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August 2017

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Glycine, *N*-(1-oxododecyl)-, sodium salt (1:1) (INCI Name: Sodium Lauroyl Glycinate)

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

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

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

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/1590	Clariant(Australia) Pty Ltd Unilever Asia Private Limited	Glycine, *N*-(1-oxododecyl)-, sodium salt (1:1) (INCI Name: Sodium Lauroyl Glycinate)	Yes	≤ 150 tonnes per annum	Ingredient in rinse off cosmetic products

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*
Skin irritation (Category 2)	H315 – Causes skin irritation
Eye damage (Category 1)	H318 – Causes serious eye damage

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

Hazard classification	*Hazard statement*
Acute Category 3	H402 – Harmful to aquatic life

Human health risk assessment
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.

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

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

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin irritation (Category 2): H315 – Causes skin irritation
  - Eye damage (Category 1): H318 – Causes serious eye damage

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

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - 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:
  - Coveralls
  - Impervious gloves
  - Safety goggles

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

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

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

Disposal

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

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - the concentration of the notified chemical exceeds 5% in rinse off cosmetic products;
  - the notified chemical is used in leave on cosmetic products.

or

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

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

(Material) Safety Data Sheet
The (M)SDS of the notified chemical in solution 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

APPLICANTS
Clariant (Australia) Pty Ltd (ABN: 30 069 435 552)
296-324 Ferntree Gully Road, Level 3, 3 Acacia Place
NOTTING HILL VIC 3168

Unilever Asia Private Limited (ABN: 29 142 738 538)
219 North Rocks Road
NORTH ROCKS NSW 2151

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: analytical data, degree of purity, impurities and import volume.

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 melting point, boiling point, density, water solubility and partition coefficient and all toxicological endpoints except *in vitro* genotoxicity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Canada (2010), Europe (2014), Japan (2006) and Philippines (2006)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Hostapon SLG (solution containing < 25% notified chemical)

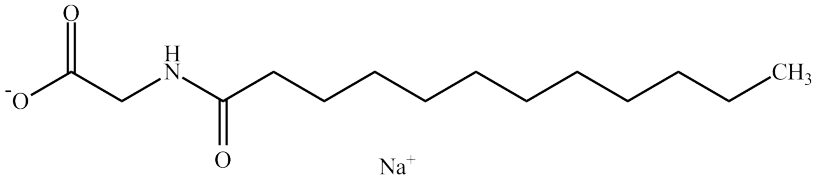
CAS NUMBER
18777-32-7

CHEMICAL NAME
Glycine, *N*-(1-oxododecyl)-, sodium salt (1:1)

OTHER NAMES
Sodium Lauroyl Glycinate (INCI name)
Reaction products of fatty acid chlorides, C8-12 (even numbered) with glycine and sodium hydroxide
Glycine, *N*-(1-oxododecyl)-, monosodium salt (9CI)
Glycine, *N*-lauroyl-, monosodium salt (7CI,8CI)
Glycine, *N*-lauroyl-, sodium salt (6CI)
N-Lauroylglycine sodium salt
Sodium *N*-lauroylglycinate
Sodium *N*-lauroylglycine
Sodium dodecanoylglycinate
Sodium β-lauroylaminoacetate

MOLECULAR FORMULA
$C_{14}\ H_{27}\ N\ O_3\ .\ Na$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
279.35 Da

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

3. COMPOSITION

DEGREE OF PURITY
40-60%

4. ANALOGUE IDENTITY

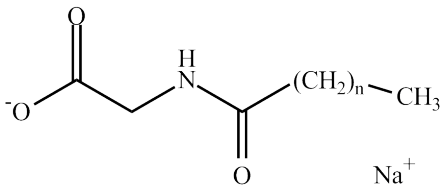
CHEMICAL NAME
Glycine, N-coco acyl derivs., sodium salt (INCI Name: Sodium cocoyl glycinate)

OTHER NAME
SCG 3028

CAS NUMBER
90387-74-9

MOLECULAR FORMULA
Unspecified

STRUCTURAL FORMULA



where n = 6-14

MOLECULAR WEIGHT
Unspecified

The notified chemical is similar to the analogue except for the proportion of fatty acids with different length of side chains. The notified chemical is a dodecanoic acid (C12) derivative with C8 and C10 impurities, whereas the analogue is a mixture of fatty acids (C8-C16) reacted with glycine. The analogue contains approximately 60% of the C12 component, which is the notified chemical.

5. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: pale yellow liquid (25% solution)

Property	Value	Data Source/Justification
Melting Point/Freezing Point	143-174 °C	Measured
Boiling Point	Not determined	Measured. The notified chemical decomposes before reaching boiling point

Property	Value	Data Source/Justification
Density	1,340 kg/m^3 at 28.1 °C	Measured
Vapour Pressure	2.3 kPa at 20 °C	(M)SDS
Water Solubility	2.0 ± 0.3 g/L at 20 °C 1.4 ± 0.2 g/L at 20 °C (active content)	Measured (critical micelle concentrations)
n-octanol Solubility	380 mg/L n-octanol at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionality, however, hydrolysis is expected to be slow in the environmental pH range (4–9) at ambient temperature.
Partition Coefficient (n-octanol/water)	log Pow = -0.57 at pH 6.8	Based on water and octanol solubilities
Surface Tension	30.3 ± 0.5 mN/m at 20 °C	Measured
Adsorption/Desorption	Not determined	Expected to adsorb to soil and sediment based on surface activity
Dissociation Constant	Not determined	The notified chemical is expected to be ionised over the environmental pH range (4–9).
Flash Point	> 100 °C	(M)SDS
Flammability	Not determined	-
Autoignition Temperature	Not determined	-
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties

DISCUSSION OF PROPERTIES
All the physical and chemical property studies were carried out on dry powder form of the notified chemical. For full details of tests on physical and chemical properties, refer to Appendix A.

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

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

6. 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 in solution at ≤ 25% concentration for reformulation in Australia into end-use cosmetic products, or in finished cosmetic products.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 150	< 150	< 150	< 150	< 150

PORT OF ENTRY
Melbourne, Sydney

IDENTITY OF RECIPIENTS
Clariant (Australia) Pty Ltd
Unilever Asia Private Limited

TRANSPORTATION AND PACKAGING
The notified chemical will be imported by air or sea in 200 L drums or 1000 L intermediate bulk containers (IBCs). The notified chemical will be transported by road from the port of entry to the warehouse for storage and

to the site of reformulation. Where imported in finished cosmetic products, these will be transported to warehouses and then to retail outlets for sale.

USE
The notified chemical will be used in rinse-off cosmetic skin and hair cleansing products. The final concentration of the notified chemical in end-use cosmetic products will be ≤ 5%.

OPERATION DESCRIPTION
The notified chemical will be imported as a component of finished cosmetic products, or at up to 25% for reformulation into cosmetic products for skin and hair cleansing. At the site of reformulation, the product containing the notified chemical will be manually transferred from either 200 L drums or 960 kg IBCs into the reformulation vessels for formulation of the final cosmetic products. Mixing and dispensing will be carried out in closed systems or under conditions designed not to generate aerosols or airborne dust. After reformulation, the final cosmetic products will be pumped into sealed holding tanks and then transferred into automated filing line via a circuit of pipes and pumps. The end-use cosmetic products will then be filled into consumer size packages of various sizes (200 – 1000 ml) for distribution to retailers. Samples will be collected at various stages during the reformulation and packaging for quality control testing.

7. HUMAN HEALTH IMPLICATIONS

7.1. Exposure Assessment

7.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Stevedores	2-3	10-15
Transport	6	260
Warehousing	6	260
Reformulation process workers	4	260
Quality assurance workers	4	260
Maintenance workers and cleaners at reformulation site	1	260
Retail workers	1	260
Professional users (e.g. beauticians)	1	260

EXPOSURE DETAILS

Transport and Storage
Transport and storage workers may come in to contact with the notified chemical at ≤ 25% concentration when handling the imported formulations in the event of a spill or rupture of container. The primary work activity undertaken by the workers will be loading and off-loading of containers. Incidental exposure to the notified chemical may occur via skin or eye during the clean-up of accidental spills.

Reformulation
Workers involved in reformulation, quality control and equipment cleaning and maintenance may come into contact with the notified chemical at concentrations up to 25% during transfer of the notified chemical in to mixing vessel, sample collection for quality control, packaging of end products, equipment cleaning and maintenance. The frequency and duration of exposure will vary depending on the role. The principal routes of exposure are expected to be dermal, ocular and inhalation.

Retail and Professional Use
Retail workers may come into contact with the notified chemical at ≤ 5% concentration when handling the end-use products in the event of a leak or rupture of container. Incidental exposure to the notified chemical may occur via skin or eye during the clean-up of accidental spills.

The notified chemical will be used in rinse-off cosmetic products. Beauty care professionals may come into contact with the notified chemical during the use of the products containing the notified chemical on customers.

The principal route of exposure will be dermal and ocular. Inhalation exposure is not expected due to the nature of product and method of application.

7.1.2. Public Exposure

There will be widespread and repeated exposure of the public to the notified chemical through the use of rinse-off cosmetic products containing the notified chemical at concentrations ≤ 5%. The notified chemical is proposed to be used in rinse-off skin and haircare cleansing products and thus the primary route of exposure will be dermal. Accidental ocular and oral exposure may also occur due to the nature of use.

7.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and the analogue 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 at 25%*	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity*	LD50 > 2,000 mg/kg bw; low toxicity
Skin irritation (*in vitro* RHE test)*	non-irritating
Rabbit, skin irritation at 25%*	irritating
Eye irritation (*in vitro* BCOP test)*	very severely irritating
Rabbit, eye irritation at 25%*	slightly irritating
Guinea pig, skin sensitisation – Magnusson & Kligman maximisation test*	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days*	NOAEL 1,000 mg/kg bw/day NOEL 250 mg/kg bw/day
Mutagenicity – bacterial reverse mutation*	non mutagenic
Genotoxicity – *in vitro* mammalian chromosome aberration test	non genotoxic
Genotoxicity – *in vitro* mammalian cell gene mutation test*	non genotoxic

* – study conducted on analogue

Toxicokinetics, metabolism and distribution

No information on toxicokinetics, metabolism and distribution was provided on the notified chemical. Based on the low molecular weight (280 Da) and derived partition coefficient (log Pow = -0.57 at pH 6.8), the notified chemical is likely cross biological membranes. The notified chemical is a surfactant and is likely to have toxicokinetics similar to other surfactants such as N-acyl derivatives of sarcosinate (CIR 2001).

Acute toxicity

An acute oral toxicity study was conducted on the analogue chemical at 25% concentration. The LD50 of the test substance was determined to be > 2,000 mg/kg body weight.

An acute dermal toxicity study was conducted on the analogue chemical. The LD50 of the test substance was determined to be > 2,000 mg/kg body weight.

Irritation

Both *in vitro* and *in vivo* skin and eye irritation studies, all conducted according to the OECD test guidelines were provided on the analogue chemical. In the *in vivo* studies at 25% concentration the test substance was irritating to the skin and slightly irritating to eyes, whereas in the *in vitro* studies the test substance at 68% indicated no skin irritation but very severe eye irritation.

Skin irritation

Erythema and oedema were seen in the *in vivo* skin irritation study at 25%. The severity of these effects alone were not sufficient for classification, however the scaling which persisted till the end of the study period (day 14) was sufficient for classification of the test substance as irritating to the skin (Category 2) under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)*, as adopted for industrial chemicals in Australia. No significant skin irritation effects were observed in the *in vitro* study at 68%. One reason for the difference may be the scope of the *in vitro* protocol, which covers the initial step of the inflammatory cascade that occurs during irritation *in vivo* (OECD, 2010), but does not measure persistence of the irritation effects, such as scaling. It is also noted that the test substance was tested as a solid in the *in vitro* study, and the very low irritation scores (similar to those of the negative control), are inconsistent with the erythema and oedema seen in the *in vivo* study and the local irritation effects observed in the acute dermal toxicity study in rats where irritation effects including slight erythema, oedema and maculated crusts were observed. This suggests that the solid form

may have had lower bioavailability in the *in vitro* study which may be due to the lower contact with test skin, underestimating the irritation potential. On a weight of evidence basis, the test substance is considered to be irritating to the skin.

Eye irritation
Eye irritation effects were observed at 25% in the *in vivo* study but were not sufficient for classification under the GHS. Irritation effects were also observed in the *in vitro* study carried out at a higher concentration of 68%, and were sufficient to classify the test substance as severely irritating (Category 1) to the eyes under the GHS. In the *in vitro* assay, the test substance was diluted to 20% as part of the test protocol. It is considered that this step (which is not part of the *in vitro* skin irritation test protocol) would maintain the bioavailability of the test substance. On the weight of evidence, the test substance is considered to be severely irritating to the eyes and classified as Category 1 for eye damage under the GHS.

Sensitisation
A skin sensitisation study using the Magnusson and Kligman method was conducted on the analogue chemical at 25% concentration. No evidence of sensitisation was observed.

Repeated dose toxicity
A 28-day repeated dose oral toxicity was conducted using the analogue chemical. The study doses were 62.5, 250 and 1,000 mg/kg bw/day. Two animals from the high dose group were found dead on day 9 and 11; the deaths were attributed to aspiration of the test substance. Microscopic effects were observed in the forestomach (ulcerations) of test animals from 1,000 mg/kg bw/day group. These effects were not seen in the recovery group suggesting they were reversible. The effects seen were local and were probably due to the irritation effects of the notified chemical. No other marked changes were noted. The No Observed Effect Level (NOEL) as 250 mg/kg bw/day, and the No Observed Adverse Effect Level (NOAEL) as 1,000 mg/kg bw/day.

Mutagenicity/Genotoxicity
The notified chemical was found to be non-clastogenic in an *in vitro* mammalian chromosome aberration test conducted using Chinese Hamster Ovary cells. The analogue chemical was negative in an *in vitro* bacterial reverse mutation study and an *in vitro* mammalian gene mutation test.

The above genotoxicity studies conducted on the notified chemical and the analogue suggest the notified chemical to be non-genotoxic.

Health hazard classification
Based on the available information on the analogue chemical, 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***
Skin irritation (Category 2)	H315 – Causes skin irritation
Eye damage (Category 1)	H318 – Causes serious eye damage

7.3. Human Health Risk Characterisation

7.3.1. Occupational Health and Safety
The notified chemical is expected to cause serious eye damage and to be irritating to the skin, based on studies conducted on a close analogue.

Reformulation
Dermal and accidental ocular exposure of workers to the notified chemical at up to 25% concentration may occur during reformulation. Due to the skin and eye irritation effects, caution should be exercised when handling the notified chemical. The notifier advised that inhalation exposure to the notified chemical is unlikely due to the processes being carried out in closed system under conditions designed not to create aerosols or air-born dust. The use of proper PPE including impervious gloves, coveralls and googles will minimise worker exposure. Therefore, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

Professional End-use
Beauty care professionals will handle the notified chemical at ≤ 5% concentration, similar to public use. Therefore, the risk to workers who regularly use products containing the notified chemical is expected to be of a similar or lesser extent than that experienced by members of the public who use such products on a regular basis. For details of the public health risk assessment see Section 7.3.2.

7.3.2. Public Health
The notified chemical has skin and eye irritation potential. The public will have widespread and repetitive exposure to the notified chemical at concentrations ≤ 5% via the dermal and perhaps ocular routes during the use of rinse-off cosmetic products.

However the skin and eye irritation effects are expected to be reduced due to the low concentration of the notified chemical (≤ 5%) and the use in rinse-off products which involve dilution and reduced skin contact time. When used in the proposed manner, the risk to the public associated with the notified chemical is not considered to be unreasonable.

8. ENVIRONMENTAL IMPLICATIONS

8.1. Environmental Exposure & Fate Assessment

8.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported into Australia as a component of cosmetic formulations or at up to 25% for reformulation in Australia. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills or leaks. In the event of spills, the product containing the notified chemical is expected to be collected 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 end-use 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. Wastes may be collected and released to sewers in a worst case scenario, or disposed of to landfill in accordance with local government regulations.

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

RELEASE OF CHEMICAL FROM DISPOSAL
A small proportion of the notified chemical may remain in 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, or to be released to sewer when containers are rinsed before recycling through an approved waste management facility.

8.1.2. Environmental Fate
Following its use in Australia, the majority of the notified chemical is expected to enter the sewer system through its use in cosmetic products, before potential release to surface waters nationwide. Based on the results of a ready biodegradability study on analogue of the notified chemical, the notified chemical is considered to be readily biodegradable (85% degradation in 28 days). In a Simulation Test on the analogue of the notified chemical, conducted according to OECD Test Guideline 303 A, the mean elimination rate of the total influent concentration of the test substance was calculated to be 97.77% in a continuously operating activated sludge unit (Dr.U.Noack-Laboratorien 2015). For details of the environmental fate studies, 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, oxides of carbon and nitrogen and salts.

The majority of the notified chemical will be released to sewer after use. A small proportion of the notified chemical may be applied to land when effluent is used for irrigation, or when sewage sludge is used for soil remediation. The notified chemical may also be disposed of to landfill as collected spills and empty container residue. Residues of the notified chemical in landfill, soil and sludge are expected to eventually degrade through biotic and abiotic processes to form water, oxides of carbon and nitrogen and salts.

8.1.3. Predicted Environmental Concentration (PEC)

A predicted environmental concentration (PEC) was calculated assuming that all of the total import volume of notified chemical will be released to sewers with removal of the notified chemical by sewerage treatment plants (STPs) calculated by SimpleTreat (European Commission, 2003). It is assumed the release of the notified chemical will occur over 365 days per annum into the total Australian effluent volume.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import Volume	150,000	kg/year
Proportion expected to be released to sewer	100%	
Annual quantity of chemical released to sewer	150,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	410.96	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	22.613	million
Removal within STP	97%	
Daily effluent production:	4,523	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	2.73	µg/L
PEC - Ocean:	0.27	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 2.73 µg/L may potentially result in a soil concentration of approximately 0.018 mg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 0.09 mg/kg and 0.18 mg/kg, respectively.

8.2. Environmental Effects Assessment

No ecotoxicity data for the notified chemical were submitted. The results from an ecotoxicological investigation conducted on the analogue of the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C. The fish toxicity study was found to be not valid and not reliable due to the test substance precipitating out from the solution during the study period, and therefore the result from the study was not used in this risk assessment.

Endpoint	*Result*	*Assessment Conclusion*
Daphnia Toxicity	EC50 = 33 mg/L	Harmful to aquatic invertebrates
Algal Toxicity	ErC50 = 89.9 mg/L	Harmful to algae

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

8.2.1. Predicted No-Effect Concentration

The predicted no-effects concentration (PNEC) has been calculated from the most sensitive ecotoxicological endpoint for aquatic invertebrates. A safety factor of 500 was used as two reliable acute ecotoxicological endpoints were available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (aquatic invertebrates)	33	mg/L
Assessment Factor	500	
PNEC:	66	µg/L

8.3. 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	2.73	66	0.041
Q - Ocean	0.27	66	0.004

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 readily biodegradable, and is not expected to be bioaccumulative. On the basis of the PEC/PNEC ratio, maximum annual importation volume and assessed use pattern in cosmetic products, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 143-174 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Measured by differential scanning calorimetry. Two samples were tested.
Test Facility	Clariant (2013a)

Boiling Point Could not be determined

Method	OECD TG 103 Boiling Point.
Remarks	Measured by differential scanning calorimetry. The boiling point of the test substance could not be determined as it decomposed before reaching the boiling point. The onset of decomposition was 290 °C.
Test Facility	Clariant (2013b)

Density 1,340 kg/m^3 at 28.1 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Determined using gas pycnometer method.
Test Facility	Clariant (2013c)

Water Solubility 2.0 ± 0.3 g/L at 20 °C
1.4 ± 0.2 g/L at 20 °C (active content)

Method	ISO 4311
Remarks	Water solubilities, reported as critical micelle concentrations, were determined from a plot of surface tension versus concentration. The surface tensions were determined by the plate method.
Test Facility	Clariant (2013d)

Fat (or n-octanol) Solubility 380 mg/L n-octanol at 20 °C

Method	OECD TG 105: Water solubility
Remarks	Guideline was adapted for n-octanol. After a preliminary test the flask method was used. Three flasks containing approximately 10 g/L test substance in n-octanol were stirred for 5 h at 30 °C. Subsequently the flasks were stirred at 20 °C for 1 day, 2 days and 3 days respectively. After each day the content of one flask was filtered through a 0.45 µm membrane filter. The content of the test substance in the n-octanol phase was determined by HPLC.
Test Facility	Clariant (2013e)

Partition Coefficient (n-octanol/water) log Pow = -0.57 at 20 °C

Method	OECD TG 105 and ISO 4311
Remarks	Calculated based on individual solubilities of the notified chemical in n-octanol (0.38 g/L) and water (1.4 g/L).
Test Facility	Clariant (2013f)

Surface Tension 30.3 ± 0.5 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions.
Remarks	Concentration: 1 g/L. Result based on the mean of duplicate measurements.
Test Facility	Clariant (2013g)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Analogue (25% concentration)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method (2001).
Species/Strain	Rat/HanRcc: WIST(SPF)
Vehicle	Purified water
Remarks - Method	No significant deviations from the OECD guidelines.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	2,000	0/3
2	3 F	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	No clinical signs were observed during the course of the study.
Effects in Organs	No macroscopic findings were recorded at necropsy.
Remarks - Results	The body weight of the animals was within the range commonly recorded for this strain and age.
CONCLUSION	The test substance is of low toxicity via the oral route.
TEST FACILITY	Harlan (2008)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Analogue (68.5% concentration)
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test (1987).
Species/Strain	Rat/RccHan: WIST(SPF)
Vehicle	Polyethylene glycol 300
Type of dressing	Semi-occlusive.
Remarks - Method	No significant deviations from the OECD guidelines. The test substance was applied for 24 hrs. The test animals were re-shaved on day 8 to facilitate the reading of the local reactions. Observations were made for 14 days after treatment.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 F & 5 M	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	Very slight to slight erythema and oedema or slightly maculated crusts were observed in 1 male and 2 female rats. Slight to moderate desquamation was observed in 2 male and 4 female rats at different intervals between test day 3 and day 14. White to yellow discolouration of the treated skin was noted in 2 male and 1 female rats on test day 2. At the end of the observation period, the only effect remaining was maculated crusts in 1 female rat.
Signs of Toxicity - Systemic	None reported
Effects in Organs	None reported
Remarks - Results	One female rat had a slight body weight loss during the first week of treatment, but regained weight until the end of observation period. For the other animals, body weight gain was as expected.

No macroscopic findings were observed at necropsy.

CONCLUSION The test substance is of low toxicity via the dermal route.

TEST FACILITY Harlan (2010)

B.3. Irritation – skin (*in vitro*)

TEST SUBSTANCE Analogue (67.9% active content) in powder form

METHOD OECD TG 439 *In vitro* Skin Irritation (2010) – Reconstructed Human *Epidermis* Test Method: EPISKIN-SM™ Reconstituted three dimensional human epidermis model

Vehicle None. The skin model was moistened with 5 µL sterile water before application of the test substance.

Remarks - Method No significant deviations from the OECD guideline, however it was not clear whether the powder was ground before use, as recommended in the TG. Phosphate buffered saline was used as negative control and 5% sodium dodecyl sulfate was used as positive control. 10mg of test substance in solid form was applied to the tissues. The exposure period was 15 mins and the post-exposure incubation duration was 42 hours. The test substance did not reduce MTT, which is used to measure the cell viability.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	1.233	100	4.8
Test substance	1.383	112.1	2.5
Positive control	0.096	7.8	0.4

OD = optical density; SD = standard deviation

Remarks - Results The relative mean viability of the test substance treated tissue was above the cut-off value (≤ 50%) for classification as an irritant. The degree of irritation shown was extremely low and was similar to the degree of irritation of the negative control.
The positive control gave satisfactory results and the optical density of the negative control was within the acceptable range, confirming the validity of the assay.

CONCLUSION The test substance was non-irritating to the skin under the conditions of the test.

TEST FACILITY BSL (2012a)

B.4. Irritation – skin

TEST SUBSTANCE Analogue (25% concentration)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 1 M, 2 F
Vehicle None
Observation Period 14 days
Type of Dressing Semi-occlusive.
Remarks - Method No significant deviations from the OECD guidelines. 0.5 mL of undiluted test substance was applied for 4 hrs. No necropsy was performed at the end of the study.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1.3	1.7	2	2	< 10 days	0**
Oedema	1	1.3	1	2	< 7 days	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
**Note comments below re scaling.

Remarks - Results	No mortality occurred and no clinical signs were observed. A well-defined erythema and very slight to slight oedema was observed in all animals 1 hour after test substance exposure. These effects had resolved by day 10 in one female and by day 7 in the other two test animals. Dry/inelastic skin was recorded 24 hours after removal of the dressing in all animals and persisted up to the 72-hour reading in the male, and to the day 7 post treatment reading in the females. Scaling of the skin was noted in all animals at 48 h or 72 hours or on day 7, and persisted to day 14 in the two female animals. All the effects except scaling reversed by day 10.
CONCLUSION	The test substance is irritating to the skin.
TEST FACILITY	Harlan (2008)

B.5. Irritation – eye (*in vitro*)

Test Substance	Analogue (67.9% active content) in powder form
Method	OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants (2009)
Vehicle	Saline (0.9% W/V sodium chloride solution)
Remarks - Method	No significant deviations from the OECD guideline. The TG suggests 10% dilution for surfactants. The chemical was tested at a dilution of 20% in saline solution, possibly to compensate for the concentration of the test substance itself (67.9%). The positive control used was 20% imidazole in saline.

Results

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0.67	0.023	1.02
*Test substance**	211.00	2.145	242.15
*Positive control**	199.67	2.021	228.97

SD = Standard deviation; IVIS = *in vitro* irritancy score
*Corrected for background values

Remarks - Results	The controls gave satisfactory results confirming the validity of the test system. The IVIS score of 242.15 was comparable to that of the positive control and indicated very severe eye irritation potential.
Conclusion	The test substance was a very severe eye irritant under the conditions of the test.
Test Facility	BSL (2012b)

B.6. Irritation – eye

TEST SUBSTANCE	Analogue (25% concentration)
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.

Species/Strain	Rabbit/New Zealand White
Number of Animals	1M, 2 F
Observation Period	7 days
Remarks - Method	No significant deviations from the OECD guidelines.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.7	2	1.7	2	< 7 days	0
Conjunctiva: chemosis	2	1.3	2	3	< 7 days	0
Conjunctiva: discharge	1	1	1	2	< 7 days	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

Remarks - Results	No mortality occurred. No clinical signs were observed during the course of the study. One female slightly lost body weight (-1.5%) from the day of application to the termination of the test. No necropsy was performed at the end of the study. Slight to moderate reddening of the conjunctivae was noted in all animals 1 to 72 hours after treatment. Slight to marked swelling of the conjunctivae (chemosis with half-closed lids) was observed in all animals 1 to 72 hours after treatment. Moderate reddening of the sclera was present in one animal 1 to 72 hours after treatment. Due to the marked swelling (with half closed lids) of the conjunctivae, the assessment of the sclera was first prevented in two animals. When assessable at the 24-hour reading, a moderate reddening of the sclera was noted. Slight to moderate ocular discharge was recorded in all animals 1 to 72 hours after treatment. No abnormal findings were observed in the treated eye of any animal 7 days after treatment, the end of the observation period for all animals. No corneal or iridial effects were observed at any of the reading times.
CONCLUSION	The test substance is slightly irritating to the eye.
TEST FACILITY	Harlan (2008)

B.7. Skin sensitisation

TEST SUBSTANCE	Analogue (25% concentration)
METHOD	OECD TG 406 Skin Sensitisation – Magnusson and Kligman Maximisation Test.
Species/Strain	Guinea pig/Dunkin Hartley CRL:(HA)BR, SPF
PRELIMINARY STUDY	Maximum Non-irritating Concentration: 5% intradermal: 5, 10, 15, 25, 50, 75% topical: 3, 5, 10, 15, 25, 50, 75, 100%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 5
Vehicle	Purified water
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-Hexylcinnamaldehyde.
INDUCTION PHASE	Induction Concentration: intradermal: 5% topical: 50%

Signs of Irritation	The expected and common findings were observed in the control and test group after the different intradermal induction applications using Freund's Adjuvant-complete (FCA) intradermally. These findings consisted of erythema, oedema, necrotising dermatitis, encrustation and exfoliation of encrustation. No erythematous or oedematous reaction was observed in the control animals treated with purified water only during epidermal induction. Discrete/patchy to moderate/confluent erythema was observed in all test animals at the 24-hour reading and in eight animals at the 48-hour reading after treatment with the test substance at 50% in purified water.
CHALLENGE PHASE	
challenge	topical: 5%
Remarks - Method	No significant deviations from the OECD guidelines.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after challenge*	
		24 h	*48 h*
Test Group	5%	0/10	0/10
Control Group	5%	0/5	0/5

Remarks - Results	No death and no clinical signs of systemic toxicity were observed in the animals during the study. No skin reactions were observed in the test and control animals when treated with either purified water only or the test substance at 5 % in purified water. The positive control, alpha-hexylcinnamaldehyde was tested to produce evidence of skin sensitisation thus confirming the sensitivity and reliability of the experimental technique.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.
TEST FACILITY	Harlan (2008)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Analogue (68.1% concentration)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Highly purified water
Remarks - Method	No significant deviations from the OECD guidelines.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 F & 5 M	0	0/10
low dose	5 F & 5 M	62.5	0/10
mid dose	5 F & 5 M	250	0/10
high dose	5 F & 5 M	1,000	2 (1 F & 1 M)/10
control recovery	5 F & 5 M	0	0/10
high dose recovery	5 F & 5 M	1,000	0/10

Mortality and Time to Death

One male rat and 1 female rat from high dose group were found dead on day 9 and 11 respectively. According to the study authors, the macroscopic changes were generally commensurate with those of dose formulation aspiration.

Clinical Observations

Daily observation

Breathing noise was noted in one male rat from high dose group on day 6. No other clinical signs were noted.

Weekly observation

No clinical signs were noted during weekly behavioural observations.

Functional observation battery

Shortness of breath was noted in 2 male rats from high dose group during the test on week 4. Locomotor activity was significantly elevated in male rats from low dose group and female rats from high dose group when compared to controls. No other significant changes in functional observation battery were observed.

Food consumption

Food consumption was reduced in female rats from high and mid dose groups. The absolute mean daily food consumption was also reduced in male rats from high and mid dose groups but when the body weights were taken into consideration to derived relative food consumptions, the food consumptions in both groups were comparable to the control group suggesting the reduction seen is due to the lower body weights of rats. This was not the case for female rats. Recovery in food intake was observed in rats from high dose recovery group during the 14-day recovery stage.

Body weights

Marginally lower mean absolute body weights were noted for rats from high dose group. These changes, however, were not considered to be test substance related by the study author as the mean body weight gain values compared favourably at all measurement intervals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology

The study authors considered that none of the parameters was test item related. A slight but statistically significant increase in haemoglobin level in female rats from the high dose group was not considered to have toxicological significance in the absence of other relevant findings. Some other effects at intermediate doses were not dose related and were discounted. In the recovery group, a slight reduction in basophil count in males was within the historical controls. A significant reduction in total leukocytes in female high dose recovery rats was attributed to an elevated control value.

Clinical chemistry

Male rats from high dose group had significantly higher phosphorous levels, higher alanine aminotransferase activity and lower lactate dehydrogenase activity. Female rats from high dose group had significantly elevated potassium, chloride and phosphorus levels. Significantly higher alanine aminotransferase activity was also noted in male rats from mid dose group. Males rats from high dose recovery group showed significantly elevated levels of urea and reduced levels of creatine kinase.

Urinalysis

No significant treatment related changes were noted in any test animals.

All the above observations were considered not to be test item related by the study author due the lack of dose response relationship or seen in only one sex or due to the values being in within the range of respective historical control values.

Effects in Organs

Changes in organ weight

Female rats from high dose group had significantly lower absolute and relative pituitary weight. Male rats from high dose group had significantly increase relative testes and epididymides weight, attributed to a low control value. Female rats from high dose recovery group had slightly but significantly lower absolute mean adrenal weight and significantly higher relative liver and lower relative ovary weight. Though significant, the changes in organ weights were considered not to be test item related by the study author due to lack of associated

microscopic changes in the organs.

Microscopic findings
Rats from high dose group showed treatment related microscopic changes in the forestomach. Acanthosis, parakeratosis, inflammation, ulceration and erosions were noted. These changes were not seen in rats from high dose recovery group suggesting the changes were reversible.
Microscopic examination also revealed some changes in the lungs of some animals from the high dose group. Amorphous material in the Broncho alveolar lumen was observed. This was considered by the study author to have occurred due to the reflux of test substance from the stomach following administration. This aspiration was considered to be cause of death of 2 animals from this group and the changes observed in other animals from this group. The changes in lungs reported were inflammation, necrosis, oedema and emphysema or collapse of the lung parenchyma. No test item related microscopic findings were reported for low and mid dose group animals.

Remarks – Results
The microscopic findings in the forestomach of test animals from high dose group were a local effect probably due irritation potential of the test substance.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 1,000 mg/kg bw/day by the study authors in this study, based on the reported adverse but reversible effects seen in forestomach of test animals from high dose group.
The No observed Effect Level (NOEL) was established as 250 mg/kg bw/day.

TEST FACILITY Harlan (2012)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE Analogue (25% concentration)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation procedure (test 1) and pre incubation procedure (test 2)

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	S9 fraction from Phenobarbital/β-Naphthoflavone induced rat liver.
Concentration Range in Main Test	a) With metabolic activation: 3-5,000 µg/plate b) Without metabolic activation: 3-5,000 µg/plate
Vehicle	Deionised water
Remarks - Method	No significant deviations from the OECD guidelines. The preliminary test was reported as test 1. Due to irregular growth in strain TA98 in test 2, no data were evaluated for the strain. This part was repeated under identical conditions and was report as part of test 2.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	≥ 2500			
Test 1		≥ 2500	> 5000	Negative
Test 2		≥ 1000	> 5000	Negative
Present	≥ 2500			
Test 1		≥ 2500	> 5000	Negative
Test 2		≥ 1000	> 5000	Negative

Remarks - Results The plates incubated with the test substance showed reduced background growth in nearly all strains with and without metabolic activation in both independent experiments.
Toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in nearly all strains in both

experiments.
No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with test substance at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).
The positive controls showed a significant increase of induced revertant colonies, thus confirming the integrity of S9-mix and the test system.

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

TEST FACILITY Harlan (2008)

B.10. Genotoxicity – *in vitro*

TEST SUBSTANCE Notified chemical (69.1% concentration)

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (1997).
Species/Strain Chinese Hamster
Cell Line CHO-K1
Metabolic Activation System S9 fraction from Phenobarbital/β-Naphthoflavone induced rat liver.
Vehicle Dimethyl sulphoxide
Remarks - Method No significant deviations from the OECD guidelines were noted, except that the values of cell aberrations and gaps were reported only for the positive controls and not for the vehicle control or the test substance.
A preliminary precipitation test was conducted to decide test substance concentrations for main study. Due to precipitation at concentrations ≥ 150 µg/mL, only low levels could be used in the study. At the concentrations tested, cytotoxicity as measured by reduction in mitotic index was low, and did not meet the desired criterion of significant reduction in mitotic index at the highest level tested.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	25, 50, 100	3 h	18 h
Test 2	25, 50, 100	18 h	18 h
Present			
Test 1	25, 50, 100	3 h	18 h

All cultures were selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 100	> 100	Negative
Test 2	> 100	> 100	Negative
Present			
Test 1	> 100	> 100	Negative

Remarks - Results The study authors reported that there was no significant increase in the percentage of aberrant cells, compared to the vehicle control (details of percentages were not provided). The positive controls showed a significant increase in number of chromosome aberrations confirming the integrity of S9-mix and the test system.

CONCLUSION The test substance was not clastogenic to Chinese hamster ovary cells treated *in vitro* under the conditions of the test.

TEST FACILITY BIONEEDS (2015)

B.11. Genotoxicity – *in vitro*

TEST SUBSTANCE Analogue (68.5% concentration)

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test (1997).
Species/Strain Mouse
Cell Type/Cell Line Lymphoma L5178Y cells
Metabolic Activation System S9 fraction from Phenobarbital/β-Naphthoflavone induced rat liver.
Vehicle Deionised water
Remarks - Method No significant deviations from the OECD guidelines. A preliminary cytotoxicity test was conducted to decide the test substance concentrations for main experiment. Severe toxicity was observed in test 2 with metabolic activation thus the experiment was repeated again.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*
Absent			
Test 1	5.9*, 11.8*, 23.5*, 47.0*, 94.0*, 141.0	4 h	48 h
Test 2	11.8*, 23.5*, 47.0*, 94.0*, 188.0*, 282.0	24 h	48 h
Present			
Test 1	11.8*, 23.5*, 47.0*, 94.0*, 188.0*, 282.0	4 h	48 h
Test 2	8.8*, 17.5*, 35.0*, 70.0*, 105.0*, 140.0	4 h	48 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 187.5	≥ 94.0	> 141.0	Negative
Test 2	≥ 187.5	≥ 94.0	> 282.0	Negative
Present				
Test 1	≥ 187.5	≥ 188.0	> 282.0	Negative
Test 2		> 105.0	> 140.0	Negative

Remarks - Results In Test 2, in the absence of metabolic activation and at the highest dose tested, one of the two cultures slightly exceeded the threshold for allowable number of mutant colonies, that determines whether the culture is considered mutagenic. It is noted that high toxicity occurred at this dose and culture. As the second culture was well below the threshold for mutagenicity, the study authors considered this effect not to be biologically relevant. None of the other cultures in either test exceeded the threshold. The positive controls showed a significant increase in number of mutant colonies confirming the integrity of S9-mix and the test system.

CONCLUSION The test substance was not clastogenic to Mouse L5178Y lymphoma cells treated *in vitro* under the conditions of the test.

TEST FACILITY Harlan (2010)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Analogue of the notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	$ThCO_2$ by potentiometric titration of $Ba(OH)_2$
Remarks - Method	The test was conducted in accordance with the test guideline above. Deviations from the guidelines reported were: 1) mineral nutrient solution was directly prepared in test vessels. 2) The titration of barium hydroxide solution was performed potentiometrically rather than with phenolphthalein indicator. 3) The biodegradation of the reference item, sodium benzoate, was not evaluated due to a leakage preventing determination of the evolved carbon dioxide.

RESULTS

Test substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
28	84.5*	N/A	Not determined

*Mean of two replicates

Remarks - Results	It was unknown if the reference compound reached the pass level of 60% within 14 days due to a leakage preventing determination of CO_2. All the other validity criteria for the test were satisfied. The percentage degradation of the toxicity control surpassed the threshold level of 25% by 7 days (36% in 7 days), indicating that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days was 84.5%. 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 test substance, and by inference, the notified chemical is considered to be readily biodegradable
TEST FACILITY	Clariant (2009a)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Analogue chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static
Species	*Danio rerio* (zebra fish)
Exposure Period	96 hours
Auxiliary Solvent	None reported
Water Hardness	59 mg $CaCO_3$/L
Analytical Monitoring	DOC
Remarks – Method	The test was conducted in accordance with the test guideline above, with no significant deviation to the test protocol reported. DOC analysis was

only conducted at the beginning of the test so no information on the stability and recovery of the test substance was reported.

RESULTS

Concentration mg/L		*Number of Fish*	*Cumulative Mortality (%)*			
Nominal	*DOC content*		*24 h*	*48 h*	*72 h*	*96 h*
Control	0.338	7	0	0	0	0
100	16.6	7	0	0	0	0

LC50	Inconclusive
NOEC	Inconclusive
Remarks – Results	The study report indicated that the test substance appeared turbid over the course of the test and sedimentation was observed after 24 h. One of the validity criteria of the test guideline is that "there must be evidence that the concentration of the substance being tested has been satisfactorily maintained". However no analytical measurement was conducted at the end of the test to confirm if the concentration of test substance had been maintained. Furthermore, it was reported that the test substance was sedimented out of solution during the study. Therefore this validity criterion was not met and the study cannot be considered reliable.

CONCLUSION Under the study conditions, the toxicity to fish of the test substance, and by inference the notified chemical, is inconclusive.

TEST FACILITY Dr.U.Noack-Laboratorien (2008)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Analogue of the notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test – Static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None reported
Water Hardness	21.4 mg $CaCO_3$/L
Analytical Monitoring	LC-MS/MS
Remarks - Method	The test substance forms calcium salts with very low solubility, removing most of the test substance from aqueous solution. To overcome this testing issue, calcium ions in test water were complexed by $Na_2EDTA.2H_2O$ (800 mg/L). It was confirmed in a preliminary range finding test that Na_2EDTA had very low toxicity to *Daphnia magna*. The test substance contains several components. The concentrations of the C12- and C14-fractions of the test substance were analytically verified with LC-MS/MS in fresh (0 h) and old media (48 h) of all concentration levels and the control. In the definitive test, four replicates containing five daphnids each were tested at each test concentration. Potassium dichromate was tested as a reference substance.

RESULTS

Concentration mg/L		*Number of D. magna*	*Percent Immobilised (mean of four replicates)*	
Nominal	*Recovery rates (%)**		*24 h*	*48 h*
160	99, 92	20	85	100
64	101, 90	20	30	90
25.6	94, 86	20	0	30
10.2	109, 94	20	0	0

4.10	83, 69	20	0	0
1.64	92, 66	20	0	0
Na_2EDTA control	< LOQ**	20	0	0

*Recovery rate of two components of the test substance in old media (48 h) compared to nominal concentration of component.
**Limits of quantification of the analytical method were 23.8 µg/L and 9.03 µg/L for two components of test substance, respectively.

EC50	33.0 mg/L at 48 hours
NOEC	10.2 mg/L at 48 hours
Remarks - Results	The study satisfied all the validity criteria of the guideline. The recovery rate of the C12- and C14-fractions of the test substance were 89-99% in fresh media (0 h) and 66-109% in old media (48 h). Effects values were based on nominal concentrations of the test substance since toxicity could not be attributed to any single component of the test substance.

CONCLUSION Notified chemical is harmful to aquatic invertebrates.

TEST FACILITY Dr.U.Noack-Laboratorien (2011a)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Analogue of the notified chemical

METHOD	OECD TG 201 Alga, Growth Inhibition Test - Static
Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 0.32 - 320 mg/L Actual*: 81 - 117% of nominal (at test start) 2 - 111% of nominal (after 72 h)
	*Recovery rate of two components of the test substance
Auxiliary Solvent	None reported
Water Hardness	0.24 mmol Ca+Mg/L
Analytical Monitoring	LC-MS/MS
Remarks - Method	No significant deviations from the test guideline were reported. A preliminary range-finding test was conducted at nominal concentration ranges of 0.01 to 100 mg/L test substance. Three replicates were tested for each test item concentration and six replicates for the control. Potassium dichromate was tested as a reference substance. The test substance contains several components. The concentrations of the C12- and C14-fractions of the test substance were analytically verified with LC-MS/MS in fresh (0 h) and old media (48 h) of all concentration levels and the control.

RESULTS

Biomass		*Growth*	
NOEC mg/L at 72 h	*EC50 mg/L*	*NOEC mg/L at 72 h*	*EC50 mg/L*
1.00	30.5	32.0	89.9

Remarks - Results	The study satisfied all the validity criteria of the guideline. The recovery rates of the C12- and C14-fractions of the test substance were 81-117% at the test start (0 h) and 2-111% after 72 h. After 72 h reduced recoveries for both components of the test substance were reported for the 32 and 100 mg/L test concentrations which were reported to be caused by the "biotic test system". Effects values were based on nominal concentrations of the test substance since toxicity could not be attributed to any single component of the test substance.

CONCLUSION	Notified chemical is harmful to algae.
TEST FACILITY	Dr.U.Noack-Laboratorien (2011b)

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