File No: LTD/1750

November 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Cyclogreen

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also 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/1750	Firmenich Pty Ltd	Cyclogreen	Yes	< 1 tonne per annum	Fragrance ingredient

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Flammable Liquids (Category 4)	H227 – Combustible liquid
Acute Toxicity (Category 4)	H302 – Harmful if swallowed
Specific Target Organ Toxicity - Repeated Exposure (Category 2)	H373: May cause damage to organs through prolonged or repeated oral exposure.

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

Xn: R22 Harmful if swallowed
Xn: R48/22 Danger of serious damage to health by prolonged exposure if swallowed

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

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

Human health risk assessment
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 at ≤ 0.05% in fine fragrances, ≤ 0.03% in other cosmetic products and ≤ 0.05% in household products, 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 assessed 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 (Category 4): H302 – Harmful if swallowed

- Specific Target Organ Toxicity - Repeated Exposure (Category 2): H373: May cause damage to organs through prolonged or repeated oral exposure.

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.

- The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the notified chemical for listing on the SUSMP.

- Due to the combustible properties of the notified chemical, the notifier should consider their obligations under the Australian Dangerous Goods Code.

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 process where possible
  - Exhaust ventilation

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

- 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 for the 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.

Public Health

- The following measures should be taken by formulators to minimise public exposure to the notified chemical:
  - The notified chemical should only be used at ≤ 0.05% in fine fragrances, ≤ 0.03% in other cosmetic products and ≤ 0.05% in household products.

Disposal

- Where reuse or recycling are unavailable or impracticable, dispose of the chemical in an environmentally sound manner in accordance with relevant Commonwealth, State, Territory and local government legislation.

Storage

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

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the concentration of the chemical exceeds or is intended to exceed 0.05% in fine fragrances, 0.03% in other cosmetic products or 0.05% in household products;

or

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

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

(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)
Firmenich Limited (ABN: 86 002 964 794)
73 Kenneth Road
Balgowlah NSW 2093

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities and additives/adjuvants.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: adsorption/desorption, dissociation constant and flammability.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Cyclogreen

MOLECULAR WEIGHT
< 200 Da

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

3. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Colourless liquid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	< -20 ± 0.5 °C	Measured
Boiling Point	215 ± 2 °C at 98.5 kPa	Measured
Density	939 kg/m^3 at 20 °C	Measured
Vapour Pressure	1.57 x 10^{-2} kPa at 25 °C	Measured
Water Solubility	0.279 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	> 10% after 28 days (40 °C, pH 2-12)	Measured
Partition Coefficient (n-octanol/water)	log Pow = 3.07	Measured
Adsorption/Desorption	log K_{oc} = 2.3-2.7	Calculated. (KOCWIN v2.0, EPI Suite v4.1: US EPA, 2011).
Dissociation Constant	Not determined	Does not contain dissociable functionalities
Flash Point	89 ± 2 °C at 101.3 kPa	Measured
Flammability	Not determined	Not expected to be a flammable based on measured flash point
Autoignition Temperature	278 °C	Measured

Property	Value	Data Source/Justification
Explosive Properties	Predicted not to be explosive	Estimated based on chemical structure
Oxidising Properties	Predicted not to be oxidising	Estimated 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 recommended for hazard classification according to the *Globally Harmonised System for the 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***
Flammable Liquids (Category 4)	H227 – Combustible liquid

4. 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 into Australia as a component of fragrance preparations at concentrations of ≤ 10%.

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, by wharf or airport

IDENTITY OF MANUFACTURER/RECIPIENTS
Firmenich Limited

TRANSPORTATION AND PACKAGING
The notified chemical will be imported into Australia as a component of fragrance preparations at ≤ 10% concentration in lacquered drums of typically 180 kg size, but the use of smaller containers down to 5 kg is also possible. 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 (≤ 0.05% notified chemical) will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a fragrance ingredient in a variety of cosmetic and household products. The content in the final consumer products will vary, with the following proposed usage concentrations: fine fragrances (≤ 0.05%), other cosmetic products (≤ 0.03%), and household products (≤ 0.05%).

OPERATION DESCRIPTION
The notified chemical will not be manufactured within Australia. The notified chemical will be imported as a component of fragrance preparations at ≤ 10% concentration for reformulation into a variety of cosmetic and household products.

Reformulation
The procedures for incorporating the imported fragrance preparations into end-use products will likely vary depending on the nature of the cosmetic and household 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 occur in enclosed environments. After being reformulated, the finished products containing the notified chemical at ≤ 0.05% concentration will be transferred via automated filling into typical consumer-sized retail packaging.

End use
The finished products containing the notified chemical may be used by consumers and may also be used in occupational settings by professionals such as hairdressers, beauticians or cleaners. Depending on the nature of the product, these could be applied in a number of ways, such as by hand, using an applicator or sprayed.

5. 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 workers	Unknown	Unknown
Mixer	4	2
Drum handling	4	2
Drum cleaning	4	2
Maintenance	4	2
Quality Control	0.5	1
Packaging	4	2

EXPOSURE DETAILS
Transport and storage workers may come into contact with the notified chemical, as a component of the imported fragrance preparations (≤ 10% concentration) or end-use products (≤ 0.05% concentration), only in the event of accidental rupture of containers.

During reformulation of the notified chemical into the final consumer products, dermal, ocular and inhalation exposure of workers (at ≤ 10% concentration) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The notifier claims this exposure is expected to be minimised due to the likely use of automated processes and PPE by workers. The notifier suggests operators will wear safety glasses, gloves, protective clothing, and respiratory protection if required.

Exposure to the notified chemical in end-use products (at ≤ 0.05% concentration) may occur in professions where the services provided involve the application of cosmetic products to clients (e.g. hair dressers, workers in beauty salons) or the use of household products in the cleaning industry. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible. Such professionals may use some PPE to minimise repeated exposure, but use is not expected. However, 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 diffuse and repeated exposure of the public to the notified chemical (at ≤ 0.05% concentration) through the widespread use of cosmetic (both rinse-off and leave-on) and household products. The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

A combined internal dose of 0.0915 mg/kg bw/day was estimated using data on typical use patterns of cosmetic and household cleaning product categories in which the notified chemical may be used (SCCS, 2010; Cadby *et al.*, 2002; SDA, 2005; specific use details of the notified chemical are considered as exempt information). This estimation assumed a worst case scenario and is for a person who is a simultaneous user of a selection of cosmetic and household products that may contain the notified chemical.

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
Rat, acute inhalation toxicity	LC50 > 5.18 mg/L/4 hour; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose by daily gavage toxicity study – 28 days	NOAEL = 30 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration	non genotoxic

Toxicokinetics.

Based on the water solubility (0.279 g/L at 20 °C), partition coefficient (log P_{OW} = 3.07) and the low molecular weight (< 200 Da) of the notified chemical, passive diffusion across the gastrointestinal (GI) tract and dermal absorption are expected to occur. The notified chemical may also be absorbed across the respiratory tract.

Acute toxicity.

In acute toxicity studies conducted in rats the notified chemical was found to be harmful by the oral route and of low toxicity by the dermal and inhalation routes.

Irritation and sensitisation.

In studies conducted in rabbits the notified chemical was found to be slightly irritating to the eyes and skin.

The notified chemical at concentrations up to 37.5% in a mouse Local Lymph Node Assay showed no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation.

Repeated dose toxicity.

A 28 day repeat dose study by oral gavage was conducted in rats with the notified chemical at dose levels of 30, 100 and 300 mg/kg bw/day. While no clinical signs of toxicity were noted, adverse effects were observed at 300 mg/kg bw/day in the heart, stomach, liver, testes, epididymis, female reproductive organs and eyes and at 100 mg/kg bw/day in the heart and liver. Adverse changes noted in the heart and liver included single cell necrosis of the myocardium and vacuolation of the myocardial fibres (females) of the heart, and hepatocellular single cell necrosis, hepatocellular hypertrophy and microvesicular hepatocellular vacuolation in the liver. At 30 mg/kg bw/day there was statistically significant higher alkaline phosphatase (ALP) activity (leading to higher potassium levels) observed in males. This finding was not considered to be adverse in nature by the study authors as it occurred in the absence of any other morphological change and did not show an apparent dose-related trend over the 30 and 100 mg/kg/day group. The NOAEL was therefore established as 30 mg/kg bw/day.

Mutagenicity/Genotoxicity.

The notified chemical was found to be non-mutagenic in a bacterial reverse mutation assay and was not clastogenic in an *in vitro* mammalian chromosome aberration test.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the 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 (Category 4)	H302 – Harmful if swallowed
Specific Target Organ Toxicity - Repeated Exposure (Category 2)	H373: May cause damage to organs through prolonged or repeated oral exposure.

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R22: Harmful if swallowed
R48/22: Danger of serious damage to health by prolonged exposure if swallowed

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
The notified chemical is slightly irritating to the eyes and skin and has the potential to cause systemic toxicity from repeated exposure.

Reformulation
Exposure of workers to the notified chemical (at ≤ 10% concentration) may occur during blending operations. While irritation effects are not expected at the proposed use concentrations, there is the potential risk of systemic effects from repeated exposure. Therefore, provided measures are in place to minimise exposure (i.e. enclosed automated process, where possible, and PPE), the risk to the health of reformulation workers from use of the notified chemical is not considered to be unreasonable.

End-use
Cleaners and beauty care professionals could potentially handle products containing the notified chemical at ≤ 0.05% concentration, similar to public use. The risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experienced by members of the general public who use such products on a regular basis. For details of the public health risk assessment see section 6.3.2.

Based on the information available, the risk to workers associated with the use of the notified chemical at ≤ 0.05% in end-use products is not considered to be unreasonable.

6.3.2. Public Health
The general public will be repeatedly exposed to the notified chemical during the use of cosmetic and household products containing the notified chemical at up to 0.05% concentration.

Local effects
The notified chemical is slightly irritating to the eyes and skin. However at the low proposed end use concentration irritation effects are not expected.

Systemic effects
The potential systemic exposure to the public from use of the notified chemical in cosmetic and household products was estimated to be 0.0915 mg/kg bw/day. Using a NOAEL of 30 mg/kg bw/day, which was derived from a 28-day repeated dose toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 328. A MOE value greater than or equal to 300 is considered acceptable to account for intra- and inter-species differences, and to account for long-term exposure (noting the NOAEL was derived from a 28-day study with adverse effects observed in the heart). Therefore the MOE is considered to be acceptable.

Therefore, based on the information available, the risk to the public associated with the use of the notified chemical at ≤ 0.05% in fine fragrances, ≤ 0.03% in other cosmetic products or ≤ 0.05% in household products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia; therefore there will be no release of the notified chemical to the environment from this activity. Environmental release during importation, transport and distribution may occur as a result of accidental spills. In the event of a spill, the notified chemical is expected to be contained and collected with an inert absorbent material and disposed of in accordance with local regulations.

During reformulation processes, limited release of the notified chemical is expected from cleaning of equipment as washings are expected to be reused. A total of up to 0.2% of the import volume is estimated to be generated as waste from residues in empty containers and spills during reformulation. Empty containers containing the notified chemical will either be recycled or disposed of through an approved waste management facility.

RELEASE OF CHEMICAL FROM USE

The majority of the notified chemical is expected to be released to sewers across Australia as a result of its use as a fragrance component in a variety of products, which are washed off the hair and skin of consumers, disposed as waste waters from domestic cleaning activities. A small percentage of the notified chemical, as residues in empty end use containers, is expected to be disposed of to landfill.

RELEASE OF CHEMICAL FROM DISPOSAL

It is expected that some of the product containing the notified chemical will remain in end-use containers. The containers are expected be disposed of through domestic garbage disposal and will enter landfill, or be subjected to recycling processes.

7.1.2. Environmental Fate

Following its use in Australia as a fragrance in cosmetics and domestic products, the majority of the notified chemical is expected to enter the sewer system before potential release to surface waters on a nationwide basis. The notified chemical is readily biodegradable and hence, it is expected to be degraded during the wastewater treatment process. Based on its soil adsorption coefficient (log K_{oc} = 2.3-2.7), some partitioning to sludge is expected. If released to surface waters, the notified chemical is expected to disperse and degrade through biotic and abiotic processes to form water and oxides of oxygen.

The notified chemical is readily biodegradable based on the biodegradation study provided. Therefore, the notified chemical is not likely to persist in the environment. The notified chemical is not considered to have potential to be bioaccumulative based on its moderate partition coefficient (log Pow = 3.07).

The notified chemical is expected to be volatile and may volatilise to air during use or STP processes. The half-life of the notified chemical in air is calculated to be 2.1 hours based on reactions with hydroxyl radicals (AOPWIN v1.92; US EPA, 2011). Therefore, in the event of release to atmosphere, the notified chemical is not expected to persist in the atmospheric compartment.

A proportion of notified chemical may be applied to land when treated sewage effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. Notified chemical residues in landfill and soil are expected to have low mobility based on its predicted adsorption coefficient (log K_{oc} = 2.3 – 2.7), and is expected to degrade to form water and oxides of carbon.

7.1.3. Predicted Environmental Concentration (PEC)

The calculation for the Predicted Environmental Concentration (PEC) is summarised in the table below. Based on the reported use in cosmetics and household cleansing products, it is assumed that 100% of the total import volume of the notified chemical is released to the sewer. The release is assumed to be nationwide over 365 days per year. It is conservatively assumed that 0% of the notified chemical will be removed during sewage treatment processes.

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 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 0.6 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 20.2 µg /kg and 40.4 µg/kg, respectively.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Daphnia Toxicity	EL50 (48 hours) = 15 mg/L*	Harmful to aquatic invertebrates
Algal Toxicity	E_rL50 (72 hours) = 6.5 mg/L*	Toxic to algae
Inhibition of Bacterial Respiration	EC50 > 1000 mg/L	Not inhibitory to microbial respiration

* Filtered Water Accommodated Fraction (WAF)

Based on the ecotoxicological endpoints for the notified chemical, it is expected to be harmful to aquatic invertebrates and toxic to 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 2; Toxic to aquatic life. Based on the acute toxicity, ready biodegradability and low bioaccumulation potential of the notified chemical, the notified chemical is not expected to be harmful to the aquatic life on long term basis and is not formally classified under the GHS.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated and is presented in the table below. The PNEC is calculated based on the endpoint for the most sensitive species (algae) for the notified chemical. An assessment factor of 1000 has been used as acute toxicity endpoints for only two trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Invertebrates).	6.5	mg/L
Assessment Factor	1000	
PNEC:	6.5	µg/L

7.3. Environmental Risk Assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.61	6.5	0.093
Q - Ocean:	0.06	6.5	0.009

The risk quotient for discharge containing the notified chemical to the aquatic environment indicates that the notified chemical is unlikely to reach ecotoxicologically significant concentrations based on its reported use pattern and annual introduction quantity. The notified chemical is readily biodegradable, therefore it is not expected to persist in surface waters, air or soils. On the basis of the PEC/PNEC ratio, maximum annual import volume and assessed use pattern in cosmetic and domestic 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 < -20 ± 5°C

Method OECD TG 102 Melting Point/Melting Range.
EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks BS4633: Method for the determination of Crystallizing Point
Test Facility Firmenich (2011)

Boiling Point 215.0 °C at 98.5 kPa

Method OECD TG 103 Boiling Point.
EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Siwoloboff method.
Test Facility Firmenich (2011)

Relative Density 939 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids.
EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Oscillating density meter.
Test Facility Firmenich (2011)

Vapour Pressure 1.57 x 10^{-2} kPa at 25 °C

Method OECD TG 104 Vapour Pressure.
EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Gas saturation method.
Test Facility Harlan (2013a)

Water Solubility 0.279 g/L at 20 °C

Method OECD TG 105 Water Solubility.
EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks Flask Method. Analysis by HPLC.
Test Facility Firmenich (2011)

Hydrolysis as a Function of pH ≥ 10% after 28 days (40 °C, pH 2-12)

Method In-house

pH	*T (°C)*	*% hydrolysis after 5 days**	*% hydrolysis after 28 days**
2	40	70	90
5	40	5	10
7	40	5	10
8.5	40	70	90
12	40	100	100

*Approximate values read from graph

Remarks Test substance (200 – 300 ppm) was dissolved in buffer solutions (types A, C, D, F and I: Reference Handbook of Chemistry and Physics) containing 1% non-ionic surfactant (Arkopal N 150) and put into storage in an oven at 40 °C over 28 days. Aliquots of test solution were extracted with organic solvent (typically cyclohexane or ethyl acetate) on a regular basis throughout the test and analysed by GC-FID. The rate of hydrolysis was approximately the same at pH 2-7 and increased markedly at pH 2, 8.5 and 12.
Test Facility Firmenich (Undated)

Partition Coefficient (n-octanol/water) log Pow = 3.07

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC method.
Test Facility	Firmenich (2011)

Flash Point 89 ± 2 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup equilibrium method
Test Facility	Firmenich (2011)

Autoignition Temperature 278 ± 5°C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	Carbolite flask heater used. No deviations from study plan.
Test Facility	Harlan (2013b)

Explosive Properties Predicted not to be explosive

Method	Structure of the test material was assessed for chemical groups that imply explosive properties.
Remarks	Estimated, based on the chemical structure of the test material
Test Facility	Firmenich (2013)

Oxidizing Properties Predicted not to be oxidising

Method	Structure of the test material was assessed for chemical groups that imply oxidising properties.
Remarks	Estimated, based on the chemical structure of the test material
Test Facility	Firmenich (2013)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 420 Acute Oral Toxicity - Fixed Dose Method.
EC Directive92/69/EEC B.1bis Acute Toxicity (Oral) Fixed Dose Method.

Species/Strain: Rat/Female Wistar (RccHan™:WIST)

Vehicle: Test item was used as supplied for 2000 mg/kg dose level tests, and as a solution in arachis oil BP for 300 mg/kg dose level tests.

Remarks - Method: Sighting test at dose levels of 300 mg/kg and 2000 mg/kg. This was followed by a group of four animals who were fasted prior to being given a single oral dose of the test item as a solution in arachis oil BP at a dose level of 300mg/kg. Observations were made at 30 minutes, 1, 2 and 4 hours and each day for 14 days.

RESULTS

Sighting Study

Dose mg/kg bw	*Administered*	*Evident Toxicity*	*Mortality*
2000	2.14 mL/kg	yes	1/1
300	10 mL/kg	no	0/1

Signs of Toxicity: No signs of toxicity were observed at the lower dosage. At the higher level (2000 mg/kg), hunched posture, ataxia, lethargy, laboured respiration, decreased respiratory rate, increased lachrymation and prostration were observed. Two days after dosing, one of the animals was euthanized due to the occurrence of clinical signs of toxicity that exceeded the severity limit.

Effects in Organs: No abnormalities were recorded at the lower dosage. At the higher level, pale liver and kidneys, and epithelial sloughing of the gastric mucosa were observed on necroscopy.
Dose level – 300 mg/kg: No abnormalities recorded.

Main Study

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
3	4F	300	0/4

Discriminating Dose: 300 mg/kg bw

Signs of Toxicity: No clinical signs of toxicity were noted

Effects in Organs: No abnormalities recorded

Remarks - Results: The LD50 of the test item was estimated to be in the range of 300-2000 mg/kg.

CONCLUSION: The notified chemical is harmful via the oral route.

TEST FACILITY: Harlan (2012a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

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

Species/Strain: Rat/Wistar strain

Vehicle: Test item was used as supplied

Type of dressing: Semi-occlusive.

Remarks - Method: Sighting test performed on two animals (one male, one female) with a 24hr exposure period to the test material. In the absence of mortalities,

additional testing was performed on eight more animals (four males, four females) with observations made at 30 minutes, 1, 2 and 4 hours and then daily for 14 days after a 24 hour exposure period to the test material

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1F/1M	2000	0/1
4	4F/4M	2000	0/4

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	None observed
Signs of Toxicity - Systemic	None observed
Effects in Organs	None observed
Remarks - Results	No evidence of erythema, oedema or other dermal reactions was observed in any of the treated animals. No abnormalities were detected at necropsy.

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

TEST FACILITY Harlan (2012b)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method

Species/Strain	Rat/ RccHan™:WIST strain
Vehicle	None
Method of Exposure	Nose-only
Exposure Period	4 hours
Physical Form	Liquid aerosol.
Particle Size	MMAD 2.76 μm; GSD 2.36
Remarks - Method	Notified chemical was aerosolized using a glass concentric jet nebulizer. A continuous supply of notified chemical was provided under pressure The inhalable fraction (proportion of aerosol less than 4 μm) was calculated as 66.9%. There were four deviations from procedure. The first occurred where the low mean achieved atmosphere concentrations were unsuitable for the purposes of the limit test. The exposure was repeated. Animals from Group 1 were necropsied one day later due to a technical error. The relative humidity within the animal room occasionally went above 70%. The deviations were not considered to affect the purpose or validity of the study.

RESULTS

Group	*Number and Sex of Animals*	*Concentration mg/L*		*Mortality*
		Nominal	*Actual*	
1	3M/3F	23.1	5.18	0/6

LC50	> 5.18 mg/L/4 hours
Signs of Toxicity	Observations were made of changes to respiratory rate (increased or decreased), ataxia, hunched posture, pilo-erection and wet fur. All animals exhibited body weight losses on day 1 after exposure, with

	reasonable weight gains recorded in all animals during the remainder of the recovery period.
Effects in Organs	No macroscopic abnormalities were detected.
Remarks - Results	No deaths occurred. Observations of hunched posture, pilo-erection and wet fur were attributed to the restraint procedure used to expose the animals to the notified chemical rather than an indication of the chemical's toxicity. During exposure, all animals exhibited a decreased respiratory rate. On removal, an increased respiratory rate and ataxia was observed in all animals. The change in respiratory rate could be attributed to the high atmosphere concentrations present during the exposure period. One day after exposure, all animals exhibited increased respiratory rate, hunched posture and pilo-erection. Observations receded over the fourteen day recovery period with animals appearing normal after day 8 to 9.
CONCLUSION	The notified chemical is of low toxicity via inhalation.
TEST FACILITY	Harlan (2013c)

B.4. Irritation - skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion EC Council Regulation No 440/2008 B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Test item used as supplied
Observation Period	3 days
Type of Dressing	Semi-occlusive.
Remarks - Method	Observations were made at 1, 24, 48 and 72 hours after patch removal. One animal was exposed to the test item for 3 minutes, 1 hour and 4 hours, with the remaining two animals exposed to the test item for 4 hours only.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0.7	0	1	< 72 h	0
Oedema	0	0	0	0	-	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal, after 4 hour exposure period

Remarks - Results	No evidence of skin irritation at 3 minute and 1 hour exposure periods was observed. Slight erythema was observed in one rabbit at 24 and 48 hours following the 4 hour exposure period (not the rabbit tested with multiple exposure periods). Skin reactions were not observed in this rabbit 72 hours after exposure to the test item.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	Harlan (2012c)

B.5. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion EC Council Regulation No 440/2008 B.5 Acute Toxicity (Eye Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	3 days
Remarks - Method	Observations were made at 1, 24, 28 and 72 hours following exposure to the test item

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	0.3	0.7	0.3	1	< 72 h	0
Conjunctiva: chemosis	0	0	0	0	-	0
Conjunctiva: discharge	0	0	0	0	-	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0.3	0	1	< 48 h	0

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

Remarks - Results	Iridial inflammation and minimal to moderate conjunctival irritation was observed in all 3 animals. Two treated eyes appeared normal at the 48-hour observation and one treated eye appeared normal at the 72 hour observation
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Harlan (2012d)

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) and United States Environmental Protection Agency Health Effects Test Guidelines OPPTS 870.2600 Skin Sensitisation March 2003
Species/Strain	Mouse/ CBA/Ca
Vehicle	Acetone/olive oil 4:1
Remarks - Method	A preliminary screening test was performed on three mice to determine the highest suitable concentration of test item that would not produce systemic toxicity or excessive local irritation. Test item concentrations were 50%, 37.5% and 25%. No signs of irritation were noted in animals treated with the test item at 37.5% and 25%. The animal treated with the test item at a concentration of 50% was humanely killed on Day 4 due to the occurrence of clinical signs of toxicity that approached the moderate severity limit. Based on the results of the preliminary screening test, groups of five mice were then treated with daily application of the test item at concentrations of 37.5%, 10% or 1%. An additional group of five mice received the vehicle alone in the same manner. ^{3}H-methyl thymidine (^{3}HTdR:80µCi/ml, specific activity 2.0 Ci/mol, ARC UK Ltd) was used to determine the proliferation response of lymph node cells. All animals were observed twice daily on days 1, 2 and 3; and on a daily basis on days 4, 5 and 6. Animals were killed 5 hours after administration of ^{3}HTdR by carbon dioxide asphyxiation followed by cervical separation.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	3501.59 (±1139.89)	NA
37.5%	5550.13 (±2658.48)	1.59
10%	4476.07 (±1898.73)	1.28
1%	4561.75 (±1866.26)	1.30
Positive Control		
50µl α-Hexylcinnamaldehyde tech., 85%	Data not provided	5.76

Remarks - Results: No deaths were recorded nor were any signs of systemic toxicity noted in the test or control animals during the main test. No visual local skin irritation or excessive irritations were noted at any dose concentration evaluated. Body weight changes were comparable to those observed in the corresponding control group animals.

CONCLUSION: There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY: Harlan (2012e)

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) and OPPTS 870.3050, Repeated dose 28-day oral toxicity study in rodents. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-00-366, 2000.

Species/Strain: Rats/ SPF-bred Wistar (han)
Route of Administration: Oral – gavage
Exposure Information: Total exposure days: 28 days
Dose regimen: daily
Post-exposure observation period: None
Vehicle: Propylene glycol
Remarks - Method: No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M/5F	0	0/10
low dose	5M/5F	30	0/10
mid dose	5M/5F	100	0/10
high dose	5M/5F	300	0/10

Clinical Observations

No clinical signs of toxicity were noted during the observation period. Salivation seen after dosing among all animals at 300 mg/kg, (and at lower incidence among most animals at 30 and 100 mg/kg) was considered to be a physiological response rather than a sign of systemic toxicity, potentially due to the taste of the test substance. The incidence of alopecia and scabs were also believed to have no toxicological significance.

Males and females dosed at 300 mg/kg showed slightly lower body weight gain and food intake throughout the

study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

At 300 mg/kg dose level, statistically significant changes in haematology parameters observed included lower white blood cell counts and platelet counts and prolonged prothrombin time in both males and females. No changes in haematology parameters were observed at the low and mid-dose levels.

Statistically significant changes in clinical biochemistry parameters included higher aspartate aminotransferase activity (males, 300 mg/kg), lower total protein level (males and females, 300 mg/kg), higher urea level (females, 100 and 300 mg/kg), and lower glucose levels (females, 300 mg/kg).

Effects in Organs

Adverse treatment related findings at 100 mg/kg and 300 mg/kg included single cell necrosis of the myocardium and vacuolation of the myocardial fibres (females) of the heart, hepatocellular single cell necrosis, hepatocellular hypertrophy and microvesicular hepatocellular vacuolation in the liver.

Adverse treatment related finding at 300 mg/kg included degeneration/depletion of germ cells and increased vacuolation of the seminiferous epithelium of the testes, mismatch of estrus stage in female reproductive organs, atrophy of the outer nuclear layer of the eyes and lymphogranulocytic inflammation, hyperplasia of the squamous epithelium, diffuse hyperkeratosis and/or ulceration of the forestomach.

Remarks – Results

Treatment related findings were present in male and female animals from 100 mg/kg, with an increased number of adverse effects observed at 300 mg/kg.

At 30 mg/kg, the only finding was higher potassium levels in males which was not considered as adverse given this change occurred in the absence of any morphological changes.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 30 mg/kg bw/day in this study, based on adverse effects observed at the middle and upper doses tested.

TEST FACILITY	WIL Research Europe (2014)

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 and the USA, EPA (TSCA) OPPTS harmonised guidelines Plate incorporation procedure (test 1) and Pre incubation procedure (test 2)
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 5-5000 µg/plate b) Without metabolic activation: 5-5000 µg/plate
Vehicle	Dimethyl sulphoxide
Remarks - Method	A preliminary toxicity test (0 - 5000 µg/plate) was performed to determine the toxicity of the test material (TA100 or WP2uvrA). Test 1: direct plate incorporation method, TA100 and TA1535 (without S9-mix only) (5 - 5000 µg/plate) and all other strains (with and without S9-mix) (50 - 5000 µg/plate). Test 2: pre-incubation method, all tester strains (5 - 5000 µg/plate). Vehicle and positive controls were used in parallel with the test material. Positive controls: i) without S9: N-ethyl-N'-nitro-N-nitrosoguandine (WP2uvrA, TA100, TA1535), 9-Aminoacridine (TA1537) and 4-

Nitroquinoline-1-oxide (TA98); ii) with S9: 2-Aminoanthracene (TA100, TA1535, TA1537, WP2uvrA) and Benzo(a)pyrene (TA98).

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	≥ 1,500	> 5000	Negative
Test 2		≥ 500	> 5000	Negative
Present				
Test 1	> 5000	≥ 5000	> 5000	Negative
Test 2		≥ 5000	> 5000	Negative

Remarks - Results

The preliminary toxicity test (0 - 5000 µg/plate) determined that the test material was non-toxic to TA100 or WP2uvrA.

No test item precipitate was observed on the plates at any of the doses tested in either the presence or absence of S9-mix.

In the range-finding test (test 1), the test item caused a visible reduction in the growth of the bacterial background lawns of several tester strains both with and/or without metabolic activation initially from 1500 µg/plate. In the main test, the test item caused a visible reduction in the bacterial background lawns initially from 500 µg/plate.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose level either with or without metabolic activation or exposure method.

The positive controls gave satisfactory responses confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY

Harlan (2011)

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 and the UK Department of Health Guidelines for Testing Chemicals for Mutagenicity.

Species/Strain: Human

Cell Type/Cell Line: Lymphocytes

Metabolic Activation System: S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver.

Vehicle: Dimethyl Sulphoxide

Remarks - Method

Vehicle and positive controls were used in parallel with the test material. (without S9: mitomycin C; with S9: cyclophosphamide).

Based on the results from the preliminary toxicity test and Test 1, the dose range of the test item in Test 2 was expanded to include the maximum recommended 10mM dose of 1682.4 µg/mL.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	40*, 80*, 160*, 240*, 320, 480	4 h	24 h
Test 2	20, 40, 80, 160*, 240*, 320*	24 h	24 h
Present			
Test 1	40, 80, 160, 320*, 480*, 960*	4 h	24 h
Test 2	52.58, 105.15, 210.3*, 420.6*, 841.2*, 1682.4*	4 h	24 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	> 210.3	≥ 240	> 240	Negative
Test 2	≥ 210.3	≥ 320	> 320	Negative
Present				
Test 1	> 1682.4	> 960	> 960	Negative
Test 2		≥ 1682.4	> 1682.4	Negative

Remarks - Results

No statistically significant increase in the frequency of cells with aberrations using a dose range that included a dose level that was outside the optimal 50% mitotic inhibition.

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

Harlan (2013)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	Not reported
Analytical Monitoring	A respirometer, CES multi-channel aerobic respirometer, was used for measurement of the consumption of oxygen.
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
3	35	3	3
7	76	7	72
28	103	28	79

Remarks - Results	All validity criteria for the test were satisfied. The reference compound, aniline, reached the 60 % pass level by day 7 indicating the suitability of the inoculum. The toxicity control exceeded 25% biodegradation within 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after the cultivation period was 103% within 28 days and satisfied the 10-day window validation criterion. Therefore, the test substance can be classified as readily biodegradable according to the OECD (301 F) guideline.
CONCLUSION	The notified chemical is readily biodegradable
TEST FACILITY	Harlan (2013f)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test – Static Test
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Not reported
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	Gas Chromatography (GC) Analysis
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. A Water Accommodated Fraction (WAF), 100% v/v, was prepared by stirring the mixture for a period of 24 hours. The mixture was then filtered through a 0.2 µm Sartorius Sartopore filter. A series of dilutions was made from this test solution to get the other eight treatment concentrations.

RESULTS

Concentration		*Number of D. magna*	*Cumulative % Immobilised*	
Nominal (% v/v saturated solution)	*Measured (mg/L)*		*24 h*	*48 h*
Control	Control	20	0	0
1.0	-	20	0	0
1.8	-	20	0	5
3.2	8.98	20	0	5
5.6	15.0	20	10	30
10	29.5	20	100	100
18	51.4	20	100	100
32	87.0	20	100	100
56	156	20	100	100
100	264	20	100	100

EL50	15 (13 – 18) mg/L at 48 hours
NOEL	7.5 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The 48-hour EL_{50} was calculated from mean measured concentrations by the maximum-likelihood probit method using the ToxCalc software.

CONCLUSION The notified chemical is harmful to aquatic invertebrates

TEST FACILITY Harlan (2012f)

C.2.2. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD

Species	*Pseudokirchneriella subcapitatus*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0, 3.2, 10, 32, and 100% v/v saturated solutions
Auxiliary Solvent	Not reported
Water Hardness	Not reported
Analytical Monitoring	Gas Chromatography (GC) Analysis
Remarks - Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported. A Water Accommodated Fraction (WAF), 100% v/v, was prepared by stirring the mixture for a period of 24 hours. The mixture was then filtered through a 0.2 µm Sartorius Sartopore filter. A series of dilutions was made from this test solution to get the other four treatment concentrations.

RESULTS

Biomass (72 h)		*Growth (72 h)*	
E_yL50 (mg/L)	*NOE_yL (mg/L)*	*E_rL50 (mg/L)*	*NOE_rL (mg/L)*
2.9	Not reported	6.5	Not reported

Remarks - Results	All validity criteria for the test were satisfied. Time-weighted mean measured test concentrations were used to calculate the end points.

CONCLUSION The notified chemical is toxic to algae

TEST FACILITY	Harlan (2013e)

C.2.3. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 100 and 1000 mg/L
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.
RESULTS	
EL50	> 1000 mg/L (loading rate)
NOEL	≥ 1000 mg/L (loading rate)
Remarks – Results	All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration
TEST FACILITY	Harlan (2012g)

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