File No: LTD/1948

March 2017

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Cyclohexanecarboxamide, 5-methyl-2-(1-methylethyl)-*N*-[2-(2-pyridinyl)ethyl]-, (1*R*,2*S*,5*R*)-*rel*-
(INCI Name: Menthane Carboxamide Ethylpyridine)

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
LTD/1948	Givaudan Singapore Pte Ltd	Cyclohexanecarboxamide, 5-methyl-2-(1-methylethyl)-N-[2-(2-pyridinyl)ethyl]-, (1*R*,2*S*,5*R*)-*rel*- (INCI Name: Menthane Carboxamide Ethylpyridine)	Yes	< 1 tonne per annum	Fragrance ingredient in oral care products

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Acute toxicity, oral (Category 4)	H302 – Harmful if swallowed

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 considered to pose/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:
  - Acute toxicity, oral (Category 4): H302 – Harmful if swallowed

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

CONTROL MEASURES

Occupational Health and Safety

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

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

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation processes:
  - Coveralls, impervious gloves, goggles

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

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

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

Disposal

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

Emergency procedures

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

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

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

(1) Under Section 64(1) of the Act; if
  - the importation volume exceeds one tonne per annum notified chemical;
  - concentration exceeds 0.1% in oral care products

or

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

- the function or use of the chemical has changed from being a fragrance ingredient in 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.

(Material) Safety Data Sheet

The (M)SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Givaudan Singapore Pte Ltd (ABN: 79 368 011 578)
Unit 34
5 Inglewood Place
BAULKHAM HILLS NSW 2153

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
EU (REACH) 2016
Japan (ISHL) 2013
China 2009

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Evercool 190

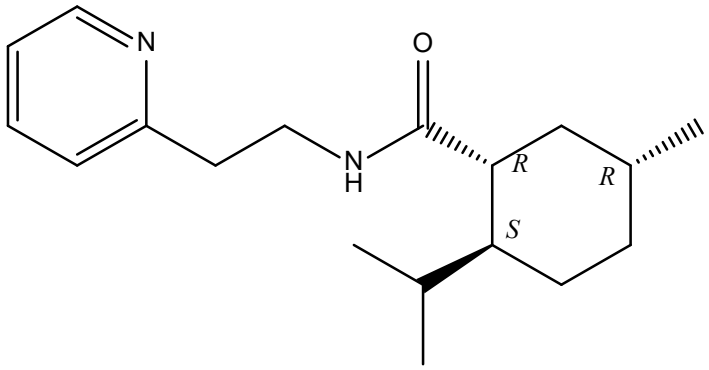
CAS NUMBER
847565-09-7

CHEMICAL NAME
Cyclohexanecarboxamide, 5-methyl-2-(1-methylethyl)-*N*-[2-(2-pyridinyl)ethyl]-, (1*R*,2*S*,5*R*)-*rel*-

OTHER NAME(S)
GR-72-1814
Menthane Carboxamide Ethylpyridine (INCI Name)
(1*R*, 2*S*, 5*R*)-N-(2-(2-pyridinyl)ethyl)-2-isopropyl-5-methylcyclohexanecarboxamide
N-(2-(pyridin-2-yl)ethyl)-3-p-menthanecarboxamide

MOLECULAR FORMULA
$C_{18}H_{28}N_2O$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
288.43 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 99%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None.

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None.

ADDITIVES/ADJUVANTS
None.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white solid

Property	Value	Data Source/Justification
Melting Point	61.5 - 82 °C	Measured
Boiling Point	346 °C at 101.3 kPa	Measured. Decomposition observed from 330 °C.
Density	1,090 kg/m^3 at 25 °C	Measured
Vapour Pressure	3 × 10^{-7} kPa at 25 °C	Measured
Water Solubility	0.145 g/L at 25 °C	Measured
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 year at 25°C at pH=4 ,7,9	Measured
Partition Coefficient (n-octanol/water)	log P_{OW} = 3.2 at 35 °C	Measured
Surface Tension	54.0 mN/m at 20 °C	Measured
Adsorption/Desorption	log K_{oc} = 3.9 and 3.0 at pH 3 and pH 9	Measured
Dissociation Constant	Not determined	Expected to be ionised under environmental conditions (pH 4-9)
Particle Size	Inhalable fraction (< 100 μm): 26.1% Respirable fraction (< 10 μm): < 0.1%	Measured
Flash Point	208 °C at 101.3 kPa	Measured
Flammability	Not highly flammable.	Measured
Autoignition Temperature	390 °C	Measured
Explosive Properties	Not determined.	Not expected to be explosive based on chemical structure.
Oxidising Properties	Not determined.	Not expected to oxidise based on chemical structure.

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported in fragrance compounds at ≤ 3.6% concentration.

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
Perth (by air), Sydney (by sea and air)

IDENTITY OF MANUFACTURER/RECIPIENTS
Givaudan Australia Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical as a component of fragrance preparations (at ≤ 3.6% concentration) will be imported into Australia in glass, lacquer-lined containers of 1, 5, 10, 25, 100 and 190 kg in size. The fragrance preparations

will be transported from the port of entry by road to the notifier's warehouse facilities for storage and then distributed to reformulation sites. The end-use products (containing the notified chemical at ≤ 0.036% concentration) will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance component in oral hygiene products. The concentration in the final consumer products will vary, but the proposed usage concentration in all products will not exceed 0.036%.

OPERATION DESCRIPTION
No manufacturing, processing, reformulation or repackaging of the notified chemical will occur at the notifier's facility. Imported products containing the notified chemical (at ≤ 3.6% concentration) will be stored at this facility until transported to customer facilities for reformulation into consumer products.

Reformulation
The procedures for incorporating the imported fragrance preparation (containing the notified chemical at ≤ 3.6% concentration) into end-use products will likely vary depending on the nature of the oral hygiene products being formulated, and may involve both automated and manual transfer steps. However, in general it is expected that the reformulation processes will involve blending operations that will be highly automated and use closed systems with adequate ventilation, followed by automated filling of the reformulated products into containers of various sizes.

End use
The finished oral hygiene products containing the notified chemical (at ≤ 0.036% concentration) will be used by consumers.

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)*
Plant operators	4	2
Cleaning and maintenance	4	2
Quality control	4	2
Packaging	4	2
End-users (professionals providing oral care services)	< 8	200

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical, as a component of the imported fragrance preparations (≤ 3.6% concentration) or end-use products (≤ 0.036% concentration), only in the event of an accidental rupture of containers.

During reformulation of the notified chemical (at ≤ 3.6% concentration) into the final consumer products, dermal, ocular and inhalation exposure of workers may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of local and general ventilation and/or enclosed systems and through the use of personal protective equipment (PPE) such as coveralls, safety glasses, face masks and impervious gloves.

Exposure to the notified chemical in end-use products (at ≤ 0.036% concentration) may occur in professions where the services provided involve the use of oral hygiene products. Such professionals may use some PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using products containing the notified chemical.

6.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of oral hygiene products (at concentrations ≤ 0.036%). The principal route of exposure will be oral, while some dermal exposure is also possible.

Data on typical use patterns

Data on typical use patterns of oral hygiene 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 receptor group. For the purposes of the exposure assessment, Australian use patterns for the product categories are assumed to be similar to those in Europe. In addition, 100% systemic exposure has been conservatively assumed based on buccal and/or gastrointestinal absorption. Using these data, the total systemic exposure for oral care products is estimated to be 0.0307 mg/kg bw/day notified chemical for young children and 0.0234 mg/kg bw/day for adults.

The contribution to dermal exposure from the proposed product categories is considered negligible due to the low concentrations of the notified chemical in these products and has therefore not been included in the exposure calculations.

Children's exposure (2-4 year old)

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Toothpaste[1]	1720	0.036	0.62[2]	**0.0307**

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)
Toothpaste[1]	2780	0.036	0.058[2]	0.0009
Mouthwash[1]	40,000	0.036	0.10	0.0225
Total				**0.0234**

C = concentration (%); RF = retention factor; assumed brushing twice daily and using mouthwash 4x/day
Daily systemic exposure = (Amount × C (%) × RF x oral absorption)/body weight (64 kg)

[1]RIVM (2006)
[3]Based on 75th percentile of amount orally ingested

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 300 - 2000 mg/kg bw; harmful
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Human, eye irritation	irritating
Mouse, skin sensitisation – Local Lymph Node Assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL = 10 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro Chromosome Aberration Test (Human Lymphocytes)	non genotoxic

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted. For dermal absorption, molecular weights below 100 Da. are favourable for absorption and molecular weights above 500 Da. do not favour absorption (ECHA, 2014). Dermal uptake is likely to be moderate to high if the water solubility is between 100-10,000 mg/L and the

partition coefficient (log P) values are between 1 and 4 (ECHA, 2014). Absorption of the notified chemical through the skin and gastrointestinal tract is expected based on the partition coefficient (3.2), water solubility (145 mg/L) and moderately low molecular weight (288.43 Da).

Acute toxicity
The notified chemical is expected to be harmful via the oral route based on a study conducted in rats where the LD50 was between 300 – 2000 mg/kg bw.

The notified chemical was of low acute dermal toxicity in a study on rats.

Irritation and sensitisation
The notified chemical is not irritating to the skin, but was slightly irritating to the eyes of rabbits and irritating to the eyes of humans.

The notified chemical was tested in human subjects at 0.005% – 0.5% concentration in shampoo. Adverse effects following exposure were recorded based on self-reporting (subjective) and objective examination by an ophthalmologist. High lacrimation values were recorded for three of the five subjects exposed to the test substance at 0.5%. A positive correlation was reported for test substance dosage and the duration and mean scores for eye irritation effects. However, as no vehicle control was used it is not possible to attribute the effects seen in the study subjects to the notified chemical, and therefore while the chemical may be an eye irritant the information is insufficient for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

The notified chemical is not expected to be a sensitiser based on the results of a local lymph node assay in mice.

Repeated dose toxicity
A NOAEL of 10 mg/kg bw/day was established for the notified chemical in a 28-day repeated dose oral dietary toxicity test in rats based on effects on the thyroid gland and liver observed in animals exposed to 50 mg/kg/day (mid-dose) and 300 mg/kg/day (high dose).

Mutagenicity/Genotoxicity
The notified chemical was non-mutagenic in a bacterial reverse mutation assay, and is not expected to be genotoxic based on the results of an *in vitro* mammalian chromosome aberration test in human lymphocyte cells.

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

Hazard classification	***Hazard statement***
Acute toxicity, oral (Category 4)	H302 – Harmful if swallowed

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is expected to be harmful following oral exposure and may be an eye irritant. Therefore, control measures are required to mitigate possible adverse health effects to the workers who may come into contact with the notified chemical.

Transport and Storage
Workers may experience dermal and accidental ocular exposure to the notified chemical (at ≤ 3.6% concentration) in the event of a discharge via spill or drum leakage. The use of PPE (e.g. impervious gloves, goggles, coveralls, hard hats and respiratory protection, if necessary) should minimise the potential for exposure. Provided adequate control measures and safe work practices are in place to minimise worker exposure, including PPE, the risk to workers from the notified chemical is not considered to be unreasonable.

Reformulation
Dermal, ocular and inhalation exposure of workers to the notified chemical (at ≤ 3.6% concentration) into the final consumer products may occur during blending operations. The notified chemical is considered to be

irritating to the eyes. In addition, harmful effects following oral, dermal exposure and/or repeated exposure to the notified chemical are possible. Therefore, caution should be exercised when handling the notified chemical during reformulation processes.

Provided that 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
Professionals providing oral care services will handle the notified chemical at up to 0.036% concentration, similar to public use. Therefore the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experience by members of the public who use such products on a regular basis. For details of the public health risk assessment see Section 6.3.2.

6.3.2. Public Health
Members of the public may experience repeated exposure to the notified chemical through the use of toothpaste and mouthwash (containing the notified chemical at ≤ 0.036% concentration). The main route of exposure is expected to be oral with some potential for accidental ocular or dermal exposure.

Irritation
The notified chemical may be irritating to the eyes. However, given the proposed use concentration (≤ 0.036%), significant irritation effects are not expected.

Repeat dose toxicity
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 0.0307 mg/kg bw/day, while the potential systemic exposure to adults from the use of the notified chemical in toothpaste and mouthwash was estimated to be 0.0234 mg/kg bw/day. Using a NOAEL of 10 mg/kg/day, which was derived from a 28 day repeated dose oral gavage toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 325.7 and 427.4 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. Based on the potential systemic exposure from the notified chemical in toothpaste and mouthwash products, an MOE value greater than or equal to 100 is also expected where the notified chemical is present at ≤ 0.1% concentration.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 0.036% in toothpaste and mouthwash products for children and adults, is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported as a component of fragrance formulations, for reformulation into finished oral care products. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills and leaks. In the event of spills, the product containing the notified chemical is expected to be collected with adsorbents, and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve blending operations that will be highly automated, and is expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. Wastes containing the notified chemical generated during reformulation include equipment wash water, residues in empty import containers and spilt materials. It is estimated by the notifier that up to 2% of the import volume of the notified chemical (or up to 20 kg) may be released from reformulation processes. These will be collected and released to sewers in a worst case scenario, or disposed of to landfill in accordance with local government regulations. Empty import containers are expected to be recycled or disposed of to landfill.

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

RELEASE OF CHEMICAL FROM DISPOSAL
It is estimated by the notifier that a maximum of 1% of the import volume of the notified chemical (or up to 10 kg), may remain in containers once the consumer products are used up. Wastes and residues of the notified chemical in empty containers are likely to either share the fate of the container and be disposed of to landfill, or be released to the sewer system when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate
Following its use in oral care products in Australia, the majority of the notified chemical is expected to enter the sewer system, before potential release to surface waters nationwide. Based on the result of the ready biodegradability study, the notified chemical is not considered readily biodegradable (0% in 28 days). For details of the environmental fate studies, please refer to Appendix C. Based on its moderate water solubility and high adsorption coefficient (log K_{OC} = 3.9), release to surface waters may not occur as partitioning to sludge and sediment is expected under environmental pH. Although the notified chemical is not readily biodegradable, it is not expected to be bioaccumulative due to its low partition coefficient (log K_{OW} = 3.2). Therefore, in surface waters the notified chemical is expected to disperse and degrade through biotic and abiotic processes to form water and oxides of carbon.

The majority of the notified chemical will be released to sewer after use. A small 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. The notified chemical may also be applied to land when disposed of to landfill as collected spills and empty container residue. The notified chemical in landfill, soil and sludge are expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)
The predicted environmental concentration (PEC) has been calculated to assume a worst case scenario, with 100% release of the notified chemical into sewer systems nationwide and no removal within sewage treatment plants (STPs).

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)	22.613	million
Removal within STP	0%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	0.61	µg/L
PEC - Ocean:	0.06	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1,000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1,500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.61 µg/L may potentially result in a soil concentration of approximately 4.04 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 20.19 µg/kg and 40.38 µg/kg, respectively.

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 = 63.2 mg/L	Harmful to fish
Daphnia Toxicity	48 h EC50 = 24.5 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	72 h EC50 = 39.4 mg/L	Harmful to algae
	72 h NOEC = 3.75 mg/L	Not harmful to algae with long lasting effects

Based on the above ecotoxicological endpoints for the notified chemical, it is expected to be harmful to fish, aquatic invertebrates and algae. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is formally classified as "Acute Category 3; Harmful to aquatic life". Based on the low chronic toxicity and low bioaccumulation potential of the notified chemical, it is not formally classified under the GHS for chronic toxicity.

7.2.1. Predicted No-Effect Concentration

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

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Daphnia, 96 h)	24.5	mg/L
Assessment Factor	100	
Mitigation Factor	1.00	
PNEC:	245	µg/L

7.3. Environmental Risk Assessment

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

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.61	245	**0.002**
Q - Ocean	0.06	245	**< 0.001**

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. Although the notified chemical is not readily biodegradable, it is expected to have a low potential for bioaccumulation. On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern in oral care products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 61.5 - 82 °C

Method OECD TG 102 Melting Point/Melting Range.
EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Capillary tube in a liquid bath.
Test Facility Givaudan (2007a)

Boiling Point 346 °C at 101.3 kPa

Method OECD TG 103 Boiling Point.
EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks Siwoloboff method. Decomposition observed from 330 °C.
Test Facility Givaudan (2008a)

Density 1090 kg/m^3 at 25 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Determined using a pycnometer
Test Facility Givaudan (2007b)

Vapour Pressure 3×10^{-7} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Vapour pressure balance.
Test Facility Huntingdon (2008a)

Water Solubility 0.145 g/L at 20 °C

Method OECD TG 105 Water Solubility.
Remarks Flask Method
Test Facility Givaudan (2008b)

Hydrolysis as a Function of pH $t_{½}$ > 1 year at 25 °C at pH 4, 7, 9

Method OECD TG 111 Hydrolysis as a Function of pH.

pH	*T (°C)*	*$t_{½}$ (years)*
4	25	> 1
7	25	> 1
9	25	> 1

Remarks HPLC method. A nominal concentration of 50 mg/L was prepared in acetone. The test was carried out at 50 °C with samples taken after 0, 2.4 and 120 hours. Less than 10% hydrolysis was observed after 120 h at 50°C at pH 4, 7 and 9 and therefore the estimated half-life at 25 °C is > 1 year.
Test Facility Givaudan (2008c)

Partition Coefficient (n-octanol/water) log Pow = 3.2 at 35 °C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks HPLC Method
Test Facility Givaudan (2007c)

Surface Tension 54.0 mN/m at 20 °C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.
Remarks Concentration: 90 % saturated
Test Facility Huntingdon (2008a)

Adsorption/Desorption log K_{oc} = 3.9 at 25 °C (pH 3)
log K_{oc} = 3.0 at 25 °C (pH 9)

Method OECD TG 121 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).
Remarks HPLC method
Test Facility Huntingdon (2008a)

Particle Size > 10 µm

Method Sieve analysis and Image analysis

Range (µm)	*Mean Mass (%)*
> 125 µm (sieved)	56.7
> 105 µm	9.7
60.0 – 105 µm	26.1
30.0 – 60.0 µm	6.6
10.4 – 30.0 µm	0.9
0.5 – 10.4 µm	< 0.1

Remarks 80.8% of notified chemical was > 75 microns based on sieve analysis (sieve aperture sizes of 400, 125, 75, 30 and 10 microns). As greater than 10% of the notified chemical was < 75 microns, image analysis was performed.
Test Facility Huntingdon (2008a)

Flash Point 208 °C at 101.3 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.
DIN 51758
Remarks Pensky-Martens method.
Test Facility Givaudan (2008d)

Flammability Not highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks Notified chemical melted at point of ignition, but no burning or smouldering was observed when the ignition source was removed. No burning or smouldering observed when notified chemical was placed in contact with a platinum wire heated to 1000 ^{0}C.
Test Facility Givaudan (2007d)

Autoignition Temperature 390 °C

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks None.
Test Facility Huntingdon (2008a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method. EC Council Directive 2004/73/EC B.1 tris Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Crl:CD (SD)
Vehicle	1% w/v aqueous methylcellulose
Remarks - Method	GLP Compliant. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	2000	3 F	3/3
2	300	3 F	0/3
3	300	3 F	0/3

LD50	300 - 2000 mg/kg bw
Signs of Toxicity	Of the Group 1 animals, 1/3 died 46 minutes after exposure with the remaining two animals euthanised on Day 2 based on the severity of adverse effects. Adverse effects were observed immediately after exposure and included piloerection, fasciculations, abnormal gait, prostration, underactivity, body tremors and partially closed eyelids (3/3 animals), salivation, gasping respiration, red staining in the mouth, fast respiration, hunched posture, flat posture, reduced body temperature, irregular respiration, reduced body tone and yellow nasal discharge (2/3 animals) and prominent eyes, post-salivation staining, closed eyelids, vocalisation, dull eyes and loose faeces seen in individual females. Animals in Groups 2 and 3 exhibited adverse effects immediately after or within 60 min of exposure. Adverse effects included piloerection, abnormal gait, underactivity (6/6 animals), hunched posture (5/6 animals), reduced body tone and partially closed eyelids (4/6 animals), reduced body temperature, convulsions, repetitive movement (head) and fast respiration (3/6 females), prostration, irregular respiration, body tremors, flat posture and vocalisation (2/6 females) and salivation, repetitive movement (whole body), lacrimation, urine staining and poor righting reflex were observed in individual females across both groups. Recovery from exposure to the notified chemical was indicated in all animals by Day 3.
Effects in Organs	Within Group 1, congestion (blood vessels injected) in the brain, stomach and duodenum and white fluid contents in the stomach were observed in the animal that died 46 minutes after exposure. Of the remaining two animals, congestion (blood vessels injected) in the subcutaneous tissue was observed, and in the brain, stomach, duodenum, small and large intestines, caecum and urinary bladder (one animal); congestion (darkened tissues/organs) in the brain (one animal) and spleen (both animals); pallor of the lungs, liver and kidneys (both animals); speckled appearance of the liver (both animals); atrophy of the spleen (one animal) and caecum (both animals); gaseous distension of the stomach (both animals) and duodenum, small and large intestines (one animal); and fluid contents (dark brown or yellow) in the duodenum and small intestines (both animals) and the stomach and large intestines (one animal) were observed. One animal in Group 2 exhibited no abnormalities, one animal exhibited

	pallor of the liver and another exhibited congestion (darkened tissues/organs) of the spleen. No abnormalities were observed in any of the animals in Group 3.
Remarks - Results	Weight loss was observed in all Group 1 animals (3/3). Over Days 8 to 15, weight loss was observed in 2/3 animals in Group 2 and only a small weight gain was observed in the remaining animal. All animals in Group 3 (3/3) gained the expected amount of body weight over the course of the study.
CONCLUSION	The notified chemical is harmful via the oral route.
TEST FACILITY	Huntingdon (2007a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Council Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/Crl:CD SD
Vehicle	1% w/v aqueous methylcellulose
Type of dressing	Semi-occlusive.
Remarks - Method	GLP compliant. No significant deviations from protocol.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1 M, 1 F	2000	0/2
2	5 M, 5 F	2000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	No animals in Group 1 exhibited skin irritation effects. Within Group 2, very slight erythema was observed in 1/5 males and 2/5 females 48 hours after exposure. Two of the animals (the male and 1 female) showed recovery by the Day 3 observation, with the effect persisting in the remaining female up to day 5 of the observation period with recovery indicated on Day 6.
Signs of Toxicity - Systemic	None observed.
Effects in Organs	No macroscopic abnormalities were observed.
Remarks - Results	Two females in Group 2 exhibited low gains in body weight during days 1 – 8 (1/5 females) and days 8 to 15 (1/5 females). All other animals gained the expected amount of body weight over the course of the study.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Huntingdon (2008b)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 2004/73/EC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 (female)
Vehicle	None

Observation Period	4 days
Type of Dressing	Semi-occlusive.
Remarks - Method	GLP compliant. No deviations from the protocol.

RESULTS

Remarks - Results	No dermal irritation was observed in any of the animals. No adverse clinical symptoms were observed.

CONCLUSION The notified chemical is non-irritating to the skin.

TEST FACILITY Huntingdon (2008c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 2004/73/EC B.5 Acute Toxicity (Eye Irritation).

Species/Strain	Rabbit/New Zealand White
Number of Animals	3 (male)
Observation Period	15 days
Remarks - Method	GLP compliant. No deviations from the protocol.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.7	1.3	2	3	< 8 days	0
Conjunctiva: chemosis	1	0.7	1	4	< 8 days	0
Conjunctiva: discharge	1	0	0.7	2	< 8 days	0
Corneal opacity	1	0.7	1	1	< 8 days	0
Iridial inflammation	0	0	0	-	-	0

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

Remarks - Results

A slight initial pain response was observed in all animals following instillation. All animals exhibited severe (2/3) to very severe (1/3) chemosis one hour after exposure, with the effect diminishing to slight (1/3 animals) to moderate (2/3 animals) at the 24 hour observation. All animals showed recovery from the effect at the 72 hour observation.

Moderate conjunctival discharge was observed in all animals one hour after exposure, persisting in 2/3 animals at the 24 hour observation. Discharge was not observed in 2/3 animals at the 24 and 48 hour observations, with the effect lessening in the remaining animal at the 48 hour observation. Conjunctival discharge was not observed in any of the animals at the 72 hour observation.

Moderate to severe conjunctival redness was observed (2/3 and 1/3 animals respectively) 24 hours after exposure with recovery indicated in all animals at the 72 hour observation where mild conjunctival redness was observed (3/3 animals).

Slight corneal opacity was observed in all animals 24 hours after exposure with recovery indicated in 1/3 animals at the 72 hour observation.

No iridial inflammation was observed in any of the animals.

All animals exhibited complete recovery at the day 8 observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Huntingdon (2008d)

B.5. Irritation – human eye

TEST SUBSTANCE Notified chemical (0.005% – 1% concentration) in shampoo

METHOD In house method

Study Design The test substance was provided to the test facility at 0.005%, 0.03%, 0.1%, 0.5% and 1% in Johnson & Johnson Baby Shampoo. A positive control (menthol) was also provided to the test facility at 0.005%, 0.03%, 0.1%, 0.5% and 1% in Johnson & Johnson Baby Shampoo.

The test facility then diluted the test substance and positive control preparations provided to 10% using demineralized water as the diluent. One drop of the diluted test substance preparation was instilled into the inferior fornix of the right or left eye of the test subject. One drop of the diluted positive control preparation was instilled into the inferior fornix of the other eye of the test subject.

Test subjects closed both eyes for 30 seconds after instillation unless pain was experienced. Subjects evaluated their response at 30 seconds, 5, 15, 30, 60 and 120 minutes post exposure using a scale of 0 to 3 (no effect to severe burning and/or stinging and/or strong itching).

Test subjects were also examined by an ophthalmologist (at 30 seconds, 5, 15, 30, 60 and 120 minutes post exposure) who evaluated the intensity of lacrimation (scale of 0 to 3 – none to intensive lacrimation), irritation of bulbar conjunctiva (scale of 0 to 3 – none to intensive red vessels, dilated), irritation of palpebral conjunctiva (scale of 0 to 3 – none to cherry to deep red) and dilation of scleral vessels (scale of 0 to 3 – none to intense dilation).

Five test subjects were tested with each concentration of test substance until all five subjects in a group discerned pain or on the decision of the ophthalmologist. Where this endpoint was reached, no higher concentration was applied. No test subject was treated with more than one dilution.

Study Group 17 F, 6 M; age range 20 - 61 years

Remarks - Method No significant deviations from the protocol.

RESULTS

Effect	*Maximum Value*				*Maximum Duration of Any Effect (minutes)*				*Maximum Value at End of Observation Period*
	0.005%	0.03%	0.1%	0.5%	0.005%	0.03%	0.1%	0.5%	
Stinging	3	3	3	3	120	120	60	> 120	1
Lacrimation	2	3	3	3	15	15	15	30	0
Irritation of bulbar conjunctiva	2	2	3	3	60	60	> 120	120	1
Irritation of palpebral conjunctiva	1	2	2	3	30	> 120	> 120	120	1
Dilation of scleral vessels	1	1	1	1	5	15	15	60	0

Remarks - Results	High lacrimation values were recorded for 3 subjects exposed to the test substance at 0.5% and no additional test subjects were exposed to this concentration. No test subjects were exposed to the test substance at 1% concentration due to the irritation effects observed at the 0.5% concentration of test substance. A positive correlation was reported for test substance dosage and the duration and mean scores for eye irritation effects. The positive control (menthol) exhibited similar results to the test substance. However a clear correlation between mean score and dosage was not observed.
CONCLUSION	The test substance is irritating to the eye under the conditions of the test.
TEST FACILITY	proDERM (2011)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)
Species/Strain	Mouse/CBA/Ca
Vehicle	Acetone:olive oil (4:1 v/v)
Preliminary study	Yes
Positive control	α-Hexylcinnamaldehyde (HCA).
Remarks - Method	GLP compliant. No protocol deviations.

RESULTS

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5 F	112.70	-
10%	5 F	163.80	1.5
25%	5 F	308.65	2.7
50%	5 F	224.20	2.0
Positive Control			
25% HCA	5 F	1815.30	16.1

Remarks - Results	No mortality was observed. In the preliminary study, cream coloured dose residue was observed on the ears following exposure on Day 1 with complete recovery observed by Day 4. All animals in the main study, including controls, exhibited greasy fur on Day 1 with recovery in all animals by Days 4 (high-dose group), 5 (vehicle control, low- and mid-dose groups) and 6 (positive control group). All animals in the mid- and high-dose groups exhibited white dose residue on the ears, with complete recovery from the effect by Day 4. No skin irritation effects were observed. All animals gained the expected body weight. The positive control confirmed the sensitivity of the test system.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY Huntingdon (2008e)

B.7. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).

Species/Strain Rat/Crl:CD(SD)
Route of Administration Oral – diet
Exposure Information Total exposure days: 28 days
Dose regimen: 7 days per week
Vehicle Certified Rodent LabDiet® 5002 (PMI Nutrition International, LLC)
Remarks - Method GLP compliant.
No significant deviations from the protocol.

Dosage levels were chosen based on findings from a previous 4 week study (with 2 week recovery period) which was unable to establish a NOAEL. Adverse effects in the liver and thyroid at all dose levels (100, 300 and 1000 mg/kg/day) were attributed to the test substance. Recovery from adverse effect was indicated in those animals examined at the end of the 2 week recovery period.

RESULTS

Group	*Number and Sex of Animals*	*Dose/Concentration mg/kg/day*		*Mortality*
		Nominal	*Actual*	
control	8 M, 8 F	0	0	0/16
low dose	8 M, 8 F	10	11 (M, F)	0/16
mid dose	8 M, 8 F	50	55 (M), 53 (F)	0/16
high dose	8 M, 8 F	300	328 (M), 306 (F)	0/16

Mortality and Time to Death
No unscheduled deaths were recorded.

Clinical Observations
No adverse clinical effects were observed that were related to exposure to the test substance. All animals gained the expected amount of body weight.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
Statistically significant higher mean levels of total protein, albumin and globulin (high-dose males), cholesterol (high-dose males and females), total triiodothyronine (total T3) (mid-dose females, high-dose males and females), thyroid stimulating hormone (TSH) (mid-dose males, high-dose males and females), and statistically significantly lower mean levels of triglycerides (high-dose group males) were attributed to exposure to the test substance.

Lower absolute monocyte counts (low- and mid-dose males), urine pH (low- and high-dose females), alanine transferase (mid- and high-dose females), aspartate transferase (high-dose females), chloride (high-dose males) and higher potassium levels (mid-dose females) were not attributed to exposure to the test substance by the study authors as a dose-response relationship was not observed and the values were not considered toxicologically important.

Effects in Organs
Higher liver weights (high-dose males and females) with centrilobular hepatocellular hypertrophy (1/8 females, high-dose group), follicular cell hypertrophy of the thyroid gland (high dose males and females) and mild or minimal follicular cell hypertrophy of the thyroid gland (2/8 mid-dose males) were attributed to exposure to the test substance.

Other findings including retinal dysplasia (3/8 males in mid- and high-dose groups, and 2/8 females in high-dose group) were not considered to be related to exposure to the test substance by the study authors as the effects were within historical control values, did not show a dose-response relationship or were not toxicologically significant.

Remarks – Results
Animals in the mid-dose group exhibited statistically significant higher total T3 and TSH levels in correlation with follicular cell hypertrophy of the thyroid gland (males) following exposure to the test substance. Animals in the high-dose group exhibited statistically significant higher total serum protein albumin, globulin, cholesterol, total T3 and TSH levels and lower triglycerides in correlation with higher liver weights, centrilobular hepatocellular hypertrophy (females) and follicular cell hypertrophy (males and females) of the thyroid gland.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 10 mg/kg bw/day in this study, based on an absence of effects on the thyroid gland and liver.

TEST FACILITY WIL (2008)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure (Test 1) and Pre incubation procedure (Test 2)

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA (pKM101)
Metabolic Activation System	S9 fraction from phenobarbital/5,6-benzoflavone induced rat liver
Concentration Range in Test 1 (Preliminary Test)	a) With metabolic activation: 5 - 5000 µg/plate b) Without metabolic activation: 5 - 5000 µg/plate
Concentration Range in Test 2	a) With metabolic activation: 50 - 5000 µg/plate b) Without metabolic activation: 50 5000 µg/plate
Vehicle	Dimethyl sulphoxide
Remarks - Method	GLP compliant. No deviations from the protocol. Positive controls: Sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), 2-nitrofluorene (TA98), 4-nitroquinoline-1-oxide [WP2uvrA (pKM101)] (absence of metabolic activation); 2-aminoanthracene (WP2uvrA (pKM101), TA100, TA1535), benzo[a]pyrene (TA98, TA1537) (presence of metabolic activation).

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5000	> 5000	> 5000	none
Test 2		> 5000	> 5000	none
Present				
Test 1	> 5000	> 5000	> 5000	none
Test 2		> 5000	> 5000	none

Remarks - Results Positive and negative controls were run concurrently and performed as expected.

No significant increase in the number of revertant colonies was observed

in the presence or absence of metabolic activation in Test 1 or Test 2.

No significant reduction of the bacterial lawn or number of revertants was observed in the presence or absence of metabolic activation in Test 1 or Test 2.

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

TEST FACILITY Huntingdon (2007b)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

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

Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	Ethanol
Remarks - Method	GLP compliant. No deviations from the protocol. Positive controls: with metabolic activation: Cyclophosphamide; without metabolic activation: Mitomycin C.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	22.5, 45.1, 90.1, 180.3, 360.5, 721.1, 1442.2, 2884.3	3	18
Test 1a	50, 100, 200, 300, 400, 500, 600, 700, 800	3	18
Test 1b	100, 200, 220, 240, 260*, 280*, 300*, 350, 400, 450	3	18
Test 2	25, 50, 100, 200, 225, 250, 275, 300	21	21
Test 2a	25*, 50*, 75, 100, 120, 140, 160*, 180, 200, 220	21	21
Present			
Test 1	22.5, 45.1, 90.1, 180.3, 360.5, 721.1, 1442.2, 2884.3	3	18
Test 1a	50, 100*, 200*, 300*, 400, 500, 600, 700, 800	3	18

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	-	≥ 1442.2	-
Test 1a	-	≥ 800	-
Test 1b	≥ 300	> 450	none
Test 2	-	≥ 300	-
Test 2a	> 160	> 220	none
Present			
Test 1	-	≥ 1442.2	-
Test 1a	> 300	≥ 800	none

Remarks - Results In the series of tests performed under Test 1, the test substance did not cause a statistically significant increase in the proportion of cells with chromosomal aberrations at any concentration in the presence or absence of metabolic activation.

In Test 2, in the absence of metabolic activation, the test substance did not

	cause a statistically significant increase in the proportion of cells with chromosomal aberrations at any concentration. An increase in the number of gap and gap type aberrations were not considered to be biologically relevant by the study authors as the increases were outside the historical control data and were not statistically significant. No increase in polyploid metaphases were observed in series of tests in Test 1 or Test 2. Positive and negative controls performed as expected in both tests.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	Huntingdon (2007c)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical

METHOD
- Inoculum: Activated sewage sludge
- Exposure Period: 67 days
- Auxiliary Solvent: None
- Analytical Monitoring: Theoretical Oxygen Demand (ThOD)
- Remarks - Method: Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	-1	7	85
14	-1	14	93
21	-1	21	95
28	-2	28	95
67	-4	67	94

Remarks - Results: All validity criteria of the test guideline were satisfied.

The percentage degradation of the reference compound (sodium benzoate) surpassed the threshold level of 60% after 7 days (85%), and attained 95% degradation in 28 days. Therefore, the tests indicate the suitability of the inoculum. The toxicity test showed no toxic effects of the test substance to the micro-organisms at the test concentration of 100 mg/L. The degree of degradation of the test substance after 28 days was -2%. Therefore, the test substance is not considered to be readily biodegradable according to the OECD (301 F) guideline.

CONCLUSION: The notified chemical is not readily biodegradable.

TEST FACILITY: Givaudan (2006)

C.2.1. Acute toxicity to fish

TEST SUBSTANCE: Notified chemical

METHOD
- Species: *Oncorhynchus mykiss* (rainbow trout)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 154 – 170 mg $CaCO_3$/L
- Analytical Monitoring: HPLC
- Remarks – Method: Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Concentration mg/L		*Number of Fish*	*Cumulative Mortality (%)*				
Nominal	*Actual*		*4 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	ND	7	0	0	14	14	14
1.94	1.65	7	0	0	0	0	0
4.27	3.64	7	0	0	0	0	0

9.39	8.39	7	0	0	0	0	0
20.7	18.3	7	0	0	0	0	0
45.5	40.4	7	0	0	0	0	0
100	90.8	7	0	100	100	100	100

LC50	63.2 mg/L (95% CI 40.4-90.8 mg/L) at 96 hours
NOEC	1.65 mg/L at 96 hours
Remarks – Results	All validity criteria of the test guideline were satisfied. The fish were exposed to the control or test conditions for a period of 96 hours with daily batch renewal of the media. The death of one control fish at 48 hours had no impact on the validity of the definitive test. The 96 h LC50 and NOEC for fish were determined to be 63.2 mg/L (95% CI 40.4-90.8 mg/L) and 1.65 mg/L, respectively, based on measured concentrations.
CONCLUSION	The notified chemical is considered to be harmful to fish.
TEST FACILITY	Huntingdon (2008f)

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	HPLC
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	ND	20	0	0
6.25	5.58	20	0	0
12.5	10.6	20	0	15
25.0	23.0	20	0	25
50.0	47.1	20	65	100
100.0	94.1	20	80	100
Control	ND	20	0	0

LC50	24.5 mg/L (95% CI 20.0-30.0 mg/L) at 48 hours
NOEC	5.58 mg/L at 48 hours
Remarks - Results	All validity criteria of the test guideline were satisfied. The test solutions were not renewed during the 48 h test period. The 48 h EC50 and NOEC for daphnia was determined to be 24.5 mg/L (95% CI 20.0-30.0 mg/L) and 5.58 mg/L, respectively, based on measured concentrations.
CONCLUSION	The notified chemical is considered to be harmful to aquatic invertebrates.
TEST FACILITY	Huntingdon (2008g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical

METHOD	OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test – Static.
Species	*Pseudokirchneriella subcapitata* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 1.94-100 mg/L Actual: 1.84-98.3 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	HPLC
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles.

RESULTS

Biomass		*Growth*	
EC50 mg/L at 72 h	NOEC mg/L	EC50 mg/L at 72 h	NOEC mg/L
15.3 (95% CI 9.36-25.1)	3.75	39.4 (95% CI 35.4-44.3)	3.75

Remarks - Results	All validity criteria of the test guideline were satisfied. The test solutions were not renewed during the 72 h test period. The 72 h EC50 and NOEC for algae was determined to be 39.4 mg/L (95% CI 35.4-44.3 mg/L) and 3.75 mg/L, respectively, based on measured concentrations.
CONCLUSION	The notified chemical is considered to be harmful to algae.
TEST FACILITY	Huntingdon (2008h)

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