File No: LTD/1878

May 2016

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

2,4-Pyridinedicarboxylic acid, 2,4-diethyl ester (INCI: Diethyl lutidinate)

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.

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/1878	L'Oreal Australia Pty Ltd	2,4-Pyridinedicarboxylic acid, 2,4-diethyl ester (INCI: Diethyl lutidinate)	Yes	≤ 1 tonne/s per annum	Cosmetic 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 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*
Irritating to eyes (Category 2A)	H319 – Causes serious eye irritation

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):
R36: Irritating to eyes

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

Based on the information available, when used in leave on and rinse off hair care cosmetic products (including pump spray products) at up to 10% concentration, 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:
  - Irritating to eyes (Category 2A): H319 – Causes serious eye irritation

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

(Material) Safety Data Sheet

The (M)SDS of the notified chemical should reflect the above mentioned hazards.

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
  - Ventilation system, including local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical during reformulation processes:
  - Avoid contact with 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:
  - Impervious gloves, eye protection, coveralls

  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

- Formulators should consider that cosmetic products containing the notified chemical should be formulated in a manner to be non-irritating.

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.

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, 2012b) or relevant State or Territory Code of Practice.

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;
- the concentration of the notified chemical exceeds or is intended to exceed 10% in hair care cosmetic products.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a hair care cosmetic ingredient, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- 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)
L'Oreal Australia Pty Ltd (ABN: 40 004 191 673)
564 St Kilda Road
MELBOURNE VIC 3004

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Spectral data, purity, use details, non-hazardous impurities, residual monomers/impurities, additives/adjuvants, and references (for *in vivo* tests) are claimed exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for: hydrolysis as a function of pH, absorption/desorption, dissociation constant, particle size and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
ECHA (2014)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
MEXORYL SBU

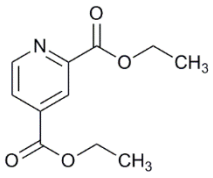
CAS NUMBER
41438-38-4

CHEMICAL NAME
2,4-Pyridinedicarboxylic acid, 2,4-diethyl ester

OTHER NAME(S)
Diethyl lutidinate (INCI)
2,4-Pyridinedicarboxylic acid, diethyl ester
Diethyl 2,4-pyridinedicarboxylate

MOLECULAR FORMULA
$C_{11}H_{13}NO_4$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
223.23 Da

ANALYTICAL DATA
Reference IR spectra were provided.

3. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: White to beige liquid/amorphous solid with a characteristic odour.

Property	Value	Data Source/Justification
Melting Point/Freezing Point	29.5 °C	Measured
Boiling Point	308.4 °C at 101.3 kPa	Measured
Density	1,286 kg/m^3 at 20.0 °C	Measured
Vapour Pressure	2.0×10^{-5} kPa at 25 °C	Measured (SDS)
Water Solubility	13.5 g/L at pH 4 at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionalities.
Partition Coefficient (n-octanol/water)	Log P_{OW} = 1.92 at pH 7.82 at 20 °C	Measured; however, the notified chemical is surface active and is expected to partition to phase boundaries.
Adsorption/Desorption	Not determined	Expected to adsorb strongly to soil and sediment based on surface active properties and potential cationicity.
Dissociation Constant	Not determined	Expected to be ionised under environmental conditions (pH 4–9).
Particle Size	Not determined	Notified chemical is liquid to semi solid under room temperature.
Flash Point	161.5 °C	Measured
Flammability	Non flammable	Measured (SDS)
Autoignition Temperature	> 400 °C	Measured
Explosive Properties	Non-explosive	Measured (SDS)
Oxidising Properties	Not determined	Notified chemical contains no functional groups that would imply oxidative properties.
Surface Tension	56.3 mN/m at 19.7 °C ± 0.1 °C	Measured

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 physio-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.

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. It will be imported into Australia both as an ingredient of hair care cosmetic products (at a maximum concentration of 10%) and as raw material (i.e. at ~100% 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
Melbourne and Sydney

IDENTITY OF MANUFACTURER/RECIPIENTS
Chimex (France)

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a mixture in finished cosmetic products in containers suitable for retail sale (≤500 g plastic/HDPE bottles or tubes) or as a raw material (in 30 kg plastic drums). The finished cosmetic products will be packaged in shipper/cartons, which in turn are arranged in pallets inside sea containers. The imported and formulated products containing the notified chemical will be transported within Australia by road. The end-use products will be packaged in containers suitable for retail sale.

USE
The notified chemical will be used as a component of leave on and rinse off hair care cosmetic products (including pump spray products) at up to 10% concentration. The notified chemical will not be used in hair dyes.

OPERATION DESCRIPTION
The notified chemical will not be manufactured within Australia. The products containing the notified chemical will be stored at this facility until they are sold and shipped to customer facilities.

Reformulation
The procedures for incorporating the notified chemical (at up to 10% concentration) into end-use products will vary depending on the nature of the formulated products and may involve both automated and manual transfer steps. However, in general, it is expected that for the reformulation process, the notified chemical will be weighed and added to the mixing tank where it will be blended with additional additives to form the finished cosmetic products. This will be followed by automated filling of the reformulated products into containers of various sizes. The blending operations are expected to be highly automated and use closed systems and/or adequate ventilation. During the formulation process, samples of the notified chemical and the finished cosmetic products will be taken for quality control testing.

Cosmetic products
The finished hair care products containing the notified chemical (at up to 10% concentration) will be used by consumers and professionals (such as beauticians and hairdressers). Depending on the nature of the product, application could be by hand, sprayed or through the use of an applicator.

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 and storage	4	12
Professional compounder	8	12
Chemist	3	12
Packers (Dispensing & Capping)	8	12
Store Persons	4	12
End Users	8	365

EXPOSURE DETAILS
Transport and storage
Transport and storage workers may come into contact with the notified chemical as a component of hair care products (at up to 10% concentration) or as a raw material (in 30 kg plastic drums) only in the event of accidental rupture of the containers.

At the notifier facility, the primary work activity undertaken by transport and warehouse workers will include the handling, loading and off-loading of pallets containing the notified chemical in end-use products or as raw material. Exposure of these workers will be limited to situations involving products sampling for quality control or, in the event of a discharge, clean up from a spill or leaking drum. If such an event occurs, a worker may be

exposed through dermal or ocular contact. The notifier states that such exposures will be minimised to the extent possible through the use of personal protective equipment (PPE) including protective coveralls and shoes.

Reformulation

During reformulation, dermal, ocular and perhaps inhalation exposure of workers to the notified chemical may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Mixing and dispensing is expected to be carried out in a closed system with flame proof mixers and pumps designed not to create aerosols or a dust hazard and earthed with static discharges. Exposure is expected to be minimised through the use of adequate ventilation, local exhaust ventilation and/or enclosed systems, and through the use of PPE (protective coveralls, chemical resistant gloves and safety glasses).

End-use

Exposure to the notified chemical in end-use products (at up to 10% 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). 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, 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 hair care products (at up to 10% concentration). The principal route of exposure will be dermal, while ocular and inhalation exposure is also possible, particularly if products are applied by spray.

Data on typical use patterns of cosmetic and household cleaning product categories in which the notified chemical may be used are shown in the following tables (SCCS, 2012). For the purposes of the exposure assessment via the dermal route, Australian use patterns for the various product categories are assumed to be similar to those in Europe. In the absence of dermal absorption data, a dermal absorption of 100% was assumed for the notified chemical. An adult bodyweight of 64 kg was used for calculation purposes.

Product type	**Amount** (mg/day)	**C** (%)	**Retention Factor (RF)** (unitless)	**Daily systemic exposure** (mg/kg bw/day)
Shampoo	10,460	10	0.01	0.1634
Conditioner	3,920	10	0.01	0.0612
Hair styling products	4,000	10	0.1	0.625
Total				**0.8497**

Daily systemic exposure = Amount × C × RF × dermal absorption /body weight.
C – Concentration of notified chemical; RF – Retention Factor.

The worst case scenario estimation using these assumptions is for a person who is a simultaneous user of all products listed in the above tables that contain the notified chemical. This would result in a combined internal dose of 0.85 mg/kg bw/day. It is acknowledged that inhalation exposure to the notified chemical from use of other hair care products by pump spray may occur. However, based on the particle size of droplets from pump sprays (typically > 100 μm) and the aggregate exposure from use of the dermally applied products, which assumes a conservative 100% absorption rate, it is sufficiently protective to cover additional inhalation exposure to the notified chemical from use of spray products.

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 > 2,000 mg/kg bw. Low toxicity.
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	irritating at 100% non-irritating at 10% concentration
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation

Rat, repeat dose dermal toxicity – 91 days.	NOAEL 750 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity–*in vitro* mammalian chromosome aberration test.	genotoxic
Genotoxicity–*in vivo* mammalian erythrocyte micronucleus test.	non genotoxic
Rat, reproductive and developmental toxicity	NOAEL 1,000 mg/kg bw/day

Toxicokinetics, metabolism and distribution
No toxicokinetic data on the notified chemical were submitted. The low molecular weight and moderate water solubility of the notified chemical indicate absorption across biological membranes may occur. However, dermal absorption may be limited due to the low partition coefficient (log Pow = 1.92). Also, the notified chemical is surface active and not likely to enhance dermal uptake. There is no evidence of skin irritation or skin sensitisation.

Acute toxicity
The notified chemical is of low acute oral toxicity based on studies conducted in rats. The notified chemical is expected to be of low acute dermal toxicity based on the results from skin irritation and repeat dose toxicity studies conducted in rabbits and rats, respectively.

Irritation and sensitisation
The notified chemical is not irritating to the skin. It is irritating to eyes at higher concentration (100%) but non-irritating at 10% concentration. Based on studies conducted on rabbits, the adverse effects were shown to be reversible by day 6 to 8. The notified chemical was not a skin sensitiser in mice (Local Lymph Node Assay).

Repeated dose toxicity
In a 91-day repeated dose dermal toxicity study in rats, the No Observed Adverse Effect Level (NOAEL) was established as 750 mg/kg bw/day. No significant clinical findings were noted during the study that could be attributed to the test substance. Test substance related effects were observed in the stomach and tongue; however, the study authors considered the microscopic findings to be non-adverse and consistent with possible unintended oral exposure.

Mutagenicity/Genotoxicity
The notified chemical was negative in a bacterial reverse mutation test. The notified chemical gave a positive result in an *in vitro* mammalian chromosome aberration test in human lymphocytes. In the chromosome aberration test a positive response occurred at the high dose only without metabolic activation for three out of four tests. There was only a slight increase in the magnitude of the chromosomal aberration compared to historical controls. A reduction in mitotic index was observed at the highest dose (with and without metabolic activation) with a larger reduction observed without metabolic activation. A negative result occurred with metabolic activation. The notified chemical was negative in an *in vivo* mouse micronucleus assay.

Overall, based on the weight of evidence, the notified chemical is not expected to be genotoxic.

Toxicity for reproduction
The No Observed (Adverse) Effect Level (NO(A)EL) for maternal toxicity and for developmental toxicity was established as 1,000 mg/kg bw/day in this study. Apart from some non-substance related minor skeletal retardation, no clinical findings could be attributed to the test substance.

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***
Irritating to eyes (Category 2A)	H319 – Causes serious eye irritation

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):

R36: Irritating to eyes

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the available information the notified chemical is an eye irritant.

Reformulation

During reformulation workers may be at risk of eye irritation effects when handling the notified chemical at 100% concentration. The notifier states that engineering controls such as enclosed and automated processes and local ventilation will be implemented where possible and appropriate PPE (coveralls, imperious gloves, eye protection and respiratory protection) will be used to limit workers' exposure.

Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

End-use

Exposure to the notified chemical in end-use products may occur in professions where the services provided involve the application of cosmetic products (at ≤ 10% concentration) to clients (e.g. hair dressers, workers in beauty salons). Such professionals may use PPE to minimise repeated exposure, and good hygiene practices are expected to be in place. If PPE is used, the exposure of such workers is expected to be of a similar or lesser extent than that experienced by consumers using the various cosmetic products containing the notified chemical.

6.3.2. Public Health

Cosmetic products containing the notified chemical at ≤ 10% concentration will be available to the public. The main route of exposure is expected to be dermal with some potential for accidental ocular or oral exposure.

Irritation

The notified chemical is an eye irritant at high concentration. However, eye irritation effects are not expected from use of the notified chemical at the proposed maximum concentration in cosmetic products.

Repeated-dose toxicity

The potential systemic exposure to the public from the use of the notified chemical in cosmetic products was estimated to be 0.84 mg/kg bw/day. Using a NO(A)EL of 750 mg/kg bw/day, which was derived from a repeated dose toxicity study on the notified chemical, the margin of exposure (MOE) was estimated to be 883. A MOE value ≥ 100 is considered acceptable to account for intra- and inter-species differences, therefore, the MOE is considered to be acceptable.

Therefore, based on the information available, the risk to the public associated with use of the notified chemical at ≤ 10% concentration in cosmetic products is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia. The notified chemical will be imported neat for reformulation into cosmetic products, or as a component of finished cosmetic formulations. Release of the notified chemical to the environment from transport and storage is unlikely, except in the case of accidental spills and leaks. In the event of spills, the notified chemical and products containing the notified chemical are 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. The process will be followed by automated filling of the formulated products into containers of various sizes suitable for retail. Wastes containing the notified chemical generated during reformulation include equipment wash water, empty import containers, and spilt materials, and may be collected and released to sewers in a worst case scenario, or disposed of to landfill.

RELEASE OF CHEMICAL FROM USE
The notified chemical is a component of rinse-off and leave-on cosmetic formulations. The formulated products will be applied to the body, and will be washed off the body with ultimate release to the sewer.

RELEASE OF CHEMICAL FROM DISPOSAL
It is estimated that a maximum of 4% (or up to 40 kg) of the notified chemical may remain in import containers after reformulation and end-use containers once the consumer products are used up. Wastes and residues of the notified chemical in empty containers are likely either to share the fate of the container and be disposed of to landfill. Wastes may also be released to sewer when containers are rinsed before recycling through an approved waste management facility.

7.1.2. Environmental Fate
Following its use in cosmetic formulations, the majority of the notified chemical is expected to enter the sewer system, before potential release to surface waters nationwide. The notified chemical is considered readily biodegradable (68.7% in 28 days). For details of the environmental fate study, please refer to Appendix C. Based on its surfactant properties, release to surface waters is unlikely as partitioning to sludge and sediment is expected under environmental pH. The notified chemical is not expected to bioaccumulate due to its surfactant properties and ready biodegradability. 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 and nitrogen.

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, or disposed of to landfill as collected spills and empty container residue. The notified chemical residues in landfill, soil and sludge are expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon and nitrogen.

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:	0.606	µg/L
PEC – Ocean:	0.061	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 the notified chemical in the applied soil in 5 and 10 years may be approximately 20.19 µg/kg and 40.39 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*
Daphnia Toxicity	48 h EC50 > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	72 h E_rC50 > 64 mg/L	Not harmful to algae up to the highest measured concentration
Inhibition of Bacterial Respiration	3 h IC50 > 1,000 mg/L	Not inhibitory to microbial respiration

Based on the ecotoxicological endpoints for the notified chemical, it is not expected to be harmful to aquatic invertebrates. In the algal toxicity test, there was a significant difference between the measured concentrations and nominal concentrations (>20%) at the end of the study period. However, the inhibitory effects observed were not considered as a biologically relevant toxic effect by the study authors. Inhibition of algal growth was significantly lower than 50% at the highest dose. The decrease in the concentration of the test material is potentially due to base catalysed hydrolysis of the ester functional groups of the notified chemical in the test system (pH was between 8.5 and 9.1). Therefore, due to the uncertainty in the algal toxicity, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical cannot be formally classified. Based on its ready biodegradability and low bioaccumulation potential, the notified chemical 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 algae as a worst case scenario. A safety factor of 1,000 was used given acute endpoints for only two trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
E_rC50 (Algae, 72 h)	> 64	mg/L
Assessment Factor	1,000	
Mitigation Factor	1.00	
PNEC:	> 64	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	0.606	> 64	**< 0.009**
Q – Ocean	0.061	> 64	**< 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. The notified chemical is considered to be readily biodegradable, and 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 cosmetic formulations, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 29.5 °C (302.7 K)

Method	OECD TG 102 Melting Point/Melting Range. EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks	The differential scanning calorimetry (thermal analysis) method was used. The test result is mean value of 2 independent tests. Estimated accuracy of the melting point measurement is ±0.5 K.
Test Facility	Harlan (2011a)

Boiling Point 308.4 °C (581.6 K) at 100.0 kPa

Method	OECD TG 103 Boiling Point. EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks	The differential scanning calorimetry (thermal analysis) method was used. The test result is mean value of 2 independent tests. Estimated accuracy of the boiling point measurement is ±0.5 K.
Test Facility	Harlan (2011b)

Density 1.286×10^3 kg/m^3 at 20.0 °C ± 0.1 °C

Method	OECD TG 109 Density of Liquids and Solids. EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks	The density was determined using a gas comparison pycnometer. The test result is mean value of 2 independent tests.
Test Facility	Harlan (2011c)

Water Solubility 13.5 g/L at pH 4 at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Shake Flask Method
Test Facility	Harlan (2011e)

Partition Coefficient (n-octanol/water) log Pow = 1.92 at pH 7.82 at 20 °C

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

Surface Tension 56.3 mN/m at 19.7 °C ± 0.1 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions. EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks	The surface tension of the notified chemical was determined in water at a concentration of about 1 g/L.. Two sets of samples were tested and each set contained a total of 6 values of surface tension (mN/m). The surface tension value, consequently, derives from mean value of 12 entries. All measurements were carried out at 20 °C with a maximum deviation of ± 0.5 °C. Based on the available data, the notified chemical is a surface active substance.
Test Facility	Harlan (2011d)

Flash Point 161 °C at 99.3 kPa

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

Autoignition Temperature > 400 °C

Method EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Test Facility Harlan (2011h)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure. EC Directive 92/69/EEC, B.1
Species/Strain	Rat/Sprague-Dawley Rj:SD (IOPS Han)
Vehicle	0.5% suspension of methylcellulose in purified water
Remarks - Method	A group of ten animals were administered a single 2,000 mg/kg oral dose of test substance and were observed for acute toxicity for 14 days. Dosing was performed through gavage. At the end of the observation period all animals were sacrificed by carbon dioxide asphyxiation and subjected to macroscopic necroscopy examination.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5M & 5F	2,000	none

LD50	>2,000 mg/kg bw
Signs of Toxicity	No evidence
Effects in Organs	No effect
Remarks - Results	*Preliminary test (2 female test animals):* 500 and 2,000 mg/kg bw doses were administered to two female test animals and were observed for 7 days. No clinical symptoms were observed for the 500 mg/kg bw dose-level. Dyspnea and piloerection were observed on day 1 in the animal given the 2,000 mg/kg bw dose. *Main experiment :* No deaths or signs of systemic toxicity were observed. All animals showed expected gains in the bodyweight over the study period and no abnormalities were noted at necroscopy.
CONCLUSION	The notified chemical if of low acute toxicity by the oral route.
TEST FACILITY	Laboratory 2 (2003a)

B.2. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC, B.4
Species/Strain	Rabbit/New Zealand White
Number of Animals	3, male
Vehicle	Water (finely ground powder was placed on moistened gauze pad)
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks - Method	A single dose of 500 mg of the test substance was placed on gauze and applied to closely-clipped skin of one flank of the rabbit for 4 hours. Cutaneous reactions were observed approximately 1, 24, 48 and 72 hours after removal of the dressing. Animals were observed for erythema and eschar formation, oedema formation and for presence of any other lesion.
Remarks - Results	Very slight erythema (grade 1) was noted in 1/3 animal at hour 1. No other cutaneous reactions were observed during the study. Mean score for both erythema and oedema at 24, 48 and 72 hour-point was 0.
CONCLUSION	The notified chemical is non-irritating to the skin.

Test Facility Laboratory 2 (2002)

B.3. Irritation – eye

TEST SUBSTANCE Notified chemical (100% concentration)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC, B.5

Species/Strain Rabbit/New Zealand White

Number of Animals 3, male

Observation Period 72 hours. However, due to persistent ocular reactions during first 72 hours in the first experiment, the observation period was extended up to their complete reversibility (day 9).

Remarks - Method The test substance (100 mg of finely ground powder) was applied to conjunctival sac of left eye. Untreated right eye served as control. Eyes were not rinsed after administration of the test item. Animals were examined at 1, 24, 48 and 72 hours post administration and were scored according to degree of positive response. These scores were then used for calculating the respective mean values.

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	3	2	2	3	8	3
Conjunctiva: chemosis	3	1.3	2	3	8	3
Corneal opacity (intensity)	1.7	1.7	2	2	5	2
Corneal opacity (area)	2	2.3	3	3	5	3
Iridial inflammation	1	0	1	1	5	1

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

Remarks - Results All test animals demonstrated ocular reaction to test substance in varying degree and the observed clinical changes were reversible under the test condition.
Corneal opacity: corneal opacity was measured and scored both for affected area and intensity of cloudiness separately. At the end of 72 hour observation period, maximum values of above-mentioned criteria were 2 and 3, respectively. Also, 2/3 test animals demonstrated positive response as scored ≥ 2 and ≥ 1.7 for respective criteria.
Iridial inflammation: At the end of 72 hour observation period, 2/3 test animals were positive for iritis and recorded score was 1.
Conjunctival redness: All animals were positive for conjunctival redness, which was reversed by day 8. At the end of 72 hour observation period, 2/3 test animals demonstrated a ≥ 2 score for the criterion.
Conjunctival oedema (chemosis): All animals were positive for conjunctival oedema, which was reversed by day 6 to 8. At the end of 72 hour observation period, 2/3 test animals demonstrated a ≥ 2 score for the criterion.

CONCLUSION The notified chemical is irritating to the eye at 100% concentration.

TEST FACILITY Laboratory 2 (2003b)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical (10% concentration)

METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 92/69/EEC, B.5
Species/Strain	Rabbit/New Zealand White
Number of Animals	3, male
Observation Period	72 hours
Remarks - Method	The test substance (10% in a 0.5% suspension of methylcellulose (0.1 ml dose-volume)) was applied to conjunctival sac of left eye. Untreated right eye served as control. Eyes were not rinsed after administration of the test item. Animals were examined at 1, 24, 48 and 72 hours post administration and were scored according to degree of positive response. These scores were then used for calculating the respective mean values.

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	0.7	0	2	2	0
Conjunctiva: chemosis	0.3	0.7	0.3	2	2	0
Corneal opacity (intensity)	0	1	0	2	2	0
Corneal opacity (area)	0	1	0	2	2	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results	All test animals demonstrated ocular reaction to test substance in varying degree and the observed clinical changes were reversible under the test condition. A slight chemosis and corneal opacity were observed but were reversed within 48 hours.
CONCLUSION	The notified chemical is non-irritating to the eye at 10% concentration.
TEST FACILITY	Laboratory 2 (2003c)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA/J (female)
Vehicle	Acetone-olive oil (4:1 v/v)
Preliminary study	No
Positive control	α-Hexylcinnamaldehyde (HCA), 25% v/v concentration in vehicle
Remarks - Method	On days 1, 2 and 3, a dose-volume of 25 µL of the control or dosage form preparations were applied to the dorsal surface of both ears. Mice were checked for clinical signs, morbidity and mortality every day. Body weight was measured at day 1 and 6. Thickness of ear was measured on day 1, 3 and 6; and irritation reaction was checked in parallel. At day 6, animals were given a single intravenous injection of 20 µCi dose of ^{3}H-TdR, 5 hours prior to they were sacrificed by cervical dislocation. Single cell suspension from auricular lymph nodes were prepared and proliferative response was measured.

RESULTS

Concentration (% w/v)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	4F	153.32	-

0.5%	4F	210.82	1.37
5%	4F	166.05	1.08
50%	4F	305.40	1.99
Positive Control	4F	1,735.81	11.32

Remarks - Results No local or systemic toxicity or notable weight changes were observed.

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

TEST FACILITY Laboratory 2 (2003d)

B.6. Repeat dose toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 411 Subchronic Dermal Toxicity: 91day Study.
Species/Strain Rat/Sprague-Dawley, CD® [Crl:CD®(SD)]
Route of Administration Dermal – non-occluded
Exposure Information Total exposure days: 91 days
Duration of exposure (dermal): 6 hours/day
Vehicle Water-Ethanol – 1:1 (w/w)
Remarks - Method No significant deviations from the OECD guidelines. Three treatment groups of 10 male and 10 female test animals were administered the test substance at respective dose levels of 250, 500, and 750 mg/kg/day by the dermal route for approximately 6 hours. During the 6 hour exposure, the site was not occluded and each animal had an Elizabethan-type collar. Following observations were made during the 91day regimen:
Morbidity, mortality, injury, and the availability of food and water: twice every day.
Detailed clinical observations: once weekly.
Functional observational battery (FOB) evaluations: during week 13 (Day 86).
Dermal irritation scoring: daily, prior to dosing.
Ophthalmoscopic examinations: during the acclimation period and prior to terminal necropsy.
Body weights and food consumption: weekly.
Blood and urine samples for clinical pathology evaluations: prior to terminal necropsy.
Necropsy examinations, organ weights and microscopic examination of tissues: at study termination

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10M & 10F	0	0
low dose	10M & 10F	250	0
mid dose	10M & 10F	500	0
high dose	10M & 10F	750	0

Mortality and Time to Death
All test animals survived until the scheduled necropsy.

Clinical Observations
No significant clinical findings could be attributed to the test substance. During weeks 9 to 13, unkempt appearance was observed in a male rat in the mid–dose group, in three males in the high–dose group, and in one female in the high–dose group. The study authors concluded that while the unkempt appearance is related to the test article, as it was not observed in animals in the control or low dose groups; the effect was considered to be non-adverse based on the lack of other test substance-related findings.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No significant findings could be attributed to the test substance

Effects in Organs
In the high dose group, 5/10 males showed microscopic findings to the limiting ridge of the non-glandular stomach. These included minimal erosion or ulcer, minimal to mild hyperplasia of the epithelium and minimal to mild hyperkeratosis. There was minimal hyperkeratosis and minimal hyperplasia of the squamous epithelium of the tongue of 2/10 male rats in the high dose group. The microscopic findings were considered to be non-adverse and consistent with possible unintended oral exposure (through transfer of the test substance to the cage).

Remarks – Results
The once daily topical administration of test article to rats at alternating application sites for 91 consecutive days did not induce any noticeable or adverse effects.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 750 mg/kg bw/day.

TEST FACILITY Laboratory 4 (2012)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Test 1: Plate incorporation procedure
Test 2: Pre incubation procedure
Species/Strain *S. typhimurium*: TA1535, TA1537, TA98, TA100, TA102
Metabolic Activation System S9 from Aroclor 1254 induced rat liver
Concentration Range in Test 1 a) With metabolic activation: 52–5,000 µg/plate
b) Without metabolic activation: 52–5,000 µg/plate
Concentration Range in Test 2 a) With metabolic activation: 492–5,000 µg/plate
b) Without metabolic activation: 492–5,000 µg/plate
Vehicle Dimethyl sulfoxide
Remarks No significant deviations from the OECD guidelines.
Positive controls:
With metabolic activation: 2-aminoanthracene (all strains)
Without metabolic activation: 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), t-butyl hyperoxide (TA102)

RESULTS

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

Remarks - No precipitate or signs of toxicity were noted at any dose level. The number of revertant colonies in the vehicle-treated control was within the normal range, and the positive controls were all mutagenic in their appropriate tester strain, confirming the validity of the test.

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

of the test.

Test Facility MDS (2003)

B.8. Genotoxicity – *in vitro*

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test.
Species/Strain *Homo sapiens*
Cell Type/Cell Line Lymphocytes from whole blood samples (primary cell culture)
Metabolic Activation System S9 from Aroclor 1254 induced rat liver
Vehicle Dimethyl sulfoxide
Remarks - Method The notified chemical was tested in two independent experiments, both with and without metabolic activation (S9 mix), obtained from rat liver previously treated with Arcolor 1254. There were two additional confirmatory experiments conducted without the S9 mix. No preliminary test was conducted. For 'without S9 mix' media, Mitomycin C was added as positive control, whereas cyclophosphamide was added in 'with S9 mix' media for the same purpose.

Metabolic Activation	*Test Substance Concentration (mM)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0.078, 0.156, 0.313, 0.625, 1.25, 2.5*, 5*, 10*	3 h	20 h
Test 2	0.625, 1.25*, 2.5*, 5*, 7.5, 10	20 h	20h
Test 3	0.156, 0.313, 0.625, 1.25, 2.5, 5, 7.5, 10	3 h	20 h
Test 4	0.313, 0.625, 1.25, 2.5, 3.33, 4.17, 5, 7.5	20 h	20 h
Present			
Test 1	0.078, 0.156, 0.313, 0.625, 1.25, 2.5*, 5*, 10*	3 h	20 h
Test 2	0.625, 1.25, 2.5, 5*, 7.5*, 10*	3 h	20 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (mM) Resulting in:*		
	Cytotoxicity in Main Test	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥2.5 mM	—	Positive
Test 2	≥ 0.625 mM	—	Positive
Present			
Test 1	≥ 10 mM	—	Negative
Test 2	≥ 1.25 mM	—	Negative

Remarks - Results The dose-level of 10 mM (corresponding to 2232.3 µg/mL) showed no precipitate in the culture medium. The notified chemical induced cytotoxicity to primary lymphocytes both in the presence and in absence of S9 mix, although decrease in mitotic index (MI) (i.e. increased cytotoxicity) was greater in absence than in presence of S9 mix.

Cells were further assessed for chromosome aberration by metaphase analysis. In the first two experiments where lymphocytes were treated with the notified chemical in absence of S9 mix, a statistically significant increase in the frequency of cells with structural chromosome aberration was noted. To check the reliability of the data, two more experiments were performed. It was observed that the test substance induces a slight but statistically significant increase in frequency of cells with structural chromosome aberrations. However, a dose-related increase in the frequency of cells with chromosome aberration was only noted in Test 4

(20 h treatment).

In presence of S9 mix, there was a slight increase in the frequency of cells with structural chromosome aberrations at the highest dose (in Test 1) which was higher than the historical controls. However, it was not a statistically significant or dose-related increase.

CONCLUSION The notified chemical was clastogenic to primary human lymphocytes treated *in vitro* under the conditions of the test (in absence of S9 mix).

TEST FACILITY CIT (2006)

B.9. Genotoxicity – *in vivo*

TEST SUBSTANCE Notified chemical

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity – Mammalian Erythrocyte Micronucleus Test.

Species/Strain Rat/Sprague-Dawley Rj:SD (IOPS Han)

Route of Administration Oral
Subcutaneous

Vehicle 0.5% methylcellulose

Remarks – Method No significant deviations from the OECD guidelines. The test substance was administered to test animals via either subcutaneous or oral route in low, mid and high dose. The top dose selection for the main experiments was based on toxicity observed in the preliminary study.

Toxicity was measured by the ratio of polychromatic erythrocytes (PE) and normochromatic erythrocytes (NE); and clastogenic response was indicated by the relevant increase of micronucleated PCEs.

Group, (route) and [number of administration]	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time Hours (after the last treatment)*
vehicle control 1 (oral) [1]	5M & 5F	0	24 h
vehicle control 2 (oral) [1]	5M & 5F	0	48 h
vehicle control 3 (subcutaneous) [2]	5F	0	24 h
vehicle control 4 (subcutaneous) [1]	5F	0	24 h
vehicle control 5 (subcutaneous) [1]	5F	0	48 h
vehicle control 6 (subcutaneous) [1]	5M & 5F	0	24 h
vehicle control 7 (subcutaneous) [1]	5M & 5F	0	48 h
low dose 1 (oral) [1]	5M & 5F	500	24 h
low dose 2 (subcutaneous) [1]	5F	1,500	24h
mid dose 1 (oral) [1]	5M & 5F	1,000	24 h
mid dose 2 (subcutaneous) [1]	5F	1,750	24 h
high dose 1 (oral) [1]	5M & 5F	2,000	24 h
high dose 1 (oral) [1]	5M & 5F	2,000	48 h
high dose 3 (oral) [1]	5M & 5F	2,000	24 h
high dose 4 (oral) [1]	5M & 5F	2,000	48 h
high dose 5 (subcutaneous) [1]	5F	2,000	24 h
high dose 6 (subcutaneous) [1]	5F	2,000	48 h
high dose 6 (subcutaneous) [2]	5F	2,000	24 h
positive control, CP (oral) [1]	5M & 5F	15	24 h
positive control, CP (oral) [1]	5F	15	24 h

CP=cyclophosphamide.

RESULTS

Doses Producing Toxicity No mortality or clinical signs were reported in the top dose-finding and main tests. Analysis of the PE/NE ratio for the treatment group and control

	group did not indicate biologically relevant evidence of toxicity.
Genotoxic Effects	There was a slight but statistically significant increase in the PE/NE ratio in the first experiment in females when given 2,000 mg/kg/day dose through oral route. Similar increase was also observed in the frequency of the MPE. Cells were identified through Giemsa staining and thereby visual characterisation of cells by microscopy. The study authors suggested the possibility that Giemsa colouration of basophilic granules may appear as micronucleus and give false positive results. To validate authenticity of the first study, DNA specific stain acridine-orange was used in the second experiment. No statistically significant increases in the frequency of MPE were observed.
Remarks - Results	The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not clastogenic under the conditions of the test.
TEST FACILITY	Laboratory 3 (2011)

B.10. Developmental toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 414Prenatal Development Toxicity Study
Species/Strain	Rat/Wistar Hannover
Route of Administration	Oral – gavage
Vehicle	0.5% Methylcellulose aqueous solution
Remarks - Method	GLP Certificate. No significant protocol deviations. The dose selection was based on the results from a preliminary study.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	25F	0	0/25
Low	25F	100	0/25
Intermediate	25F	300	0/25
High	25F	1,000	0/25

Mortality and Time to Death

No animal died during the study. Alopecia and hypotrichosis were observed in a total of 6 animals from different groups. None of these findings had a dose–related response and were considered incidental.

Effects on Dams

Daily clinical observations during the gestation period did not reveal any treatment-related clinical signs of systemic toxicity. There were no premature births or dead foetuses. No treatment-related clinical findings were observed at the necroscopy of the dams.

Effects on Foetus

No treatment-related effects were seen on the mean foetal weight, incidences of major abnormalities and number of foetuses with one or more minor external and visceral abnormalities.

Minor abnormalities in relation to skeletal retardation were noted in certain groups. For instance, foetal incidence of sternebrae not ossified was significantly higher in the low dose group, but lower in the high dose group when compared to the control group. A statistically significant increase in foetal incidence of interparietal bone with incomplete ossification was noted in animals from low dose group when compared to control. The study authors concluded that certain abnormalities were incidental and there was foetal incidence of supraoccipital bone incomplete ossification which was regarded as non-adverse since the retardation was an

isolated finding that could have resumed to normal after birth.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) for maternal toxicity and for developmental toxicity was established as 1,000 mg/kg bw/day.

TEST FACILITY Laboratory 1 (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 B Ready Biodegradability: CO2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon (TOC)
Remarks - Method	Conducted in accordance with the test guidelines above, and in compliance with GLP standards and principles. No significant deviation in protocol was reported.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	4.2	2	36.8
7	16.0	7	68.5
14	62.3	14	82.0
28	76.5	28	86.7

Remarks - Results	All validity criteria for the test were satisfied. The percentage degradation of the reference compound, sodium benzoate, surpassed the threshold level of 60% by 7 days (68.5%) and reached 86.7% degradation by 28 days. Therefore, the test indicates the suitability of the inoculums. The test substance attained 76.5% degradation by 28 days. As the test substance is surface active, the 10-day window is not applicable. Therefore, the test substance is considered to be readily biodegradable according to the OECD (301 B) guideline.
CONCLUSION	The notified chemical is readily biodegradable.
TEST FACILITY	Harlan (2010c)

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 and Reproduction Test – Semi-static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 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. The definitive test was conducted at nominal concentrations of 4.6, 10, 22, 46 and 100 mg/L of the test substance. A total of 20 daphnids (5 daphnids/replicate across 4 replicates) were used. No significant deviation in protocol was reported.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	20	0	0
4.6	(Not determined)	20	0	0
10	(Not determined)	20	0	0
22	(Not determined)	20	0	0
46	38.6	20	0	0
100	86.1	20	10	10

EC50	> 100 mg/L at 48 hours
NOEC	46 mg/L at 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The test solutions were renewed every 24 h during the 48 h test period. The actual concentrations of the test substance were measured at 0 and 48 hours during the 48 h test period. The 48 h EC50 and NOEC for daphnia were determined to be > 100 mg/L and 46 mg/L, respectively, based on nominal concentrations.

CONCLUSION Under the study conditions, the notified chemical is not considered to be harmful to aquatic invertebrates.

TEST FACILITY Harlan (2010a)

C.2.2. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test.

Species	*Pseudokirchneriella subcapitata* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 1–100 mg/L Actual: 0.31–64 mg/L
Auxiliary Solvent	None
Water Hardness	24 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	The definitive test was conducted at nominal concentrations of 1.0, 3.2, 10, 32, and 100 mg/L of the test substance. No significant deviation in protocol was reported.

RESULTS

Biomass		*Growth*	
E_bC50	*NOE_bC*	*E_rC50*	*NOE_rC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 64	1.3	> 64	1.3

Remarks - Results	All validity criteria for the test were satisfied. The actual concentrations of the test substance were measured at 0 and 72 hours within the 72 h test period. The deviation from the nominal concentrations was greater than 20%. The test solutions were not renewed during the 72 h test period. The 72 h E_rC50 and NOEC were determined to be >64 and 1.3 mg/L, respectively, based on measured concentrations.

CONCLUSION Under the study conditions, the notified chemical was not harmful to algae up to the highest measured concentration tested.

TEST FACILITY Harlan (2010b)

C.2.3. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Aerated activated sludge from a synthetic sewage feed.
Exposure Period	3 hours
Concentration Range	Nominal: 1,000 mg/L Actual: Not determined
Remarks – Method	No significant deviation in protocol was reported. 3,5-Dichlorophenol was used as the reference control. The respiration rate was determined by measurement of Biochemical Oxygen Demand during the test after 3 hours of exposure.
RESULTS	
IC50	> 1,000 mg/L at 3 hours
NOEC	Not determined
Remarks – Results	All validity criteria for the test were satisfied. No significant inhibition of respiration rates were observed. The 3 h IC50 was determined to be > 1,000 mg/L, based on nominal concentrations.
CONCLUSION	The notified chemical is not inhibitory to microbial activity.
TEST FACILITY	Harlan (2010d)

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