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February 2013

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Distearyl Ether

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 Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of Sustainability, Environment, Water, Population and Communities.

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
STD/1430	L'Oreal Australia Pty Ltd	Distearyl ether	No	≤ 10 tonnes per annum	Component of rinse-off and leave-on cosmetic products

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

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

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

Environmental risk assessment
On the basis of the limited aquatic exposure, expected low hazard and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following isolation and 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 while handling 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.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical 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 concentration of the notified chemical exceeds or is intended to exceed 5% concentration in leave-on or rinse-off cosmetics;
- the notified chemical is proposed to be used in aerosol spray cosmetics;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of cosmetic products, or is likely to change significantly;
- the amount of chemical being introduced has increased from 10 tonnes per annum, 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 and products containing the notified chemical, provided by the notifier were 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
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: spectral data, degree of purity, impurities, use details, import volume, site of manufacture and analogue details.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: all physico-chemical endpoints except for melting point and water solubility, acute dermal toxicity, and all ecotoxicological endpoints.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Cutina STE

CAS NUMBER
6297-03-6

CHEMICAL NAME
Octadecane, 1,1'-oxybis-

OTHER NAME(S)
Distearyl ether (INCI name)
Dioctadecyl ether

MOLECULAR FORMULA
$C_{36}H_{74}O$

STRUCTURAL FORMULA
$CH_3(CH_2)_{17}O(CH_2)_{17}CH_3$

MOLECULAR WEIGHT
523 Da

3. COMPOSITION

DEGREE OF PURITY > 80%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Off white powder

Property	Value	Data Source/Justification
Melting Point	62.8 °C	Measured
Boiling Point	532 °C at 101.3 kPa	Estimated. EPI suite V 4.0 (Adopted Stein and Brown method.
Density	980 kg/m^3 at 20 °C	(M)SDS
Vapour Pressure	8.41 x 10^{-10}kPa at 25 °C	Estimated. EPI suite V 4.0 (Modified Grain method
Water Solubility	< 5× 10^{-5} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Significant hydrolysis is not expected under environmental conditions due to the lack of hydrolysable functionalities and its limit water solubility
Partition Coefficient	log Pow = 16.76	Estimated using KOWWIN v1.68, EPI

(n-octanol/water)		v.4.10 (US EPA 2011).
Adsorption/Desorption	log K_{oc} = 10.11	Estimated using KOCWIN v2.00, EPI v.4.10 (US EPA 2011).
Dissociation Constant	Not determined	The notified chemical does not contain functional groups that are expected to dissociate under typical environmental conditions
Particle Size	Not determined	Introduced as pellets
Flash Point	259 °C	Analogue 1/(M)SDS
Flammability	Not expected to be flammable	Based on flash point
Autoignition Temperature	Not expected to auto ignite	Analogue 1/(M)SDS
Explosive Properties	Not Explosive	Contains no functional groups that imply explosive properties
Oxidising Properties	Not expected to be oxidising	No functional groups that imply oxidising properties

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in finished cosmetic products at ≤ 5% concentration or in the neat form (> 80% purity) as pellets.

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-10	1-10	1-10	1-10	1-10

PORT OF ENTRY
Sydney
Melbourne

TRANSPORTATION AND PACKAGING
Products containing the notified chemical will be shipped to Australia by sea in containers suitable for retail sale (plastic bottles ≤ 500 mL). The products are packed in dozens inside a shipper, with multiple shippers per pallet and multiple pallets per container. Individual orders are then shipped by road to major retailer warehouses.

USE
The notified chemical will be used in leave-on and rinse-off cosmetic products at ≤ 5% concentration.

OPERATION DESCRIPTION
The notified chemical will be imported as a component of finished cosmetic products at ≤5% concentration. The notified chemical may also be imported in the neat form (i.e. at > 80% purity) for formulation of cosmetic products within Australia.

Formulation of cosmetic products
The notified chemical will be stored in a raw materials store room. The notified chemical will be weighed and added to a flame proof mixing tank. Mixing will be highly automated and occur in a fully enclosed environment.

Prior to and after the formulation process, samples of the notified chemical will be taken for quality control testing using a spatula.

End-use
The finished cosmetic products containing the notified chemical at ≤5% concentration will be used by consumers and professionals (such as workers in beauty salons). Application of products could be by hand or through the use of an applicator.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	4	12
Professional compounder	8	12
Chemist	3	12
Packers (Dispensing and 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 in the neat form (> 80% purity) or as a component of cosmetic products (≤ 5%) only in the event of accidental rupture of containers.

Formulation of cosmetic products
During formulation of cosmetic products from the neat notified chemical, dermal, ocular and inhalation exposure of workers to the notified chemical (at > 80% concentration) may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. Exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems and through the use of personal protective equipment such as coveralls, safety glasses and impervious gloves.

End-use
Exposure to the notified chemical (at ≤ 5%) in end-use products may occur in professions where the services provided involve the application of cosmetic and personal care products to clients (e.g. workers in beauty salons). Such professionals may use some personal protective equipment (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 (at ≤ 5% concentration) through the use of cosmetic products. The principal routes of exposure will be dermal and oral (through the use of lip products), while ocular exposure is also possible.

Data on typical use patterns of product categories in which the notified chemical may be used are shown in the following table (SCCS, 2010). Australian use patterns for the various product categories are assumed to be similar to those in Europe and an adult bodyweight of 60 kg has been used for calculation purposes. In addition, 100% absorption has been assumed for the dermal and oral route. The table below shows factors relevant for the dermal route, which is the main contributor to exposure for all product types except lipstick. Exposure to lipstick products is mainly through ingestion.

Product type	Amount (mg/day)	C (%)	RF	Daily systemic exposure (mg/kg bw/day)
Body lotion	7820	5	1	6.52
Face cream	1540	5	1	1.28
Hand cream	2160	5	1	1.80
Deodorant (non-spray)	1500	5	1	1.25
Liquid Foundation	510	5	1	0.43
Lipstick, lip salve	57	5	1	0.05
Shower gel	18670	5	0.01	0.16
Hand wash soap	20000	5	0.01	0.17
Shampoo	10460	5	0.01	0.09
Hair conditioner	3920	5	0.01	0.03
Total				**11.76**

C = concentration; 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 table that contain the notified chemical. This would result in a combined internal dose (*via* dermal and oral routes) of 11.76 mg/kg bw/day.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and acceptable analogues of the notified chemical (Analogues 1 and 2) 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 > 5000 mg/kg bw; low toxicity
Rat, acute dermal toxicity (Analogue 1)	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Evaluation of skin irritation potential using the EPISKIN Reconstituted Human Epidermis Model	non irritating
Rabbit, eye irritation	non-irritating
Guinea pig, skin sensitisation – non-adjuvant test	no evidence of sensitisation
Rat, repeat dose oral toxicity – 90 days (Analogue 1).	NOAEL 1000 mg/kg bw/day
Rat, repeat dose oral toxicity – 90 days (Analogue 2).	NOAEL 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity in vitro mouse lymphoma (Analogue 2)	non genotoxic
Genotoxicity – in vitro Chinese hamster (Analogue 2)	non genotoxic
Genotoxicity–in vitro human lymphocytes (Analogue 1)	non genotoxic
Rat, parental developmental toxicity (Analogue 1)	NOAEL 1000 mg/kg bw/day
Rabbit, parental developmental toxicity (Analogue 1)	NOAEL 1000 mg/kg bw/day
Toxicity to reproduction- one generation (Analogue 1)	NOAEL > 450 mg/kg bw/day

Toxicokinetics, metabolism and distribution.
Given the relatively low molecular weight of the notified chemical, absorption across biological membranes is possible. However, it may be limited by the low water solubility and expected high partition co-efficient of the notified chemical.

Acute toxicity.
Based on studies conducted on the notified chemical and an analogue (Analogue 1) in rats, the notified chemical is of low acute oral and dermal toxicity. No acute inhalation toxicity studies were submitted for the notified chemical. The notified chemical has an estimated low vapour pressure (8.41×10^{-10}kPa at 25 °C); therefore inhalation exposure is not expected unless aerosols are formed.

Irritation and sensitisation.
Based on studies conducted on the notified chemical in rabbits and an *in vitro* skin irritation study, the notified chemical is not irritating to the skin or eyes.

There was no evidence of sensitization in a study conducted in guinea pigs (Buehler test) for the notified chemical.

Repeated Dose Toxicity.
No repeated dose toxicity studies were provided for the notified chemical. In 90-day repeated dose toxicity studies conducted on Analogue 1 and 2 in rats, no adverse effects were observed up to the highest dose tested of 1000 mg/kg bw/day.

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in a bacterial reverse mutation study and Analogues 1 and 2 gave negative results in a range of *in vitro* genotoxicity studies. The notified chemical is therefore not expected to be genotoxic.

Toxicity for reproduction.
There are no toxicity for reproduction data available for the notified chemical. Studies on an analogue chemical (Analogue 1) have been conducted. In a prenatal developmental toxicity study in rats treatment related effects noted were ptylaism in the mothers and reduced ossification of the frontal bone of foetuses in the 1000 mg/kg bw/day treatment group, which were not considered as adverse effects by the study authors. The same study conducted in rabbits found that 1000 mg/kg bw/day resulted in a transient, minor to slight decrease in food consumption and body weight gain. Given the transient and minor nature of these effects, the NOAEL has been established by NICNAS to be 1000 mg/kg bw/day in this study. Further, a one generation toxicity to reproduction study determined that fertility, mating behaviours, gestation and parturition of parent animals were not affected even at the highest dose-level (450 mg/kg bw/day) under the conditions of the experiment. No adverse effects were noted in the 1st filial generation.

Overall, based on studies conducted on Analogue 1, the NOAEL for the notified chemical for reproductive and developmental toxicity is determined to be 1000 mg/kg bw/day.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on toxicity studies, the notified chemical is essentially non-hazardous. However, slight toxicity (decreased food consumption and weight loss) has been observed with an analogue chemical (Analogue 1) at the high dose level only (1000 mg/kg bw/day) in a parental developmental toxicity study.

Compounders and laboratory staff involved in the formulation of cosmetic products may come in contact with the neat notified chemical (> 80% purity). Exposure is expected to be limited during product formulation by the engineering controls and PPE used, and the enclosed and automated processes. Given the low hazardous nature of the notified chemical and the control measures in place to limit exposure, the notified chemical is not

considered to pose an unreasonable risk to reformulation workers.

Beauty care professionals will handle the notified chemical at ≤ 5% concentration in cosmetic products, similar to public use. Therefore, the risk for beauty care professionals 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 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 use of the notified chemical at ≤ 5% concentration in cosmetic products is not considered to be unreasonable.

6.3.2. Public Health

At the proposed use concentration of ≤ 5% notified chemical in cosmetic products, acute toxicity effects are not expected. Based on a range of repeated dose toxicity studies on analogue chemicals, the notified chemical is determined to have a NOAEL of 1000 mg/kg bw/day.

Repeat dose toxicity potential was estimated by calculation of the margin of exposure (MoE) of the notified chemical using the worst case exposure scenario of 11.76 mg/kg bw/day (see Section 6.1.2) and a NOAEL of 1000 mg/kg bw/day. A MoE value greater ≥ 100 is considered acceptable to account for intra- and inter-species differences. Using the abovementioned NOAEL, a MoE of 85 was estimated. Thus, in light of the conservative parameters used, the risk to the public associated with the use of the notified chemical at ≤ 5% in cosmetics 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 is not manufactured in Australia; therefore, there is no release to the environment from this activity. Environmental release during importation, transport and distribution may occur as a result of accidental breakage and 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.

The notified chemical may be reformulated in Australia into a variety of cosmetic products. During formulation and mixing, release of notified chemical to the environment is expected to be negligible as these processes occur in a closed system in industrial settings. Typical wastes generated during reformulation that may contain the notified chemical include reformulation equipment washings, empty import containers and spilt materials. Due to its limited water solubility, concentrated notified chemical in wastes from reformulation activities are likely to be present in non-aqueous waste streams and are expected to be disposed of either directly to landfill, or via licensed waste contractors, in accordance with local regulations. Some of the notified chemical may be released to sewers in dilute aqueous rinsate.

RELEASE OF CHEMICAL FROM USE

Formulated products containing the notified chemical are expected to be applied to skin and hair. It is expected that the majority of the annual import volume will be washed off the skin and hair and released to the sewer following consumer use.

RELEASE OF CHEMICAL FROM DISPOSAL

Expired product and residues of the notified chemical in the empty consumer containers (up to 3% of the annual import volume) are likely either to share the fate of the container and be disposed of to landfill, or be washed to sewer when containers are rinsed before recycling.

7.1.2. Environmental Fate

The majority of the notified chemical is expected to be disposed of to sewer following its use in cosmetic products. As it has low water solubility (< 0.05 mg/L), estimated high n-octanol/water partition coefficient (log Pow = 16.76) and soil adsorption coefficient (log K_{oc} = 10.11), it is estimated that the notified chemical will be efficiently removed from effluent by adsorption to sediment and sludge in sewage treatment plants, with

the sludge eventually disposed of to landfill or re-used for soil remediation. A small proportion of the notified chemical may be discharged to surface waters in treated effluent. However, the notified chemical is expected to degrade rapidly in water based on its high biodegradability (60% over 28 days). In landfill or in soil, the notified chemical is expected to have low mobility, due to its low water solubility and high soil adsorption coefficient. The notified chemical is not expected to be readily bioavailable due to its anticipated high n-octonal/water partition coefficient. It is not likely to bioaccumulate based on the measured bioconcentration factor for an analogue chemical (BCF = 92 ± 19) and the predicted bioconcentration factor for the notified chemical (BCF = 3.16) (BCFBAF v.3.01, EPI v.4.10, US EPA 2011). Therefore, the notified chemical is expected to degrade biotically and abiotically to form water and oxides of carbon in landfill, soil or water. For the details of the environmental fate studies refer to Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

Since most of the chemical will be washed into the sewer, under a worst case scenario assuming no removal of the notified chemical in the sewage treatment plant (STP), the Predicted Environmental Concentration (PEC) on release of sewage effluent on a nationwide basis would be 6.06 µg/L in rivers and 0.61 µg/L in oceans.
However, a more realistic exposure scenario includes mitigation by 89% removal of the notified chemical via degradation and absorption to sediment and sludge during STP processes. Therefore, the resultant PEC in sewage effluent on a nationwide basis are estimated as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	10,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	10,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	27.4	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	89%	**Mitigation**
Daily effluent production:	4,523	ML
Dilution Factor - River	1	
Dilution Factor - Ocean	10	
PEC - River:	0.61	µg/L
PEC - Ocean:	0.06	µg/L

Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 47.86 mg/kg (dry wt). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.319 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 1.59 mg/kg and 3.19 mg/kg, respectively. However, due to the biodegradability of the notified chemical, these calculated values represent theoretical maximum concentrations only.

7.2. Environmental Effects Assessment

No ecotoxicity data for the notified chemical were submitted. The results from an ecotoxicological investigation conducted on a mixture, containing Analogue 1, are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	**Result*	*Assessment Conclusion*
Acute Toxicity		
Fish (rainbow trout)	LC50 (96 h) > 10 mg/L	Not expected to be harmful to fish up to the limit of its solubility
Daphnia	LC50 (48 h) > 6.9 mg/L	Not expected to be harmful to aquatic invertebrates up to the limit of its solubility
Algal	E_rC50 (72 h) > 0.012 mg/L	Not expected to be harmful to algae up to the limit of its solubility in water
Chronic Toxicity		

Fish (Carp)	NAOEC (28 d)** ≥ 0.06 mg/L	Not expected to have effects on fish growth up to the limit of its solubility
Daphnia	NOEC (21 d) ≥ 0.009 mg/L	Not expected to have long lasting effects on aquatic invertebrates up to the limit of its solubility in water
Algal	NOEC ≥ 0.012 mg/L	Not expected to have long lasting effects on algae up to the limit of its solubility
Inhibition of Bacterial Respiration	EC50 (3 h) > 100 mg/L	Not expected to inhibit microbial respiration

*The reported endpoint is based on test conducted on a mixture containing two components. Both components have low water solubility and the values are close to each other. The actual concentration of the test substance was based on the mean values determined for two components of test substance.
** No adverse observed effect concentration (NAOEC)

The ecotoxicological endpoints measured for the analogue chemical indicates that the notified chemical is not harmful to the aquatic organisms up to the limit of its water solubility, which is supported by the endpoints estimated for the notified chemical using Quantitative Structure-Activity Relationships (QSARs). The notified chemical is not expected to be bioavailable based on its very high predicted log Pow of 16.76. Therefore, no effects on aquatic biota are predicted for the notified chemical at its water saturation concentration (ECOSAR, v1.00, US EPA, 2011).

Classification should only be based on toxic responses observed in the soluble range and, therefore, the notified chemical cannot be formally classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) has not been calculated for the notified chemical as no effects to aquatic organisms are expected up to its limit of solubility in water.

7.3. Environmental Risk Assessment

The Risk Quotient, Q (= PEC/PNEC), has not been calculated since a PNEC is not available. The potential for exposure of the notified chemical to the aquatic environment is limited given it is expected to be efficiently removed from effluent during sewage treatment processes. The notified chemical is also not expected to be bioavailable to aquatic organisms in surface waters based on its intrinsic hydrophobicity. On the basis of the limited aquatic exposure, expected low hazard and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 62.8 °C

Method Differential scanning calorimetry

Test Facility SGS (2011)

Water Solubility $< 5 \times 10^{-5}$ g/L at 20 °C

Method EC Council Regulation No 440/2008 A.6 Water Solubility

Remarks Water solubility was determined for the notified chemical using Column Elution Method. The test substance (87.5 mg) was mixed with sand (2.51 g) and acetone (10 mL). The mixture was homogenized. Acetone was removed by evaporation. In this way, the sand was coated with the test substance. The coated sand was transferred into the micro column for swelling. The pump was started 2 hours later and elution fractions were collected and measured. The concentration of the notified chemical in fractions was determined by GC-MSD method.

Test Facility Henkel (2011)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity.
Species/Strain	Rat/Sprague Dawley
Vehicle	Mineral oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	5F/5M	5000	0/10

LD50	> 5000 mg/kg bw
Signs of Toxicity	There were no mortalities during the study. Slight piloerection and loss of motor activity was observed in 5 animals up to 5 hours post treatment.
Effects in Organs	No effects in organs were observed.
Remarks - Results	The individual weight of all animals was normal.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Evic-Ceba (1997a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	A mixture containing Analogue 1 (concentration unknown)
METHOD	OECD TG 402 Acute Dermal Toxicity.
Species/Strain	Rat/Sprague Dawley
Vehicle	None
Type of dressing	Semi-occlusive.
Remarks - Method	The test sites were moistened with Arachis oil B.P. prior to treatment with the test substance.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	No signs of irritation were noted.
Signs of Toxicity - Systemic	No signs of systemic toxicity were noted.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	No toxicologically significant effects were observed.
CONCLUSION	The test substance and, by inference, the notified chemical are of low toxicity via the dermal route.
TEST FACILITY	Safepharm (1990)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Vehicle	Distilled water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations
RESULTS	
Remarks - Results	No reactions were recorded for any animal at any time point.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	Evec-Ciba (1997b)

B.4. Irritation – skin (In vitro)

TEST SUBSTANCE	Notified chemical
METHOD	Similar to OECD TG 439: In Vitro Skin Irritation: Reconstituted Human Epidermis Test Method
Remarks - Method	Evaluation of Skin Irritation Potential using the EPISKIN Reconstituted Human Epidermis Model. The principle of the assay is based on the measurement of cytotoxicity in reconstituted human epidermal cultures following topical exposure to the test material. The irritation potential of the test substance was assessed by applying the notified chemical undiluted onto the surface of 3 EpiSkin reconstructed human epidermis (RHE) units for 15 mins. The notified chemical was then washed from the surface of the EpiSkin units which were incubated for a recovery period of approximately 42 hrs at 37 °C. Triplicate Episkin samples with 25 µL of negative (Phosphate Buffered Saline (PBS)) and positive (Sodium Dodecyl Sulfate (SDS) 5%) controls were treated similarly. After incubation, the tissues were transferred to the MTT (2H-tetrazolium, 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-, bromide (1:1)) filled wells and incubated for 3 hours before being transferred to micro tubes containing 500 µL isopropanol for extraction of formazan crystals out of the MTT loaded tissues. The amount of extracted formazan was determined spectrophotomically at 570 nm in duplicate. Data are presented in the form of % viability (MTT reduction in the test material treated tissue relative to negative control tissues). IL-1α released in the culture medium was determined using the ELISA assay. The procedure was performed in duplicate using two different lots of reconstructed skin. Positive (Sodium Dodecyl Sulfate) and negative (Phosphate Buffered Saline) controls were also run in triplicate. Criteria for in vitro interpretation:

If mean tissue viability is ≤ 50% the test substance is considered an Irritant
If mean tissue viability is > 50% and IL-1α RM release is < 50 pg/mL the test substance is considered a Non Irritant

In the OECD TG 439, measurement of IL-1α release is not required and is thus not part of the criterion to consider a test substance an non-irritant.

RESULTS

	% cell viability*	IL-1α (pg/mL)*	Conclusion
Negative Control	100	-	Predicted non irritant
Positive Control	6.65	-	Predicted irritant
Test Substance	100	20.2	Predicted non irritant

* Average from tests on 2 different lots of reconstructed skin

Remarks - Results: The results suggest that the test substance is a non-irritant. The results of the concurrent positive control confirm the validity of the test.

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

TEST FACILITY: Episkin (2007)

B.5. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 females
Observation Period: 72 hours
Remarks - Method: No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.3	0.3	0.3	2 (1 h)	< 48 h	0
Conjunctiva: chemosis	0.3	0	0	1	< 48 h	0
Conjunctiva: discharge	0	0	0	2 (1 h)	0	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	1 (1 h)	0	0

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

Remarks - Results: At the 1-hour observation period, Grade 2 conjunctival redness and discharge was observed in all tested animals and slight chemosis in one of them. Iris congestion was also noticed in the three rabbits.

At the 24 hour observation period, a slight redness remains visible in the three animals with the persistence of slight chemosis in one animal.

All signs of irritation were resolved at the 48-hour observation period.

CONCLUSION: The notified chemical is non-irritating to the eye.

TEST FACILITY: Evic-Ceba (1997c)

B.6. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation – Buehler Test
Species/Strain	Guinea pig/Hartley Albino
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 50%
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
INDUCTION PHASE	Induction Concentration: topical: 50%
Signs of Irritation	Not reported.
CHALLENGE PHASE	
1st challenge	topical: 20% and 50%
Remarks - Method	The test substance was diluted in mineral oil. For the challenge phase, all test and control animals were treated at different sites with the vehicle alone and the test substance at 20 and 50% dilution in mineral oil.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after: 1st challenge*	
		24 h	*48 h*
Test Group	0%	0/19	0/19
	20%	0/19	0/19
	50%	0/19	0/19
Control Group	0%	0/10	0/10
	20%	0/10	0/10
	50%	0/10	0/10

Remarks - Results	One animal of the test group was found dead on Day 4. This mortality was reported by the study authors of not being attributable to the test substance but to a lung pathology. No signs of irritation were observed in the control and test group animals challenged with 20% and 50% of the test substance. The vehicle alone also did not produce any signs of irritation. Body weight changes were normal.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Evic-Ceba (1997d)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents. EC Directive 88/302/EEC B.26 Sub-Chronic Oral Toxicity Test: 90-Day Repeated Oral Dose Study using Rodent Species.
Species/Strain	Rat/ Crl:CD (SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days

	Dose regimen: 7 days per week
	Post-exposure observation period: 6 weeks
Vehicle	Sunflower oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10M/10F	0	0/20
low dose	10M/10F	100	0/20
mid dose	10M/10F	300	0/20
high dose	10M/10F	1000	0/20
control recovery	5M/5F	0	0/20
high dose recovery	5M/5F	1000	0/20

Mortality and Time to Death

There were no test substance related mortalities during this study.

Clinical Observations

A slight increase in spontaneous motility was observed in males receiving 1000 mg/kg bw/day test substance; however this was not determined to be statistically significant. No other treatment related changes were observed by physical examination, sensory reactivity assessment or motor skills. There were no adverse bodyweight or food consumption effects in either males or females.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

An increase in mean corpuscular volume was noted in females receiving 300 mg/kg bw/day, however this was not considered to be statistically significant. Urinary pH was slightly decreased in females treated with 300 or 1000 mg/kg bw/day. This effect was considered to be spontaneous and not treatment related.

Effects in Organs

Increased lobular pattern was noted in males and females at all doses of the test article. These changes were noted in two non-treatment animals and so were considered not to be treatment related. Increased liver and kidney weights were noted in male and female animals treated at 1000 mg/kg bw/day test article. No changes were noted in the rats in the recovery group treated with the same dose. These changes were therefore considered to be adaptive and non-adverse.

A statistically significant increase in lymphocytes and histiocytes was observed in the trachea of females in the 1000 mg/kg bw/ day group. This was regarded to be an incidental finding.

Remarks – Results

The data show that the test substance, administered orally, was well-tolerated at up to 1000 mg/kg/day and did not induce significant adverse effects.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study, based on no adverse effects observed at the highest dose tested.

TEST FACILITY LPT (2009)

B.8. Repeat dose toxicity

TEST SUBSTANCE A mixture containing Analogue 1 (~47%)

METHOD OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Species/Strain	Rat/ Wistar:Han
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days

	Dose regimen: 7 days per week Post-exposure observation period: 15 days
Vehicle	0.5% aqueous methylcellulose
Remarks - Method	The dose-levels were determined on the basis of results of a previous 4-week study in which no major effects were noted. A constant dosage – volume of 5 ml/kg/administration was used. Dose not adjusted for concentration of Analogue 1.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10M/10F	0	0/20
low dose	10M/10F	100	0/20
mid dose	10M/10F	300	0/20
high dose	10M/10F	1000	0/20
control recovery	10M/10F	0	0/20
high dose recovery	10M/10F	1000	0/20

Mortality and Time to Death

There were no test substance related mortalities during this study.

Clinical Observations

No treatment related changes were observed by physical examination, sensory reactivity assessment or motor skills. There were no adverse bodyweight or food consumption effects in either males or females.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

A slightly lowered leucocyte count associated with lower lymphocyte count was noted in males at all test substance dose. Since the differences were small and not dose-related, they were considered to be of no toxicological significance.

Dose-related lowered potassium levels were noted in females in all treatment groups. However, since the differences were slight and not observed in males this was not considered to be of toxicological significance.

Effects in Organs

No test substance related effects were noted in organs.

Remarks – Results

The data show that the test substance, administered orally, was well-tolerated at up to 1000 mg/kg/day and did not induce significant adverse effects.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study, based on no adverse effects observed at the highest tested dose.

TEST FACILITY CIT (2002a)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure: Test 1 and Test 2 without metabolic activation Pre incubation procedure: Test 2 with metabolic activation only
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA (pKM101), WP2 (pKM101)

Metabolic Activation System	S9 fraction from rat liver induced with Aroclor 1254
ConcentrationRange in Main Test	a) With metabolic activation: Test 1: 5-500 µg/plate Test 2: 1.5-150 µg/plate b) Without metabolic activation: Test 1: 5-500 µg/plate Test 2: 1.5-150 µg/plate
Vehicle	Tetrahydrofuran (THF)
Remarks - Method	Due to low solubility, the notified chemical was dissolved in THF at a maximum concentration of 60 mg/mL. Further, due to the relative toxicity of THF, the volume of the solvent used in the test was 20 µl/plate in all assays without pre-incubation and 10 µl/plate in all assays with pre-incubation, rather than 100 µl/plate. The highest dose selected was based on the solubility of the test substance. In first assay, at 500 µg/plate, a heavy precipitate prevented colony scoring. Consequently, the maximum dose was limited to 150 µg/plate in the second assay.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 500	> 500	≥ 50	Negative
Test 2		> 150	≥ 150	Negative
Present				
Test 1	> 500	> 500	≥ 50	Negative
Test 2		> 150	≥ 150	Negative

Remarks - Results	No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test substance, either with or without metabolic activation. All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	IPL (1998)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test. EC Directive 2000/32/EC B.17 Mutagenicity - In vitro Mammalian Cell Gene Mutation Test.
Species/Strain	Mouse
Cell Type/Cell Line	Mouse/ L5178Y lymphoma cells
Metabolic Activation System	S9 mix from rat livers induced with Aroclor 1245
Vehicle	Acetone
Remarks - Method	Methyl methanesulphonate (without metabolic activation) and 3-methylcholanthrene (with metabolic activation) were used concurrently as the positive controls.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				

Test 1	1.56, 3.13, 6.25, 12.5, 25	3 h	2-3 days	11-12 days
Test 2	1.56, 3.13, 6.25, 12.5, 25	24 h	2-3 days	11-12 days
Present				
Test 1	1.56, 3.13, 6.25, 12.5, 25	3 h	2-3 days	11-12 days
Test 2	1.56, 3.13, 6.25, 12.5, 25	3 h	2-3 days	11-12 days

*All 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	≥ 25			
Test 1		≥ 25	> 25	Negative
Test 2		≥ 25	> 25	Negative
Present	≥ 25			
Test 1		≥ 25	> 25	Negative
Test 2		≥ 25	> 25	Negative

Remarks - Results: The test substance did not induce any statistically significant increases in the mutant frequency at any tested concentration in each exposure group with and without metabolic activation.

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

CONCLUSION: The test substance and, by inference, the notified chemical are not clastogenic to mouse lymphoma cells treated in vitro under the conditions of the test.

TEST FACILITY: LPT (2008)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE: Analogue 2

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

Species/Strain: Chinese hamster
Cell Type/Cell Line: V79 cell line
Metabolic Activation System: S9 mix from rat liver induced with phenobarbital/β-naphthoflavone
Vehicle: Acetone
Remarks - Method: Ethyl methanesulfonate (without metabolic activation) and cyclophosphamide (with metabolic activation) were used concurrently as the positive controls.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	2.5*, 5.0*, 10.0*, 15.0, 20.0, 25.0	4 h	18 h
Test 2	1.3, 3.8, 5.0*, 7.5*, 10.0, 15.0*	18 h	18 h
Test 3	5.0, 7.5, 10.0*, 15.0	28 h	28 h
Present			
Test 1	18.8, 37.5, 75, 150*, 300*, 600*	4 h	18 h
Test 2	18.8, 37.5, 75*, 150*, 300*, 600	4 h	28 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	≥ 10			
Test 1		≥ 10	> 25	Negative
Test 2		≥ 15	> 15	Negative
Test 3		≥ 10	> 15	Negative
Present	≥ 625			
Test 1		≥ 600	≥ 19.5*	Negative
Test 2		≥ 300	≥ 19.5*	Negative

* Test substance reported to float on the surface of the culture medium in the preliminary toxicity test. This was not reported in the main test.

Remarks - Results

In both experiments, in the absence and presence of metabolic activation, no biologically relevant increase in the number of cells with structural aberrations was observed. However, two statistically significant differences were observed in Test 2 in the presence of S9 mix at 28 hours after treatment with 150 and 300 μg/mL (2% aberrant cells exclusive gaps for each). The significance was caused by the low response (0% aberrant cells, exclusive gaps) of the solvent control. The observed effects were within the laboratory's historical control data range (0-4% aberrant cells, exclusive gaps) and were regarded as being biologically irrelevant.

CONCLUSION

The test substance and, by inference, the notified chemical are not clastogenic to V79 cells treated in vitro under the conditions of the test.

TEST FACILITY

RCC (2001)

B.12. Genotoxicity – in vitro

TEST SUBSTANCE

A mixture containing Analogue 1 (48.8%)

METHOD

OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line: Human lymphocytes

Metabolic Activation System: S9 mix from rat liver induced with Aroclor 1254

Vehicle: DMSO

Remarks - Method

The dose concentration was adjusted for the concentration of Analogue 1.

The positive controls used in the study were ethyl methanesulphonate (without metabolic activation) and Cyclophosphamide (with metabolic activation).

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	7.8*, 15.63*, 31.25*, 62.50*, 125, 250, 500, 1000	20 h	20 h
Test 2	7.8*, 15.63*, 31.25*, 62.50*, 125, 250, 500, 1000	20 h	20 h
Test 3	7.8*, 15.63*, 31.25*, 62.50*, 125,250, 500, 1000	44 h	44 h
Present			
Test 1	7.8, 15.63, 31.25, 62.50, 125*, 250*, 500*, 1000*	4 h	20 h
Test 2	7.8, 15.63, 31.25, 62.50, 125*, 250*, 500*, 1000*	4 h	20 h
Test 2	7.8, 15.63, 31.25, 62.50, 125*, 250*, 500*, 1000*	4 h	44 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic	*Test Substance Concentration (μg/mL) Resulting in:*

Activation	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	≥ 125	≥ 500	Negative
Test 2	≥ 125	≥ 500	Negative
Test 2	≥ 125	≥ 500	Negative
Present			
Test 1	> 1000	≥ 500	Negative
Test 2	> 1000	≥ 500	Negative
Test 2	≥ 125	≥ 500	Negative

Remarks - Results: No significant increases in chromosomal aberrations were seen in any treatment groups either with or without metabolic activation.

The positive controls caused statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the test system and the efficacy of the S9 mix.

CONCLUSION: The test substance and, by inference, the notified chemical are not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.

Test Facility: Safepharm (1991a)

B.13. Developmental toxicity

TEST SUBSTANCE: A mixture containing Analogue 1 (47%)

METHOD: OECD 414 Prenatal Development Toxicity Study

Species/Strain: Rat/ Crl:CD(SD)

Route of Administration: Oral – gavage

Exposure Information: Exposure days: day 6 to day 17 *post-coitum* inclusive
Post-exposure observation period: 3 days

Vehicle: Corn oil

Remarks - Method: Dose not adjusted for concentration of Analogue 1.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	24	0	0/24
low dose	24	100	0/24
mid dose	24	300	0/24
high dose	24	1000	0/24

Mortality and Time to Death

There were no unscheduled deaths in any group during the study.

Effects on Dams

Ptyalism was observed in females in the 1000 mg/kg bw/day. This finding, although attributed to treatment with the test substance, was not considered to be an adverse effect.

No total resorptions or evidence of abortion were noted in any group. Body weights and food consumption were similar in all groups throughout the study. Post mortem observations revealed no treatment related effects in any treated animals.

Effects on Foetus

Mean number of corpora lutea, implantation sites, resorptions, live foetuses and pore- and post implantation losses were similar in control and treated groups. Additionally, no treatment related external or soft tissue anomalies or malformations were observed in any group.

Reduced ossification of the frontal bone was noted in the 1000 mg/kg bw/day treatment group. This finding was not determined to be an adverse effect by the study authors as this was the only reported finding of skeletal ossification.

Remarks - Results
No treatment related adverse effects were observed in any treatment or control groups.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study for both maternal and embryofetal effects, based on no adverse effects observed at the highest dose tested.

TEST FACILITY CIT (1998a)

B.14. Developmental toxicity

TEST SUBSTANCE A mixture containing Analogue 1 (47%)

METHOD OECD 414 Prenatal Development Toxicity Study
Species/Strain Rabbit/New Zealand White
Route of Administration Oral – gavage
Exposure Information Exposure days: day 7 to day 18 *post-coitum* inclusive
Post-exposure observation period: 10 days
Vehicle 0.5% methylcellulose
Remarks - Method Dose not adjusted for concentration of Analogue 1.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	20	0	0/20
low dose	22	100	2/22
mid dose	22	300	2/22
high dose	23	1000	3/23

Mortality and Time to Death
There were no test substance related deaths in any group during the study. A total of two animals from the low dose group, two from the mid dose group and three females from the high dose group died during the experiment. Effects noted at necropsy included dilation and firmness of the lung, red contents of the trachea or lung. These effects were attributed to regurgitation of the test substance and difficult dosing and were not considered to be related to toxicity of the test substance.

Effects on Dams
There were no treatment-related clinical signs or abortions in any group. There was a decrease in body weight gain noted in the 1000 mg/kg bw/day group during the treatment period (Day 7 to 19) that was considered to be test substance related as it was associated with a slight decrease in food consumption. No other anomalies were noted in macroscopic *post-mortem* examination.

Effects on Foetus
No treatment related effects were noted in numbers of pre- and post-implantation loss, live foetuses or sex ratio. There was a low incidence of malformation noted in all groups. A cleft palate was recorded in the control group and exencephaly or spina bifida was recorded for 2 foetuses in the 100 mg/kg bw/day group. In the 300 mg/kg bw/day group, an umbilical hernia or omphalocele were recorded in two foetuses. In the 1000 mg/kg bw/day group, three foetuses presented exophthalmia, omphalocele or a domes head. Due to the low incidence of these findings and absence of statistical significance, these malformations were not considered to be test substance related.

Remarks - Results
No test substance related embryofoetal or teratogenic effects were observed. There was a transient, minor to slight decrease in food consumption and body weight gain in the 1000 mg/kg bw/day group. Given the

transient and minor nature of these effects, the NOAEL has been established by NICNAS to be 1000 mg/kg bw/day in this study.

CONCLUSION
The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study for both maternal and embryofetal effects, based on no adverse effects observed at the highest dose tested.

TEST FACILITY CIT (2002b)

B.15. Toxicity to reproduction – one generation study

TEST SUBSTANCE A mixture containing Analogue 1 (47%)

METHOD OECD 415 One generation Reproduction study
Species/Strain Rat/Sprague-Dawley Crl:CD(SD) IGS BR
Route of Administration Oral – gavage
Exposure Information Exposure period - female: 15 days before mating, during mating period (max. 21 days), during pregnancy, and until final sacrifice (after day 21 post-partum)
Exposure period - male: 71 days before mating, during mating (max. 21 days) and until sacrifice (after delivery of females)
Vehicle Corn oil
Remarks – Method Dose not adjusted for concentration of Analogue 1.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	12M/24F	0	0
low dose	12M/24F	50	0
mid dose	12M/24F	150	0
high dose	12M/24F	450	0

Mortality and Time to Death
There were no unscheduled deaths in any group during the study.

Effects on Parental (P) animals
Ptyalism was noted in males with a dose-related incidence. There were no adverse bodyweight or food consumption effects in either males or females before or after mating or during lactation. Ptyalism was noted with a dose-related during the treatment period for male animals. This effect was not considered an adverse effect.

The fertility indices, duration of gestation and the number of implantation sights for treated animals were similar to the control group.

Macroscopic post-mortem examination revealed no treatment related effects.

Microscopic examination of genital organs and pituitary glands revealed no treatment related effects.

Effects on 1st Filial Generation (F1)
The sizes of litters at birth and during lactation were similar in control and treatment groups. Body weight at birth and body weight gain for pups in treatment groups were similar to those of the control group. In addition, the sex-ratio was unaffected in treated groups.

Macroscopic post-mortem examination revealed no treatment related effects.

Remarks – Results
Fertility, mating behaviours, gestation and parturition of parent animals were not affected at any dose-level under the conditions of this experiment. Further, no adverse effects were noted in the 1st filial generation.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) for paternal and maternal toxicity and the No Observed Effect Level for effects on fertility and pre- and post natal development was > 450 mg/kg bw/day.

TEST FACILITY CIT (1998b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Activated sludge from wastewater treatment plant
Exposure Period	28 days
Auxiliary Solvent	Disponil NP 9.5 EO 5 PO
Analytical Monitoring	Chemical oxygen demand
Remarks - Method	Biodegradability of the notified chemical was determined at two concentration levels with solvent control for each concnetraiton level. The test was conducted according to the guideline above without significant deviation from the protocol reported.

RESULTS

Day	*Test substance* *% Degradation (ThOD)*		*Day*	*Sodium benzoate* *% Degradation (ThOD)*
	2 mg test substance/L	*5 mg test substance/L*		
7	27	25	7	69
14	47	26	14	73
21	50	33	21	84
28	60	41	28	85

Remarks - Results	The degree of degradability at 28 days was determined to 60% and 41%, respectively, for two test concentrations. However, biodegradation of at least 60% was not reached within a 10-day window. Therefore, the notified chemical is not readily biodegradable. All validity criteria for the test were satisfied.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	Henkel (1992)

C.1.2. Ready biodegradability

TEST SUBSTANCE	A mixture containing Analogue 1 (47%)
METHOD	OECD TG 301 D Ready Biodegradability: Closed Bottle Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	Chloroform
Analytical Monitoring	Chemical oxygen demand (COD)
Remarks – Method	The test substance was dissolved in chloroform to give a stock solution of 560 mg/10ml. Aliquots of this stock solution were placed on filter paper and the chloroform was removed by evaporation. The filter paper containing the test substance was placed in each test bottle prior to filling with inoculated medium. Filter paper controls and positive control of aniline were prepared in the same manner. Sodium benzoate standards were prepared in water solution.

RESULTS

Test substance		*Aniline*		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*	*Day*	*% Degradation*
5	13	5	73	5	72
15	32	15	76	15	97
28	31	28	78	28	95

Remarks – Results: Percentage of biodegradation was calculated based on oxygen depletion divided by COD for the test substance. Percentage of biodegradation for aniline and sodium benzoate were calculated based on oxygen depletion divided by concentration of substance × ThOD NO_3. All validity criteria for the test were satisfied.

CONCLUSION: The test substance and, by inference, the notified chemical are not readily biodegradable

TEST FACILITY: Safepharm (1991b)

C.1.3. Bioaccumulation

TEST SUBSTANCE: A mixture containing Analogue 1 (47%)

METHOD: OECD TG 305 Bioconcentration: Flow-through Fish Test.

Species: Bluegill sunfish (*Leopomismacrochirus*)
Exposure Period: Exposure: 28 days Depuration: 28 days
Auxiliary Solvent: Ethylene glycol monomethyl ether
Concentration Range: Nominal: 2 and 20 µg/L for low dose and high dose, respectively, for the ^{14}C-labelled Analogue 1.
Actual: average of 1.56 and 9.02 µg/L for low dose and high dose, respectively, for the ^{14}C-labelled Analogue 1.
Analytical Monitoring: Packard liquid scintillation counters
Thin layer chromatography
Remarks - Method: Analogue 1, contained in the test mixture, was radioactively labelled in the test. The test was conducted in accordance with the above guidelines. Two dose levels were prepared from the stock solution of ^{14}C-labelled Analogue 1 by dilution in ethylene glycol monomethyl ether to a daily amount of 5 mg/25 mL (high dose of 20 µg/L) and 0.5 mg/25 mL (low dose of 2 µg/L). The fish were continuously exposed to the ^{14}C-labelled Analogue 1 at an average low dose concentration of 1.56 µg/L and an average high dose concentration of 9.02 µg/L for 28 days at 22-23 °C , pH 8.2-8.5 and an oxygen concentration of 7.3-8.6 mg/L. During 28 days depuration (29 – 56 days) the respective values were 22-23 °C, 8.2-8.5 and 7.4-8.3 mg/L. The fishes were weighed and solubilised at 50 °C for 24-48 days and the fish lipid residue was measured by liquid scintillation counting.

RESULTS
Bioconcentration Factor: Average 92 ± 19 in whole fish.
CT50: 9 to 20 days
Remarks - Results: Plateau levels were reached after 14 days for both low dose and high dose, with a depuration half-life of 9 and 20 days, respectively. Based on the total radioactivity levels in exposure water and the plateau levels in fish, the Bioconcentration Factor (BCF) value amounted to 55 for low dose and to 75 for the high dose. Based on the kinetic data, the BCF values were calculated to be 82 for the low dose and 85 for the high dose. The BCF value for the ether component in Analogue 1 in fish was consider to be 74 ± 13, the mean values of the above values (55, 75, 82 and 85). Taking into account the actual concentration of the Analogue 1 in the exposure water, the average BCF in fish for Analogue 1 is calculated to be 92 ± 19. When based on lipid content the overall lipid

BCF amounted to 5320 ± 1580 µg/L.

Validity criteria for the test were satisfied with no significant deviation from the guidelines.

CONCLUSION Analogue 1 and, by inference, the notified chemical have limited potential for bioaccumulation

TEST FACILITY RCC (2005a)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE A mixture containing Analogue 1 (47%)

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi-static.

Species	Rainbow trout (*Oncorhynchusmykiss)*
Exposure Period	96 hours
Auxiliary Solvent	1% Tween 80-tetrahydrofuran
Water Hardness	50 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography
Remarks – Method	Tween 80 was used during the range-finding experiment, however, the use of 1% Tween 80-tetrahydrofuran was found to give the best testable dispersion of the test substance in water. Test substance (1.0 g) was dispersed in 1% Tween 80-tetrahydrofuran and the volume was adjusted to 10 ml. The test solutions were prepared by dilution of the above stock solution. Tests were conducted for one test concentration of 10 mg/L in duplicate, the highest test concentration that could be prepared due to the limited solubility of the test substance in water and auxiliary solvent. The fish test media were renewed daily. The concentration of test substance was verified by chemical analysis at 0, 24 and 96 hours.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*				
Nominal	*Actual*		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	-	20	0	0	0	0	0
10	9.52-10.1	20	0	0	0	0	0

LC50 > 10 mg/L at 96 hours

Remarks – Results The LC50 value was calculated in terms of nominal test concentrations as the measured test concentration from analysis at 0, 24 and 96 hours were close to the nominal concentration. Validity criteria for the test were satisfied with no significant deviation from the guidelines.

CONCLUSION The test substance and, by inference, the notified chemical are not harmful to fish up to the limit of their water solubilities

TEST FACILITY Safepharm (1991c)

C.2.2. Growth test for fish

TEST SUBSTANCE A mixture containing Analogue 1 (47%)

METHOD OECD TG 215 Fish, Juvenile Growth Test – Semi-Static

Species	Carp *(Cyprinuscarpio)*
Exposure Period	28 days

Auxiliary Solvent	None
Water Hardness	Total hardness 150 ± 20 mg $CaCO_3$/L
Analytical Monitoring	Gas liquid chromatography with Flame Ionisation detection
Remarks - Method	The test substance was ground down to fine particles and directly added to the test flask to prepare the test solutions at the loading rate of 200 mg/L. Two 72-hour preliminary assays were carried out to check the stability of the test substance for solutions at the limit of solubility and at the loading rate of 200 mg/L. Based on the results of the stability assays, chemical analysis was not undertaken during the toxicity test performed at the loading rate of 200 mg/L. The solubilised fraction was found to be stable at 0.062 mg/L at 0 hour and 0.069 mg /L at 72 hours. Test solutions were changed every 2 or 3 days.

RESULTS

Concentration mg/L		*Number of Fish*	*Growth rate on 28 days*	*Weight increase on 28 days (%)*
Nominal	*Actual*			
0	-	40	5.29	110
200	0.06	40	5.98	131

NAOEC (growth rate)	≥ 0.06 mg/L at 28 days
Remarks - Results	No mortality or sub-lethal effects were observed in any replicate of the control and test solutions throughout the test. The 28-day LC50 was calculated to be greater than 0.06 mg/L. The test was conducted on Carp, which is not on the list of fish species recommended by the test guideline above. Therefore, these results should be treated with cautions. Validity criteria for the test were satisfied with no significant deviation from the guidelines.
CONCLUSION	The test substance and, by inference, the notified chemical are not harmful to fish up to the limit of their water solubilities.
TEST FACILITY	CIT (2000a)

C.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	A mixture containing Analogue 1 (47%)
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test - Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	1% Tween 80-tetrahydrofuran
Water Hardness	50 mg $CaCO_3$/L
Analytical Monitoring	Gas chromatography
Remarks - Method	Test substance (1.0 g) was dispersed in 1% Tween 80-tetrahydrofuran and the volume was adjusted to 10 ml. One test concentration of 10 mg/L, the highest concentration that could be prepared due to the limited solubility of the test substance in water and auxiliary solvent, was prepared by dilution of the above stock solution. The concentration of test substance was verified by chemical analysis at 0 and 48 hours.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	-	40	0	0
10	6.77-10.31	40	0	0

EC50	> 6.9 mg/L at 48 hours
NOEC	≥ 6.9 mg/L at 48 hours
Remarks - Results	The test concentration at 48 hours decreased significantly (more than 20%). This decrease was concluded to be due to adherence of the test substance to the test vessels. Therefore, for the worst case scenario, the mean measured test concentration of 6.9 mg/L at 48 hours is suggested to be taken as the true exposure level. Validity criteria for the test were satisfied with no significant deviation from the guidelines.
CONCLUSION	The test substance and, by inference, the notified chemical are not harmful to aquatic invertebrates up to the limit of their water solubilities
TEST FACILITY	Safepharm (1991d)

C.2.4. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	A mixture containing Analogue 1 (47%)
METHOD	OECD TG 211 *Daphnia magna* reproduction test
Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	None
Water Hardness	Total hardness 238-289 mg $CaCO_3$/L
Analytical Monitoring	Gas liquid Chromatography with Flame Ionisation detection
Remarks - Method	The test substance was ground down to fine particles and directly added to the test flask to prepare the stock solution with a loading rate of 1 mg/L, well above its solubility in the test media. The stock solution was agitated for 20-24 hours, followed with filtration. The filtered solution, corresponding to the limit of solubility of the two components in the test substance, was used as the highest test concentration of 9.00 mg/L. Other test solutions with a lower concentration were prepared by dilution of this saturated solution. Daphnia test solutions were changed every 2 or 3 days. Immobilization of adults and number of dead and living neonates were recorded daily throughout the test.

	Day 21		
Loading rate (mg/L)	*Mean Percent % Adult Survival*	*Mean Number of Living Offspring Produced per female – cumulative*	*Total number of offspring produced per female (living and dead offspring)*
Control	83.3	67.4	68.6
0.09	91.6	63.4	63.5
0.285	91.6	64.7	65.3
0.90	91.6	63.8	64
2.85	83.3	67.2	67.3
9.00	100	67.9	68.3

EC50 (reproduction)	> 0.009 mg/L at 21 days (dissolved fraction of the test substance in test media)
NOEC	≥ 0.009 mg/L at 21 days
Remarks - Results	The solubilised fraction of test substance was stable under the toxicity text experimental conditions for at least 3 days. Validity criteria for the test were satisfied with no significant deviation from the guidelines.
CONCLUSION	The test substance and, by inference, the notified chemical do not have long term effects to aquatic invertebrates up to the limit of their water solubilities.

TEST FACILITY CIT (2000b)

C.2.5. Algal growth inhibition test

TEST SUBSTANCE A mixture containing Analogue 1 (47%)

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	*Pseudokirchneriellasubcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 200 mg/L Actual: 0.07 mg/L
Auxiliary Solvent	None
Water Hardness	34 ± 17 mg $CaCO_3$/L
Analytical Monitoring	Gas liquid chromatography
Remarks - Method	The test substance was ground down to fine particles and directly added to the test flask to prepare the test solutions at the loading rate of 200 mg/L. Two 72-hour preliminary assays were carried out to check the stability of the test substance for solutions at the limit of solubility and at the loading rate of 200 mg/L. Based on the results of the stability assays, chemical analysis was not undertaken during the toxicity test performed at the loading rate of 200 mg/L. The solubilised fraction of test substance was stable in algal test media under the toxicity text experimental conditions for at least 72 hours.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
< 0.07	< 0.07	> 0.07	< 0.07

Remarks - Results Validity criteria for the test were satisfied with no significant deviation from the guidelines.

CONCLUSION The test substance and, by inference, the notified chemical are not harmful to algae up to the limit of their water solubilities.

TEST FACILITY CIT (2000c)

C.2.6. Algal growth inhibition test

TEST SUBSTANCE A mixture containing Analogue 1 (47%)

METHOD OECD TG 201 Alga, Growth Inhibition Test.

Species	*Pseudokirchneriellasubcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1 mg/L Actual: 0.012 mg/L
Auxiliary Solvent	None
Water Hardness	34 ± 17 mg $CaCO_3$/L
Analytical Monitoring	Gas liquid chromatography with Flame Ionisation detection
Remarks - Method	The test substance was ground down to fine particles before weighing to prepare a stock solution with a loading rate of 1 mg/L, well above the solubility limit of the test substance. This stock solution was agitated for 23 hours followed with filtration to remove the undissolved fraction. This filtered solution, corresponding to the limit of solubility (LS) of the test substance under the experimental conditions, was used in the limit test.

Two other test solutions with lower concentration were prepared by dilution of this saturated LS solution.

The test substance was stable for at least 72 hours under experimental conditions. The concentration of test substance in the LS solution was therefore measured only at the beginning of the assay.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC mg/L*	*E_rC50 mg/L at 72 h*	*NOEC mg/L*
> 0.012	≥ 0.012	> 0.012	≥ 0.012

Remarks - Results: Validity criteria for the test were satisfied with no significant deviation from the guidelines.

CONCLUSION: The test substance and, by inference, the notified chemical are not harmful to algae with no long term effects up to the limit of their water solubilities.

TEST FACILITY: CIT (2000d)

C.2.7. Inhibition of microbial activity

TEST SUBSTANCE: A mixture containing Analogue 1 (47%)

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test.

Inoculum: Activate Sludge
Exposure Period: 3 hours
Concentration Range: Nominal: 1, 3.16, 10, 31.6 and 100 mg/L
Actual: not determined
Remarks – Method: Conducted according to the guidelines above with no significant deviations from the protocol.

RESULTS
EC50: > 100 mg/L
NOEC: Not determined
Remarks – Results: All validity criteria for the test were satisfied. No significant inhibition of respiration rate of the sludge was recorded at 100 mg/L for the test substance. The IC50 for reference substance of 3,5-dichlorophenol is determined to be 13 mg/L, which was in the required range.

CONCLUSION: The test substance and, by inference, the notified chemical are not expected to inhibit microbial respiration

TEST FACILITY: CIT (1997)

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