the test substance. Due to the irritation effects, three subjects wore less than 9 induction patches. Although a subject inadvertently kept the challenge patches on for 48 hours, no irritation symptom was observed. During induction, mild erythema (grade 1) was observed in 58 subjects with a further 6 subjects showing stronger reactions. One subject showed strong erythema with oedema and five subjects showed mild erythema with papules. During challenge, four subjects showed mild erythema with three further subjects showing stronger reactions. One subject showed mild erythema with oedema at 48 hours that regressed to mild erythema at 96 hours. The second subject showed mild erythema with papules at 48 hours and 96 hours. The third subject showed moderate spreading erythema with oedema at 48 hours that progressed to strong erythema with oedema at 96 hours. These three subjects took part in a rechallenge with three other subjects that did not show a reaction during challenge (as control). One subject showed mild spreading erythema at 48 hours regressing to mild erythema at 96 hours. The second subject showed moderate spreading erythema with oedema at 48 hours regressing to mild erythema at 96 hours. The third subject showed strong spreading erythema with oedema and papules at 48 hours which progressed to strong spreading erythema with oedema and vesicles at 96 hours. None of the 3 control subjects developed reactions at rechallenge. The reactions in two of the subjects appear to subside; however, the increase in severity of the reaction observed in the third subject confirms a sensitisation reaction in this subject.
CONCLUSION	The notified chemical at 3% concentration was sensitising under the conditions of the test.
TEST FACILITY	Exempt Information (3)

B.7. Repeat Dose Oral Toxicity – Rats

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Sprague-Dawley/CD
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days

Vehicle	Polyethylene glycol 400
Remarks – Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5M/5F	0	0/10
Low Dose	5M/5F	15	0/10
Mid Dose	5M/5F	150	0/10
High Dose	5M/5F	1,000	0/10
Control Recovery	5M/5F	0	0/10
High Dose Recovery	5M/5F	1,000	0/10

Mortality and Time to Death

No unscheduled mortalities were observed during the main study.

Clinical Observations

Ataxia, hunched posture and tiptoe gait were observed in a high dose female on day 17. A high dose male showed piloerection on day 7.

Increased salivation was observed intermittently in high dose males and females from day 5 onwards immediately after dosing and was generally short-lived.

Red or brown staining around the mouth, noisy respiration and wet fur were observed intermittently in several high dose males and females from day 4 onwards.

These finding were no longer evident in the recovery group.

All animals showed expected bodyweight gain during the study. Females (all treatment groups) showed reduced mean food intake (3-14% reduction than control group), except for first week mean food intake was 8% higher than the control group.

Laboratory Findings – Clinical Chemistry, Haematology

The following statistically significant changes were observed in treated animals:

- Reduction in mean total leucocyte count in low dose males and low and high dose females
- Reduction in mean neutrophil level in low, mid and high dose males and females
- Increase in mean albumin/globulin ratio in low dose males
- Increased mean monocyte level in mid dose females
- Increased mean total plasma protein content in high dose recovery males
- Reduction in mean plasma urea content (19.5% reduction than the control females) in low dose females
- Increase in mean alkaline phosphatase level in low dose females
- Reduction in mean alanine aminotransferase content in mid and high dose females
- Reduction in mean potassium level in all treated females
- Reduction in mean lymphocyte count in high dose recovery males
- Increase in mean alanine aminotransferase level in high dose recovery males

The study authors stated these observations were not treatment related and/or the values were not abnormally high or low, therefore these effects were not considered to be toxicologically significant.

Effects in Organs

All five high dose males showed a speckled appearance of the kidney and one of the high dose males also showed pale kidney. Three out of five mid dose males showed a slightly speckled appearance of the kidney.

The following statistically significant changes were observed:

- Increase in mean relative kidney weight in high dose males
- Increase in mean relative liver weight in high dose males and females

- Increase in mean relative spleen weight in high dose recovery females.
- Reduction in mean absolute testicular weight in high dose males

A high dose recovery male showed dark and enlarged spleen, however, no abnormalities were observed.

Centrilobular hepatocyte enlargement and a reduced severity of periportal vacuolation were observed in mid and high dose males. All five control recovery males (four males had minimal and one male had slight) and one high dose recovery males (minimal) showed periportal vacuolation and this effect was not observed in control males or in female groups.

Remarks – Results
Speckled kidneys were observed in all high dose males and slightly speckled kidneys in 3/5 mid dose males but was not evident in the recovery group animals or in treated females. The study authors stated the toxicological significance of these findings were unclear because no associated histopathological lesions were observed in the kidneys during necropsy and no evidence of kidney dysfunction based on clinical chemistry parameters were observed during necropsy. However, high dose males showed statistically significant increased mean relative kidney weight compared to control males, As no associated change in kidney weight in mid dose males were observed, the speckled appearance in the kidney in mid dose males was not considered to be toxicologically significant by the study authors.

CONCLUSION
The NOAEL was established as 150 mg/kg bw/day by the study authors, based on effects observed in the kidney.

TEST FACILITY Exempt Information (14)

B.8. Genotoxicity – Bacteria

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 471 Bacterial Reverse Mutation Test
Plate incorporation procedure

Species/Strain *Salmonella typhimurium*: TA1538, TA1535, TA1537, TA98, TA100

Metabolic Activation System S9 mix from Aroclor 1254 induced rat liver

Concentration Range in Main Test
Test 1
With or without metabolic activation: 10, 33, 333, 1,000 and 5,000 µg/plate

Test 2
a) With metabolic activation: 15, 50, 150, 500, 1,500 and 5,000 µg/plate
b) Without metabolic activation: 15, 50, 150, 500 and 1,500 µg/plate

Vehicle Dimethyl sulfoxide (DMSO)

Remarks – Method A preliminary test was not conducted.

Negative controls: distilled water and DMSO

Positive control:
With metabolic activation: 2-aminoanthracene
Without metabolic activation: 2-nitrofluorene (TA1538 and TA98), 9-aminoacridine (TA1537) and sodium azide (TA1535 and TA100).

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	Not investigated	≥ 1,000	> 5,000	Negative
Test 2	Not investigated	≥ 500	> 1,500	Negative
Present				
Test 1	Not investigated	≥ 5,000	> 5,000	Negative

Test 2	Not investigated	≥ 1,500	> 5,000	Negative

Remarks – Results

No statistically significant increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any concentration level, with or without metabolic activation.

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

CONCLUSION

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

TEST FACILITY

Exempt Information (4)

B.9. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE

Notified chemical

METHOD

Similar to OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test

Species/Strain: Human
Cell Type/Cell Line: Peripheral blood lymphocytes
Metabolic Activation System: S9 mix from Aroclor 1254 induced rat liver
Vehicle: DMSO
Remarks – Method: Vehicle control: DMSO
Positive control: mitomycin and cyclophosphamide

A preliminary study was conducted, but no details were provided.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	10*, 30*, 100*	24 h	24 h
Test 2	10*, 30*, 100*	24 h	24 h
Present			
Test 1	30*, 100*, 300*	24 h	24 h
Test 2	30*, 100*, 300*	24 h	24 h

*Cultures selected for metaphase analysis

RESULTS

Metabolic	*Test Substance Concentration (µg/mL) Resulting in:*			
Activation	*Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	No information	≥ 100	Not stated	Negative
Test 2	No information	≥ 100	Not stated	Negative
Present				
Test 1	No information	≥ 300	Not stated	Negative
Test 2	No information	≥ 300	Not stated	Negative

Remarks – Results

Cytotoxicity was observed as a reduction in mitotic index at the highest test concentrations on an average of 32% without metabolic activation and 49% with metabolic activation.

No statistically significant increases were noted in chromosome aberrations, 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 clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Exempt Information (5)

B.10. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test (2016)
Species/Strain	Chinese hamster
Cell Type/Cell Line	V79
Metabolic Activation System	S9 mix from phenobarbital/ß-naphthoflavone induced rat liver
Vehicle	DMSO
Remarks – Method	A preliminary toxicity test was conducted at a concentration range of 16-2,050 µg/mL (both with or without S9 mix). positive control: without S9 mix: ethylmethane sulfonate with S9 mix: 7,12-dimethylbenz(a)anthracene

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	4*, 8*, 16*, 32*, 64*, 96, 128	4 h	7 days	8 days
Test 2	32, 48*, 64*, 72.3*, 80.6*, 88*	4 h	7 days	8 days
Present				
Test 1	8*, 16*, 32*, 64*,128, 192, 256	4 h	7 days	8 days
Test 2	32, 64*, 80.6*, 96*, 112*, 128*	4 h	7 days	8 days

*Cultures selected for metaphase analysis

RESULTS

Metabolic	*Test Substance Concentration (µg/mL) Resulting in:*			
Activation	*Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 128.1	≥ 64	> 128	Negative
Test 2		≥ 88	> 88	Negative
Present				
Test 1	≥ 256.3	≥ 128	≥ 128	Negative
Test 2		> 128	≥ 128	Negative

Remarks – Results	A statistically significant increase in mutant colonies was observed at a concentration of 112 µg/mL with metabolic activation (test 2). The study authors stated this effect was not considered biologically relevant as it was not reproduced at any other concentrations tested. No biologically relevant increase in the number of mutant colonies was observed at any concentration, with and 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 Chinese hamster V79 cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Exempt Information (1)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon
Remarks – Method	No significant deviations were noted. Sodium benzoate was used as the reference substance.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	0	1	4
6	43	6	65
14	61	14	80
29*	87	29*	81

*Day 29 values corrected to include any carry-over of CO_2 detected in absorber 2 on Day 29.

Remarks – Results	All validity criteria were met. Inorganic carbon was < 5% in the test sample at the beginning of the test, the CO_2 levels in the inoculum blank did not exceed 40 mg/L and the difference in extremes did not exceed 20% at any time throughout the test.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	Exempt Information (15)

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	*Oncorhynchus mykiss*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	GC
Remarks – Method	No deviations were noted. The test concentrations for the main study were based on the results of a preliminary range finding test.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	-	10	0	0	0	0	0
1.8	1.86	10	0	0	0	0	0
3.2	3.43	10	0	0	0	0	0
5.6	5.30	10	0	0	0	0	0

10	10.8	10	0	0	0	0	0
18	16.8	10	0	10	10	10	10

LC50	13 mg/L at 96 hours determined by moving average method.
NOEC	3.2 mg/L at 96 hours
LOEC	5.6 mg/L at 96 hours
Remarks – Results	All validity criteria were met. Dissolved oxygen was maintained at > 60% and test concentrations were analytically validated.

CONCLUSION The notified chemical is harmful to fish

TEST FACILITY Exempt Information (16)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	270 mg $CaCO_3$/L
Analytical Monitoring	GC
Remarks – Method	No deviations were noted. The test concentrations for the main study were based on the results of a preliminary range finding test.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
1.0	1.01	20	0	0
1.8	1.73	20	0	0
3.2	3.19	20	0	0
5.6	4.72	20	0	0
10	9.83	20	0	0
18	16.5	20	0	0
32	25.3	20	0	7
56	46.1	20	7	13
100	84.9	20	20	20

LC50	62 mg/L at 24 hours 42 mg/L at 48 hours
NOEC	18 mg/L at 48 hours
Remarks – Results	All validity criteria were met. Dissolved oxygen was maintained at > 3mg/L in all vessels, pH was maintained within 1.5 units and temperature was maintained at 21 °C.

CONCLUSION The notified chemical is harmful to aquatic invertebrates

TEST FACILITY Exempt Information (17)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE Notified chemical

METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours

Concentration Range	Nominal: 2.4 mg/L Actual: 83 mg/L
Auxiliary Solvent	None
Water Hardness	15 mg $CaCO_3$/L
Analytical Monitoring	GC-FID
Remarks – Method	No protocol deviations were noted. The test concentrations for the main study were based on the results of a preliminary range finding test.

RESULTS

Growth rate		*Yield*	
ErC50 (mg/L at 72 h)	*NOEC*	*EyC50 (mg/L at 72 h)*	*NOEC*
39	2.4	18	2.4

Remarks – Results	All validity criteria were met. In the control sample, there was a growth factor of 142, a section-by-section < 35% and the specific growth rate coefficient of variation was < 7%. A positive control using potassium dichromate was run 2 months prior to the main test which indicated a 72 hour ErC50 of 1 mg/L which is within the expected range (0.92 – 1.46 mg/L)
CONCLUSION	Notified chemical is harmful to algal growth.
TEST FACILITY	Exempt Information (2)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 100 mg/L
Remarks – Method	The following protocol deviation was noted: A single concentration was used instead of the usual minimum of 3 due to the limited water solubility of the test substance. 3, 5-Dichlorophenol was used as a control substance.
RESULTS	
IC50	> 100 mg/L
NOEC	> 100 mg/L
Remarks – Results	All validity criteria were met. The oxygen consumption in the control samples was maintained at > 20 mg/g of sludge and the 3 hour EC50 of 3,5-dichlorophenol was 8 mg/L which is within the expected range (5-25 mg/L).
CONCLUSION	Notified chemical is not harmful to bacterial respiration up to 100 mg/L.
TEST FACILITY	Exempt Information (18)

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