File No.: LTD/2097

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Carbonic acid, 2-hydroxypropyl (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(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/2097	Colgate-Palmolive Pty Ltd	Carbonic acid, 2-hydroxypropyl (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*
Eye irritation (Category 2)	H319 – Causes serious eye irritation

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

Hazard Classification	*Hazard Statement*
Acute (Category 2)	H401 - Toxic to aquatic life
Chronic (Category 3)	H412 - Harmful to aquatic life with long lasting effects

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

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  – Eye irritation (Category 2A): H319 – Causes serious eye irritation

  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

- 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 exposure to aerosols 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;

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-hydroxypropyl (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl ester

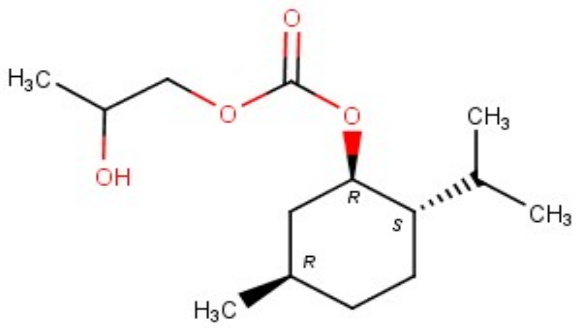
CAS NUMBER
260781-16-6

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

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

MOLECULAR FORMULA
$C_{14}H_{26}O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
258.36 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless to yellow liquid

Property	*Value*	*Data Source/Justification*
Melting Point	< -21 °C	Measured
Boiling Point	Decomposes without boiling at 150 °C	Measured
Density	1,014 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.363×10^{-5} kPa at 25 °C	Measured
Water Solubility	0.0888 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	pH 4 < 10% after 5 days at 50 °C pH 7 $t_{1/2}$ 179 hours at 50 °C pH 7 $t_{1/2}$ (estimated): 3,300 hours at 25 °C pH 9 $t_{1/2}$ = 49.6 hours at 25 °C	Measured
Partition Coefficient (n-octanol/water)	log Pow = 3.72 at 20 °C	Measured
Surface Tension	50.2 mN/m at 19 °C	Measured
Adsorption/Desorption	log K_{oc} = 2.325	Calculated (KOCWIN v2.00 US EPA, 2012)
Dissociation Constant	Not determined	Does not contain dissociable functional groups
Flash Point	153 °C	Measured
Flammability	Not determined	Not highly flammable based on measured flash point
Autoignition Temperature	276 °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.

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 liquid 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 (1.363×10^{-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 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
Skin irritation – rabbit	slightly irritating
Skin irritation – human (n = 51)	non-irritating at 1%
Eye irritation – rabbit	irritating
Skin sensitisation – guinea pig, maximisation test	no evidence of sensitisation
Skin sensitisation – HRIPT (n = 107)	no evidence of sensitisation at 1%

Endpoint	*Result and Assessment Conclusion*
Skin sensitisation – HRIPT (n = 106)	no evidence of sensitisation at 0.5%
Repeat dose oral toxicity – rat, 28 days	NOAEL = 150 mg/kg bw/day*
Mutagenicity – bacterial reverse mutation assay	non mutagenic
Genotoxicity – *in vitro* mammalian cell gene mutation test	non mutagenic
Genotoxicity – *in vitro* mammalian cell micronucleus test	non clastogenic

*established by the study authors

Toxicokinetics
Given the low molecular weight (258.36 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 inhalation toxicity data were provided.

Irritation and Sensitisation
In a skin irritation study, the notified chemical was found to be slightly irritating to the skin of rabbits. Slight erythema was observed in all 4 treated animals, which resolved in three animals at the 48 hour observation but persisted in one animal at the 72 hour observation. No observations were made beyond 72 hours. In a 48 hour human closed patch test, the notified chemical at 1% concentration was found to be non-irritating.

The notified chemical was irritating to eyes based on a study conducted in four rabbits. All four animals showed moderate conjunctival irritation and slight corneal opacity, with one animal showing slight iridial inflammation. All signs of irritation were resolved at the day 7 observation. Based on the results of this study, the notified chemical warrants classification as a Category 2 eye irritant according to the GHS.

The notified chemical (at 100% topical induction and challenge concentration) was not a skin sensitiser in a guinea pig maximisation test. In two human repeated insult patch tests (HRIPT), the notified chemical at 0.5% and 1% concentration in diethyl phthalate/ethanol (3:1) did not elicit a positive sensitisation response in more than 100 subjects at each test concentration.

Repeated Dose Toxicity
A repeated dose oral 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 kidneys. Speckled kidneys were observed in all dose groups which was still evident in the recovery group animals. In high dose animals, this was associated with increased mean kidney weights (14.5% increase than control group) which remained slightly elevated at the end of the recovery period (6% increase than control recovery group). As no associated kidney weight changes were observed in the low and mid dose groups, the macroscopic kidney changes in the low and mid dose group in isolation were not considered to be toxicologically significant by the study authors.

The mean calcium levels in blood were statistically significantly higher in high dose males and in mid dose females, but not in the recovery groups.

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* micronucleus 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*
Eye irritation (Category 2)	H319 – Causes serious eye irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the toxicological information provided, the notified chemical is an eye irritant and is slightly irritating to the skin. Systemic effects on kidneys may occur at high concentrations.

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) is 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 and oral 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 an eye irritant and slightly irritating to the skin. However, effects are not expected from the use of products containing the notified chemical at the proposed low use concentration 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. The notified chemical will be imported as a component of finished cosmetic products or as a raw material and blended into finished cosmetic products. Release of the notified chemical is expected to be < 1% and will be from spills during the transport, storage and product reformulation of the notified chemical. Accidental spills and equipment washings are to be collected for disposal, in accordance with 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 is expected to enter the sewer system before potential release to surface waters on a nationwide basis. A ready biodegradation test determined that the notified chemical is readily biodegradable (78% degradation after 28 days). For details of the biodegradability study refer to Appendix C.

The notified chemical is expected to be effectively removed at sewage treatment plants (STPs) due to its ready biodegradability. A proportion of the notified chemical may be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. The notified chemical residues in landfill and soils are expected to have medium mobility based on its soil adsorption coefficient. The notified chemical is not expected to be bioaccumulative based on its measured partition coefficient (log Pow = 3.7) and the modelled bioconcentration factor (BCF) of 182. In the aquatic and soil compartments, the notified chemical is expected to degrade through biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)
The use pattern will result in most of the notified chemical being washed into the sewer. The predicted environmental concentration (PEC) has been calculated assuming the realistic worst-case scenario with 100% release of the notified chemical into sewer systems nationwide over 365 days per annum. 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 this scenario, and therefore no removal of the notified chemical during sewage treatment processes is assumed. The 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	200.0	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 = 24 mg/L	Harmful to fish
Daphnia Toxicity	EC50 = 8.8 mg/L	Toxic to aquatic invertebrates
Algal Toxicity	ErC50 =21 mg/L	Harmful to algae

Inhibition of Bacterial Respiration	NOErC = 0.9 mg/L EC50 > 62.5 mg/L	Not inhibitory to microbial activity at the highest tested concentration

Based on the above ecotoxicological endpoints, the notified chemical is expected to be acutely toxic to aquatic invertebrates and harmful to algae (acute and chronic). Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), notified chemical is formally classified as "Acute Category 2; Toxic to aquatic life" and "Chronic Category 3; Harmful to aquatic life with long lasting effects".

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) was calculated using the most sensitive endpoint for the notified chemical (*Daphnia magna*, EC50 = 8.8 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		
EC50 (Daphnia)	8.8	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC	88.00	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q – River	0.56	88	< 0.01
Q – Ocean	0.06	88	< 0.01

The risk quotient for discharge of treated effluents containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations in surface waters, based on its maximum annual importation quantity. 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 < -21 °C

Method	Commission Directive 92/69/EEC A.1 Melting/Freezing Temperature
Remarks	No evidence of solidification other than increased viscosity observed down to -21 °C
Test Facility	Exempt Information (13)

Boiling Point Decomposes without boiling at 150 °C

Method	Commission Directive 92/69/EEC A.2 Boiling Temperature
Remarks	Determined using distillation method
Test Facility	Exempt Information (13)

Density 1,014 kg/m^3 at 20 °C

Method	Commission Directive 92/69/EEC A.3 Relative Density
Remarks	Determined using a pycnometer
Test Facility	Exempt Information (13)

Vapour Pressure 1.363×10^{-5} kPa at 25 °C

Method	Commission Directive 92/69/EEC A.4 Vapour Pressure
Remarks	Effusion method
Test Facility	Exempt Information (14)

Water Solubility 0.0888 g/L at 20 °C

Method	EC Council Regulation No 440/2008 A.6 Water Solubility
Remarks	Flask Method
Test Facility	Exempt Information (13)

Hydrolysis as a Function of pH

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

pH	*T (°C)*	*$t_{½}$ (hours)*
4	50	< 10% after 5 days
7	50	179
7	25	3,300 (estimated)
9	25	49.6

Remarks	The notified chemical is expected to hydrolyse under environmental conditions, increasing at higher pH levels.
Test Facility	Exempt Information (13)

Partition Coefficient (n-octanol/water) log Pow = 3.72 at 23 °C

Method	EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	Flask Method
Test Facility	Exempt Information (13)

Surface Tension 50.2 mN/m at 19 °C

Method	Commission Directive 92/69/EEC A.5 Surface Tension
Remarks	Concentration: 5.41×10^{-2} g/L
Test Facility	Exempt Information (13)

Flash Point 153 °C

Method	Commission Directive 92/69/EEC A.9 Flash Point
Remarks	Closed cup method
Test Facility	Exempt Information (15)

Autoignition Temperature 276 °C

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

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	A dose rang-finding study was conducted. No protocol deviations.

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

*dose range-finding study

LD50	> 2,000 mg/kg bw
Signs of Toxicity	Two males showed ataxia at the 2 and 4 hour observations. The symptom was resolved at the day 1 observation.
Effects in Organs	No abnormalities were observed at necropsy.
Remarks – Results	All animals showed expected bodyweight gain during the study.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Exempt Information (11)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (1981)
Species/Strain	Rat/Sprague-Dawley
Vehicle	Nil
Type of dressing	Semi-occlusive
Remarks – Method	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	No signs of toxicity were observed.
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.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Exempt Information (12)

B.3. Skin Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion (1992)
Species/Strain	Rabbit/New Zealand White (SPF)

Number of Animals	4 F
Vehicle	Diethyl phthalate
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks – Method	Animals were treated with 1%, 5%, 10%, 25% 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	0.7	1	0.3	1	> 72 h	1
Oedema	0	0	0	0	0	n/a	0

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

Remarks – Results	No signs of irritation were noted with the test substance at 1%, 5%, 10% and 25% concentration. At 100% concentration, slight (grade 1) erythema was observed in 3/4 animals at the 1 hour observation and in the fourth animal at the 48 hour observation. The symptom was resolved in 3 animals at the 72 hour observation with slight erythema persisting in one animal at the 72 hour observation. No observations were made beyond 72 hours.
CONCLUSION	The notified chemical slightly irritating to the skin.
TEST FACILITY	Exempt Information (8)

B.4. Skin Irritation – Human Closed Patch Test

TEST SUBSTANCE	Notified chemical (1% concentration)
METHOD	Human closed patch test
Study Design	Patches containing 0.2 mL of the test substance were applied for 48 hours. Application sites were evaluated immediately after patch removal and at 72 hours after application.
Study Group	37 F, 16 M; age range 16-74 years
Vehicle	Diethyl phthalate/ethanol (3:1) (information supplied by notifier)
Remarks – Method	Occluded. The test substance was spread on a 1.9 cm × 1.9 cm patch.
RESULTS	
Remarks – Results	51/53 subjects completed the study. Two subjects discontinued the study for reasons unrelated to the test substance. No adverse responses were noted.
CONCLUSION	The notified chemical at 1% concentration was non-irritating under the conditions of the test.
TEST FACILITY	Exempt Information (2)

B.5. 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	4 F

Observation Period	7 days
Remarks – Method	No protocol deviations.

RESULTS

Lesion	*Mean Score**				*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4			
Conjunctiva – Redness	2.7	3	3	3	3	< 7 days	0
Conjunctiva – Chemosis	2.3	1.3	1.7	2.3	4	< 7 days	0
Conjunctiva – Discharge	1.3	1	0.7	1	3	< 7 days	0
Corneal Opacity	1	1	1	1	1	< 7 days	0
Iridial Inflammation	0	0	0	0.3	1	< 7 days	0

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

Remarks – Results	Slight (grade 1, in 1 animal) to moderate (grade 2, in 3 animals) redness was observed in all 4 animals at the 1 hour observation. Three animals showed severe (grade 3) and 1 animal moderate redness at the 24 hour observation. The redness was severe in all 4 animals at the 72 hour observation. Slight (grade 1) to minimal (grade 2) chemosis was observed in all 4 animals at the 1 hour observation. Minimal (in 1 animal), moderate (in 2 animals) and severe (grade 4, in 1 animal) chemosis was observed at the 24 hour observation. The symptom was reverted to minimal to slight in all 4 animals at the 48 and 72 hour observations. All animals showed moderate (grade 2) discharge at the 1 hour observation and the symptom was elevated to severe (grade 3) in 2 animals at the 24 hour observation. One animal showed slight discharge at the 48 and 72 hour observations. No discharge was observed in the other 3 animals at the 48 hour observation. Slight corneal opacity (grade 1) was observed in one animal at the 1 hour observation and in all 4 animals at the 24 to 72 hour observations. One animal showed slight iridial inflammation (grade 1) at the 24 hour observation. No iridial inflammation was observed in the other 3 animals. All eyes appeared normal at the day 7 observation.
CONCLUSION	The notified chemical is irritating to the eye.
TEST FACILITY	Exempt Information (9)

B.6. Skin Sensitisation – Guinea Pig Maximisation Test

TEST SUBSTANCE	Notified chemical	
METHOD	OECD TG 406 Skin Sensitisation – Guinea Pig Maximisation Test (1992)	
Species/Strain	Guinea pig/Albino/SPF	
PRELIMINARY STUDY	Maximum non-irritating concentration: intradermal: not determined (discrete erythema was observed at up to 5%) topical: 100%	
MAIN STUDY		
Number of Animals	Test Group: 10 F	Control Group: 5 F
Vehicle	Arachis oil	
Positive Control	Not conducted	

INDUCTION PHASE	Induction concentration: intradermal: 5% topical: 100%
Signs of Irritation	Discrete erythema was observed in test group animals at intradermal induction sites with 5% test substance in arachis oil. No signs of irritation were observed in control and test group animals following topical induction.
CHALLENGE PHASE	
1st Challenge	topical: 100%
2nd Challenge	not conducted
Remarks – Method	All test and control group animals were treated with 10% sodium lauryl sulphate the day before topical induction.

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	100%	0	0	-	-
Control Group	100%	0	0	-	-

Remarks – Results	No skin reactions were noted in control and test group animals. No unscheduled mortalities were observed during the study. All animals showed expected bodyweight gain during the study.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Exempt Information (10)

B.7. Skin Sensitisation – Human Volunteers

TEST SUBSTANCE	Notified chemical (1% concentration)
METHOD	Repeated insult patch test with challenge
Study Design	Induction procedure: patches containing 0.2 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.
Study Group	84 F, 31 M; age range 18-79 years
Vehicle	Diethyl phthalate/ethanol (3:1) (information supplied by notifier)
Remarks – Method	Occluded. The test substance was spread on a 1.9 cm × 1.9 cm patch.
RESULTS	
Remarks – Results	107/115 subjects completed the study. Eight subjects discontinued (during induction) participating in the study for reasons unrelated to the test substance. Barely perceptible erythema (grade 0.5) was observed in a male at the 9th induction. No other skin reactions were observed in test subjects during induction or challenge.

CONCLUSION	The notified chemical was non-sensitising under the conditions of the test.
TEST FACILITY	Exempt Information (3)

B.8. Skin Sensitisation – Human Volunteers

TEST SUBSTANCE	Notified chemical (0.5% concentration)
METHOD	Repeated insult patch test with challenge
Study Design	Induction procedure: patches containing 0.2 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.
Study Group	82 F, 31 M; age range 17-76 years
Vehicle	Diethyl phthalate/ethanol (3:1) (information supplied by notifier)
Remarks – Method	Semi-occluded. The test substance was spread on a 2.5 cm × 2.5 cm patch.
RESULTS	
Remarks – Results	106/113 subjects completed the study. Six subjects discontinued (five subjects during induction and one subject during challenge) with the study for reasons unrelated to the test substance. No skin reactions were observed during induction or challenge.
CONCLUSION	The notified chemical was non-sensitising under the conditions of the test.
TEST FACILITY	Exempt Information (1)

B.9. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
Species/Strain	Rat/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	Arachis oil BP
Remarks – Method	In a dose-range finding study, 24 (3M/3F/dose) rats (Sprague-Dawley/CD) were orally (gavage) exposed to the notified chemical at 0, 150, 400 and 1,000 mg/kg bw/day for 14 consecutive days. No mortalities were observed during the study. Increased salivation was observed immediately after dosing from day 3 in all low, mid and high dose animals (both sexes). Red or brown staining around the mouth was observed sporadically from day 2-14 in mid and high dose animals. A high dose animal showed wet fur on day 4 and 5. No abnormalities were observed at necropsy. All animals showed expected bodyweight gain during the study.

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 study.

Clinical Observations

In high dose animals clinical observations included increased salivation immediately after dosing from day 3 onwards associated with wet fur and red-brown staining around the mouth, and on two isolated occasions, noisy respiration. Increased salivation was observed on two occasions (immediately after dosing on day 14 in one male and day 13 in one female) in mid dose animals. No clinical signs of toxicity were observed in low dose animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

The following statistically significant changes were observed in treated animals, although the study authors considered these are not toxicologically important:

- Increased mean corpuscular volume in high dose males
- Increased mean platelet count in low and mid dose males and mid dose females
- Increased mean neutrophil count in high dose recovery females
- Increased mean potassium in high dose males
- Increased mean calcium levels in high dose males (4.1% increase compared to control male group) and mean calcium in mid dose females (9.2% increase compared to control female group). Statistically not significant increase in mean calcium levels in all treatment groups, except for high dose recovery female group, compared to control groups was observed.
- Reduction in mean chlorine level in high dose males
- Reduction in mean urea concentration in high dose recovery females
- Increased mean glucose level in high dose females
- Increase in mean creatinine level in mid dose females
- Reduction in mean bilirubin level in high dose recovery females

Effects in Organs

Eosinophilic globules were observed in the proximal tubular epithelium in the kidney in mid and high dose males and in high dose recovery males, although with reduced severity and incidence. The study authors stated the lesion is characteristic of a condition known as hydrocarbon nephropathy which is specific to the male rat following administration of diverse group of hydrocarbons (Alden, 1986), and therefore this finding is not relevant to humans. Statistically significant increase in absolute mean kidney weight in high dose males and increased relative mean kidney weight in high dose recovery males were observed. The study authors stated this effect was confined to males and therefore probably associated with the presence of eosinophilic globules in the proximal tubular epithelium.

Speckled or slightly speckled kidneys were observed in low dose (in 1 male and 2 females), mid dose (in all 5 males and a female), high dose (in all 5 males and 4 females) and high dose recovery (in 3 males and 1 female) groups. Five high dose males and a high dose female showed pale or slightly pale kidneys. One low and one mid dose males showed hydronephrosis (swelling of kidney due to build-up of urine) in the right kidney.

A statistically significant increase in relative and absolute liver weights were observed in high dose males and females. Increased liver weights were not observed in high dose recovery animals.

The following statistically significant organ weight changes were also reported:

- Reduction in mean relative heart weight in high dose males and mid dose females
- Reduction in mean relative spleen weight in high dose females
- Reduction in mean relative brain weight in low and high dose females

These findings were not considered of toxicological significance based on no dose relationship or the values were within the range normally expected for rats of this strain and age.

Remarks – Results
Speckled kidneys were observed in all dose groups which was still evident in the recovery group animals. In high dose animals, this was associated with increased liver weights and a slight but not significant increase in kidney weights which remained slightly elevated at the end of the recovery period. As no associated liver and kidney weight changes were observed in the low and mid dose groups, the macroscopic kidney changes in the low and mid dose group in isolation were 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 kidneys.

TEST FACILITY Exempt Information (16)

B.10. 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
a) With metabolic activation: 50, 150, 500, 1,500 and 5,000 µg/plate
b) Without metabolic activation: 15, 50, 150, 500, 1,500 and 5,000 µg/plate

Test 2
a) With metabolic activation: 15, 50, 150, 500 and 1,500 µg/plate
b) Without metabolic activation: 5, 15, 50, 150, 500 and 1,500 µg/plate
Vehicle Dimethyl sulfoxide (DMSO)
Remarks – Method A preliminary test was not conducted.

Vehicle, control and positive control studies were conducted in parallel with the main study.

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	≥ 500	> 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	> 1,500	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 dose 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 (7)

B.11. 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 16.1-2,056 µg/mL with an exposure period of 4 hours (both with and 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*, 48*, 64	4 h	7 days	8 days
Present				
Test 1	16*, 32*, 64*,128*, 192, 256	4 h	7 days	8 days
Test 2	80*, 120*, 160*, 200, 240, 260	4 h	7 days	8 days

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 64.3	≥ 48	> 64	Negative
Present				
Test 1	≥ 257	≥ 192	≥ 192#	Negative
Test 2		≥ 120	≥ 200#	Negative

#Phase separation

Remarks – Results	A significant increase in mutation frequency was observed at 64 µg/mL with metabolic activation. However as it was not reproduced at any other, even higher, concentrations and there was no dose dependent increase, it was not considered biologically relevant by the study authors. 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 (4)

B.12. Genotoxicity – *In Vitro* Mammalian Cell Micronucleus Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 487 Mammalian Cell Micronucleus Test (2014)
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 mix from phenobarbital/ß-naphthoflavone induced rat liver
Vehicle	DMSO
Remarks – Method	A preliminary toxicity study was conducted. positive control: without S9 mix: mitomycin C (pulse treatment) and demecolcin (continuous treatment) with S9 mix: cyclophosphamide

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	13.4, 23.4*, 40.9*, 71.6*, 125, 219, 384, 671, 1,175, 2,056	4 h	40 h
Test 2	2.6, 4.5, 8.0, 13.9, 24.4*, 42.6*, 74.6*, 131, 229, 400	20 h	40 h
Present			
Test 1	13.4, 23.4, 40.9, 71.6*, 125*, 219*, 384, 671, 1,175, 2,056	4 h	40 h
Test 2	82.9, 108, 140*, 182*, 237*, 308, 400	4 h	40 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				
Test 1	≥ 219	≥ 219	≥ 219[#]	Negative
Test 2		≥ 131	≥ 400[#]	Negative
Present				
Test 1	≥ 384	≥ 384	≥ 384[#]	Negative
Test 2		≥ 308	> 400	Negative

[#]Phase separation

Remarks – Results	In test 1 with metabolic activation a statistically significant increase (1.3% increase) in the number of micronucleate cells was observed at the highest evaluated concentration (219 µg/mL), which was above the historical control range of 0.08-1.2%. However, in the confirmatory test (test 2), no significant increase in the number of micronucleate cells was observed and the values (0.2-0.25%) were below the historical control range. As the effect was not reproducible it was considered as biologically irrelevant by the study authors. 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)

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 from sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	CO_2 evolution and total organic carbon
Remarks – Method	The test was conducted according to good laboratory practice (GLP) principles with no significant protocol deviations.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
3	3	3	37
14	69	14	80
28	78	28	83

Remarks – Results	All validity criteria for the test were satisfied. The mean biodegradation of notified chemical was 78% during the 28 day window. The test substance is, therefore, considered to be readily biodegradable.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	Exempt Information (17)

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 EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - Semi-static
Species	*Oncorhynchus mykiss* (rainbow trout)
Exposure Period	96 hrs
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography
Remarks – Method	The test was conducted according to the good laboratory practice (GLP) principles with no significant protocol deviations. Test solutions were prepared in dechlorinated tap water and renewed every 24 hours during the test.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	<LOD	10	0	0	0	0	0
3.2	1.45	10	0	0	0	0	0

5.6	2.47	10	0	0	0	0	0
10	5.12	10	0	0	0	0	0
18	10.8	10	0	0	0	0	0
32	ND	10	7	10	10	10	10

LC50	24 mg/L at 24 hours 24 mg/L at 96 hours
NOEC	5.6 mg/L at 96 hours
Remarks – Results	The dissolved O_2 concentrations throughout the test were ≥ 9.9 mg/L (> 96% oxygen saturation in fresh water at 14°C; U.S. Geological Survey, 2011) in all the test concentrations levels and the control. All validity criteria were met except the measured concentrations of parent test material were <80% of nominal. The LC50 values based on measured concentrations were considered inappropriate as the toxicity cannot be attributed to the parent or the hydrolysis product alone, but a combination of the two. Therefore, the LC50 values were based on nominal concentrations.
CONCLUSION	The notified chemical is harmful to fish.
TEST FACILITY	Exempt Information (18)

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 test EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia – Static test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	270 mg $CaCO_3$/L
Analytical Monitoring	GC
Remarks – Method	The test was conducted according to good laboratory practice (GLP) principles. A saturated solution of 100 mg/L was prepared and diluted to obtain the test concentrations.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h [acute]*	*48 h [acute]*
Control		20	0	0
1.0		20	0	0
1.8		20	0	0
3.2		20	0	0
5.6		20	0	3
10		20	7	13
18		20	16	19
32		20	20	20
56		20	20	20
100		20	20	20

*Total Daphnia in duplicates

EC50	12 mg/L at 24 hours 8.8 mg/L at 48 hours
NOEC	5.6 mg/L at 24 hours 3.2 mg/L at 48 hours

Remarks – Results	All validity criteria were satisfied. The dissolved O_2 concentration was ≥ 7.9 mg/L at all test concentrations and the control. Measured concentrations of parent material and its hydrolysis products were 82.9 to 98% and 1.7 to 6.6% of the nominal, respectively. The EC50 values were based on nominal concentrations.
CONCLUSION	The notified chemical is toxic to *Daphnia magna*.
TEST FACILITY	Exempt Information (19)

C.2.3. Algal Growth Inhibition Test

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test EC Council Regulation No 266/2016 C.3 Algal Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1:320, 1:100, 1:32, 1:10, 1:3.2 and undiluted filtrate in mg/L Actual: 0.601, 2.04, 6.44, 21.2, 67.2 mg/L
Auxiliary Solvent	None
Water Hardness	15 mg $CaCO_3$/L
Analytical Monitoring	
Remarks – Method	The test was conducted according to good laboratory practice (GLP) principles with no significant protocol deviations. Mean measured concentrations were calculated as the arithmetic mean of the measured concentrations. At the end of the test, 83 to 99 % of the initially measured concentrations were detected.

RESULTS

Biomass		*Growth*	
EyC50 (mg/L at 72 h)	*NOEC (mg/L)*	*ErC50 (mg/L at 72 h)*	*NOErC (mg/L)*
6.7	0.55	21	0.9

Remarks – Results	All the validity criteria were met. In control, the biomass increased by a factor of 164 over 72 hours. The ErC50 and EyC50 values were calculated on the basis of the geometric mean measured loading rates. The analytical samples of the lowest test concentration (dilution 1:320) were not analysed.
CONCLUSION	The notified chemical is toxic to *algae*.
TEST FACILITY	Exempt Information (6)

C.2.4. Inhibition of Microbial Activity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 62.5 mg/L Actual: 62.5 mg/L
Remarks – Method	The test was conducted according to the good laboratory practice (GLP) principles with no significant protocol deviations.

RESULTS	
EC50	> 62.5 mg/L (the highest concentration under tested conditions)
Remarks – Results	All validity criteria were satisfied. The treatment mixture dosed with 62.5 mg/L of the notified chemical had a respiration rate of 0.44 mg O_2/L/hr showing there was no significant uptake or release of oxygen resulting from reactions as compared to the control. The EC50 was > 62.5 mg/L, the highest concentration tested.
CONCLUSION	The notified chemical does not inhibit microbial activity.
TEST FACILITY	Exempt Information (20)

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