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AUSTRALIAN INDUSTRIAL CHEMICALS INTRODUCTION SCHEME (AICIS)

PUBLIC REPORT

Chemical in Mackam® LSB-50

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals Act 2019 (the IC Act) and Industrial Chemicals (General) Rules 2019 (the IC Rules) by following the Industrial Chemicals (Consequential Amendments and Transitional Provisions) Act 2019 (the Transitional Act) and Industrial Chemicals (Consequential Amendments and Transitional Provisions) Rules 2019 (the Transitional Rules)*. The legislations are Acts of the Commonwealth of Australia. The Australian Industrial Chemicals Introduction Scheme (AICIS) 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 Agriculture, Water and the Environment.

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SUMMARY

The following details will be published on our website:

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1723	Solvay Interox Pty Ltd	Chemical in Mackam® LSB-50	Yes	≤ 300 tonnes per annum	Component of liquid dishwashing detergents

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Eye Irritation (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 2	H401 – Toxic 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 assessed chemical is not considered to pose an unreasonable risk to the health of workers.

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

Environmental Risk Assessment
On the basis of the PEC/PNEC ratio, the assessed chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The assessed chemical should be classified as follows:
  – Eye irritation: H318 ─ Causes serious eye damage

  The above should be used for products/mixtures containing the assessed chemical, if applicable, based on the concentration of the assessed chemical present.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the assessed chemical during reformulation processes:
  - Enclosed/automated processes
  - Local exhaust ventilation

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the assessed 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 assessed chemical during reformulation processes:
  - Protective clothing
  - Impervious gloves
  - Safety goggles
  - Respiratory protection, if inhalation exposure may occur

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

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

- If products and mixtures containing the assessed 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.

Storage

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

Public Health

- The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the assessed chemical for listing on the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP).

- Formulators should take into account the potential for the assessed chemical to cause serious eye damage when manufacturing consumer products containing the assessed chemical.

Emergency procedures

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

Disposal

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

Regulatory Obligations

Specific Requirements to Provide Information
This risk assessment is based on the information available at the time of the application. The Executive Director may initiate an evaluation of the chemical based on changes in certain circumstances. Under Section 101 of the IC Act the applicant of the assessed chemical has post-assessment regulatory obligations to provide information to AICIS when any of these circumstances change. These obligations apply even when the assessed chemical is listed on the Australian Inventory of Industrial Chemicals (the Inventory).

Therefore, the Executive Director of AICIS must be notified in writing within 20 days by the applicant or other introducers if:

- the final use concentration of the assessed chemical exceeds 1% in liquid dishwashing detergents;
- the function or use of the chemical has changed from a component of liquid dishwashing detergents, 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 chemicals on human health, or the environment.

The Executive Director will then decide whether an evaluation of the introduction is required.

Safety Data Sheet
The SDS of the product containing the assessed chemical provided by the applicant was reviewed by AICIS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND APPLICATION DETAILS

APPLICANT
Solvay Interox Pty Ltd (ABN: 70 000 882 137)
20-22 McPherson Street
BANKSMEADOW NSW 2019

APPLICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year)

PROTECTED INFORMATION (SECTION 38 OF THE TRANSITIONAL ACT)
Data items and details taken to be protected information include: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, use details and identity of analogue chemical.

VARIATION OF DATA REQUIREMENTS (SECTION 6 OF THE TRANSITIONAL RULES)
Schedule data requirements are varied for vapour pressure, water solubility, partition co-efficient, absorption/desorption, dissociation constant, hydrolysis as a function of pH, explosive properties, oxidising properties, and for all human health toxicity endpoints.

PREVIOUS APPLICATION IN AUSTRALIA BY APPLICANT(S)
None

APPLICATION IN OTHER COUNTRIES
Canada, EU, Japan, New Zealand, Taiwan and USA

2. IDENTITY OF CHEMICAL

MARKETING NAME
Mackam® LSB-50 (product containing the assessed chemical at ≤ 50% concentration in aqueous solution)

MOLECULAR WEIGHT
< 500 g/mol

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

3. COMPOSITION

DEGREE OF PURITY
> 75%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: white powder

Property	*Value*	*Data Source/Justification*
Melting Point	55.0 °C	Measured
Boiling Point	311.6 °C at 101.3 kPa	Measured
Density	1,304 kg/m^3 at 20 °C	Measured
Vapour Pressure	2.3 x 10^{-11} kPa at 20 °C	Measured. Analogue chemical 1
Water Solubility	> 500 g/L at 20 °C	Estimated from the assessed chemical concentration in commercial aqueous products
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionality; however, significant hydrolysis is not expected in the environmental pH range of 4-9

Property	*Value*	*Data Source/Justification*
Partition Coefficient (n-octanol/water)	log Pow = 2.1 at 25 °C	Measured. Analogue chemical 1
Surface Tension	38.5 mN/m	Measured
Adsorption/Desorption	Not determined	Expected to bind strongly to charged particles in soil based on its amphoteric characteristics
Dissociation Constant	Not determined	Amphoteric salt and expected to be dissociated in the environmental pH range of 4-9
Particle Size	Not determined	Introduced in aqueous solution
Flash Point	> 120 °C at 101.3 kPa	Measured
Flammability	Not pyrophoric	Measured
Autoignition Temperature	396 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Non-oxidising	Measured. Analogue chemical 1

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

Reactivity
The assessed 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 assessed chemical is not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF ASSESSED CHEMICAL (100%) OVER NEXT 5 YEARS
The assessed chemical will not be manufactured in Australia. It will be imported into Australia mostly as a component of finished products at ≤ 1% concentration. The assessed chemical may also be imported in a product at ≤ 50% concentration in aqueous solution, for reformulation into finished products.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 300	≤ 300	≤ 300	≤ 300	≤ 300

PORT OF ENTRY
Major ports in Australia

IDENTITY OF RECIPIENTS
Solvay Interox Pty Ltd

TRANSPORTATION AND PACKAGING
The product containing the assessed chemical at ≤ 50% concentration in aqueous solution will be imported in bulk (18,000 L), 250 L plastic drums or 1,000 L intermediate bulk containers (IBCs). The finished products containing the assessed chemical at ≤ 1% concentration will be packaged in various container sizes and types, typically 300 mL to 2 L plastic bottles or pouches. Transportation within Australia will be predominantly by road.

USE
The assessed chemical will be used as a component of liquid dishwashing detergents at ≤ 1% concentration.

OPERATION DESCRIPTION

Reformulation
The assessed chemical at ≤ 50% concentration will typically be pumped into a closed blending tank via transfer lines under local exhaust ventilation. After blending with other components, the finished liquid dishwashing detergents containing the assessed chemical at ≤ 1% concentration will be transferred via automatic filling machines into appropriate containers for retail sale.

End-use
The liquid dishwashing detergents containing the assessed chemical at ≤ 1% concentration will be used by professional kitchen workers and the general public. The liquid dishwashing detergents will be dispensed from wall-mounted dispensers contained within pouches, or from a bottle, and will be used for manual washing of dishes.

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 warehouse workers	2-3	24
Reformulation	4-8	200
End-use		
– Retail staff	2-8	300
– Kitchen workers	8-12	300

EXPOSURE DETAILS

Transport and Storage
Transport and storage workers may come into contact with the assessed chemical (at ≤ 50% concentration as introduced for reformulation, or ≤ 1% concentration in finished products), only in the unlikely event of an accidental breach of the product packaging.

Reformulation
Dermal and possible accidental ocular exposure to the assessed chemical (at ≤ 50% concentration) may occur during connection and disconnection of transfer lines, quality control, and cleaning and maintenance of equipment. Based on the expected low vapour pressure of the assessed chemical (2.3×10^{-11} kPa at 20 °C for an analogue chemical), inhalation exposure to the assessed chemical is not expected unless aerosols are formed. The applicant states that exposure is expected to be minimised through the use of local exhaust ventilation and enclosed automated processes, and personal protective equipment (PPE) by workers such as protective clothing, eye protection, impervious gloves and appropriate respiratory protection.

End-use
Exposure of professional kitchen workers to the assessed chemical (at ≤ 1% concentration) in end-use products may occur during measuring and dispensing of the liquid dishwashing detergent. The principal route of exposure will be dermal, while ocular exposure is also possible. Such professionals may use some PPE (gloves and protective clothing) to minimise repeated exposure

6.1.2. Public Exposure
Dermal and possible accidental ocular exposure of the public to liquid dishwashing detergents containing the assessed chemical at ≤ 1% concentration may occur through spills and splashes during handling.

Data on typical use pattern of dishwashing liquid (ACI, 2010) in which the assessed chemical will be used is shown in the following table. In the absence of dermal absorption data, a dermal absorption (DA) of 100% was assumed

for the assessed chemical (ECHA, 2017). For calculation purposes, a lifetime average female body weight (BW) of 64 kg (enHealth, 2012) was used.

Household products (Direct dermal exposure)

Product type	*Frequency (use/day)*	*C (%)*	*Contact Area (cm^2)*	*Product Usage (g/cm^3)*	*Film Thickness (cm)*	*Time Scale Factor*	*Daily systemic exposure (mg/kg bw/day)*
Dishwashing liquid	3	1.0	1980	0.009	0.01	0.03	**0.0025**

Daily systemic exposure = Frequency × C × Contact Area × Product Usage × Film Thickness × Time Scale Factor × DA/ BW
(C = maximum intended combined concentration of the assessed chemicals; DA = dermal absorption; BW = body weight)

Using these assumptions results in an internal dose of 0.0025 mg/kg bw/day of the assessed chemical.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the assessed chemical and a structurally similar analogue chemical (analogue chemical 1) are summarised in the following table. For details of the studies, refer to Appendix B.

Endpoint	***Result and Assessment Conclusion***
Acute oral toxicity* – rat	LD50 = 2,950 mg/kg bw; low toxicity
Acute oral toxicity* – rat	LD50 > 830 mg/kg bw
Acute dermal toxicity* – rat	LD50 > 2,000 mg/kg bw; low toxicity
Skin irritation* – rabbit	slightly irritating at 41.5%
Eye irritation[#] – *in vitro* HET-CAM	moderately irritating at 0.5%
Eye irritation* – rabbit	severely irritating at 41.5%
Skin sensitisation* – guinea pig, maximisation test	no evidence of sensitisation at 42%
Repeat dose oral toxicity* – rat, 90 days	NOAEL (systemic) = 66.7 mg/kg bw/day
Mutagenicity* – bacterial reverse mutation	non mutagenic
Genotoxicity* – *in vitro* chromosome aberration test in human lymphocytes	non clastogenic
Genotoxicity* – *in vitro* gene mutation test in mouse lymphoma cells	non mutagenic
Genotoxicity* – *in vivo* mouse micronucleus test	non clastogenic
Combined repeated dose oral toxicity with reproduction/developmental screening* – rat	NOAEL (systemic) = 100 mg/kg bw/day NOAEL (reproductive/developmental) = 300 mg/kg bw/day
Prenatal developmental toxicity* – rat	NOAEL (maternal) = 66.7 mg/kg bw/day NOAEL (embryotoxicity, foetotoxicity and teratogenicity) = 600 mg/kg bw/day

*Study conducted on analogue chemical 1
[#]Study conducted on assessed chemical

Toxicokinetics,
Based on the low molecular weight of the assessed chemical (< 500 g/mol) and partition coefficient (log Pow = 2.1 at 25 °C), absorption across biological membranes may occur.

Acute Toxicity
No acute toxicity studies were provided of the assessed chemical. Analogue chemical 1 was found to be of low acute oral and dermal toxicity in rats.

Irritation and Sensitisation
No skin irritation study was provided of the assessed chemical. An aqueous product containing analogue chemical 1 at 42% concentration was found to be slightly irritating to the skin in a study conducted in rabbits.

The eye irritancy potential of the assessed chemical at 0.5% concentration was determined to be moderately irritating in an *in vitro* Hen's Egg Test on Chorio-Allantoic-Membrane (HET-CAM). The study authors state that

the hen's egg is more sensitive to liquid irritants than the rabbit eye and as such the study authors suggest the *in vitro* test results indicate a moderate eye irritation potential *in vivo* at 1% concentration for the assessed chemical.

An aqueous product containing analogue chemical 1 at 41.5% concentration was found to be severely irritating to eyes in a study conducted in rabbits. Conjunctival irritation (grade 2 to 3), corneal opacity (grade 1) and iridial inflammation (grade 1) were observed in all animals at the 24, 48 and 72 hour observation periods. Some corneal opacity and conjunctival irritation persisted at up to the day 14 observation. All signs of irritation were resolved at the end of the study period (day 21), except for conjunctival chemosis (grade 1) in one animal. Based on the results of this study, analogue chemical 1, and by inference the assessed chemical, warrants classification as a Category 1 eye irritant according to the GHS criteria.

No skin sensitisation study was provided of the assessed chemical. An aqueous product containing analogue chemical 1 at 42% concentration (administered topically at 100% induction and challenge concentrations) was not a skin sensitiser in a guinea pig maximisation test (GPMT).

Repeated Dose Toxicity
No repeated dose toxicity data was provided of the assessed chemical.

In a 90-day repeated dose oral (gavage) toxicity study in rats, analogue chemical 1 was administered at 66.7, 200 or 600 mg/kg bw/day. A No Observed Adverse Effect Level (NOAEL) was established as 66.7 mg/kg bw/day in this study, based on gastric irritation, multifocal hyperkeratosis and increased kidney weights observed at ≥ 200 mg/kg bw/day.

In a combined repeated dose toxicity study with the reproductive/developmental toxicity screening test in rats (OECD TG 422), analogue chemical 1 was administered by gavage at 30, 100 or 300 mg/kg bw/day. A No Observed Adverse Effect Level for systemic toxicity was established as 100 mg/kg bw/day in this study, based on the histopathological findings on the lungs, trachea and kidneys at 300 mg/kg bw/day.

Mutagenicity/Genotoxicity
No mutagenicity/genotoxicity studies were provided of the assessed chemical. Analogue chemical 1 was not mutagenic in a bacterial reverse mutation assay. Analogue chemical 1 was neither mutagenic in a gene mutation test in mouse lymphoma cells nor clastogenic in a chromosomal aberration test in human lymphocytes. Analogue chemical 1 was also found to be non clastogenic *in vivo* in a mouse micronucleus assay.

Toxicity for Reproduction
No reproduction toxicity data was provided of the assessed chemical.

In the combined repeated dose toxicity study (OECD TG 422), a NOAEL was established as 300 mg/kg bw/day for analogue chemical 1, the highest dose tested in this screening study for reproductive/developmental effects.

In a prenatal developmental toxicity study in rats, analogue chemical 1 was administered by gavage in dams at 66.7, 200 or 600 mg/kg bw/day on GD 6–19. A NOAEL for maternal systemic effects was established as 66.7 mg/kg bw/day in this study, based on significant maternal toxicity and thickened stomach mucosa at ≥ 200 mg/kg bw/day. A NOAEL for prenatal developmental toxicity was established as 600 mg/kg bw/day, the highest dose tested. However, incidences of retarded foetuses (runts) and retarded ossification were statistically significantly increased at this dose level were considered to be due to maternal toxicity.

Health Hazard Classification
Based on the available information for analogue chemical 1, the assessed chemical is a hazardous chemical according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The hazard classification applicable to the assessed chemical is presented in the following table.

Hazard Classification	***Hazard Statement***
Eye Irritation (Category 1)	H318 ─ Causes serious eye damage

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the available information, the assessed chemical is severely irritating to eyes and slightly irritating to skin. Adverse systemic effects and local irritation effects could be expected following repeated exposure (with doses above 66.7 mg/kg bw/day).

Reformulation

Exposure of workers to the assessed chemical (at ≤ 50% concentration) may occur during transfer and blending operations. Provided that adequate control measures are in place to minimise worker exposure, including the use of enclosed and automated processes and PPE (protective clothing, eye protection, impervious gloves and respiratory protection, if inhalation exposure may occur), the risk to workers from use of the assessed chemical is not considered to be unreasonable.

End-use

Professional kitchen workers will handle the assessed chemical (at ≤ 1% concentration), similar to public use. However, the low use concentration in the end use products may not cause severe eye irritating effects if used according to the label directions with PPE, as recommended for professional workers.

6.3.2. Public Health

Members of the public may experience repeated exposure to the assessed chemical at ≤ 1% concentration in liquid dishwashing detergents. The main route of exposure is expected to be dermal with some potential for accidental ocular exposure.

Local effects

The assessed chemical is a severe eye irritant and has been shown to be moderately irritating at 0.5% concentration. Accidental ocular exposure to the assessed chemical in liquid dishwashing detergents is possible with spray detergents. Therefore consumer products should be labelled with appropriate safety directions for users, if the end use product formulations could cause irritation to eyes.

Systemic effects

The potential systemic exposure to the public from the use of the assessed chemical in liquid dishwashing detergents was estimated to be 0.0025 mg/kg bw/day (see Section 6.1.2). Using a NOAEL of 66.7 mg/kg bw/day established from a 90-day repeated dose oral toxicity study for analogue chemical 1, the margin of exposure (MOE) was estimated to be 26,680. A MOE value greater than or equal to 100 is considered acceptable to account for intra and inter-species differences.

Therefore, when used at ≤ 1% concentration in liquid dishwashing detergents with warnings on the label for any potential risks and safety directions for use, the assessed chemical is not considered to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The assessed chemical will be imported into Australia mostly as a component of finished liquid dishwashing products. The assessed chemical may also be imported as a component in aqueous solution, for reformulation into finished products in fully automated and enclosed systems. The applicant estimates up to 2% of the import volume of the assessed chemical may be contained in wastewater generated from reformulation equipment cleaning. This will be collected and reused in subsequent batches as far as practicable, but approximately 0.5% (as estimated by the applicant) may be released to sewer after on-site treatment. The applicant estimates up to 1% of the import volume of the assessed chemical may be released from spills and leaks which will be collected for disposal to landfill, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The liquid dishwashing detergents containing the assessed chemical will be used by professional kitchen workers and the general public. Therefore, the release of the assessed chemical from use will primarily be to sewers across Australia.

RELEASE OF CHEMICAL FROM DISPOSAL

Empty containers containing up to 2% of the import volume of the assessed chemical as estimated by the applicant, will be disposed of to landfill, in accordance with local government regulations.

7.1.2. Environmental Fate

Following its use in liquid dishwashing products, the majority of the assessed chemical is expected to enter sewers across Australia. The ready biodegradability test conducted on the assessed chemical indicates that it is not readily biodegradable, but shows inherent biodegradability in freshwater (62% degradation over 28 days in OECD 310 test, 10 day window criterion was not met). Therefore, the assessed chemical is expected to be removed effectively through biodegradation at sewage treatment plants (STPs) and only a very small portion of the assessed chemical may be released to surface waters. This is supported the applicant's submission (Olkowska *et al*., 2014), which shows surface active agents like the assessed chemical could be removed ≥ 90% through STPs. The biodegradability in seawater test conducted on the assessed chemical indicates that it is also biodegradable in seawater (61% degradation over 28 days in OECD 306 test). Thus, any assessed chemical release to surface waters and seawater is expected to further biodegrade. For details of the biodegradability studies, refer to Appendix C.

A minor proportion of the assessed chemical may be disposed of to landfill as collected spill and container residues. A small proportion of the assessed chemical may also be applied to land when effluent is used for irrigation or when sewage sludge is used for soil remediation, or disposed of to landfill. The assessed chemical residues in landfill and soils are expected to have very low mobility based on its amphoteric and surface active characteristics. The assessed chemical is not expected to bioaccumulate based on its log Pow of 2.10 and its biodegradability. In the aquatic and soil compartments, the assessed chemical is expected to eventually degrade through biotic and abiotic processes to form water and oxides of carbon, nitrogen, and sulphur.

7.1.3. Predicted Environmental Concentration (PEC)

The use pattern will result in most of the assessed chemical being washed into the sewer. The predicted environmental concentration (PEC) has been calculated assuming the realistic worst-case scenario with 100% release of the assessed chemical into sewer systems nationwide over 365 days per annum. The extent to which the assessed chemical is removed from the effluent in STP processes based on the properties of the assessed chemical has not been considered for this scenario, and therefore no removal of the assessed chemical during sewage treatment processes, is assumed.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	300,000	kg/year
Proportion expected to be released to sewer	100	%
Annual quantity of chemical released to sewer	300,000	kg/year
Days per year where release occurs	365	days/year
Daily chemical release:	821.92	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0	%
Daily effluent production:	4,877	ML
Dilution Factor – River	1.0	
Dilution Factor – Ocean	10.0	
PEC – River:	21.9	µg/L
PEC – Ocean:	2.19	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 assessed 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 21.9 µg/L may potentially result in a soil concentration of approximately 0.146 mg/kg. No accumulation of the assessed chemical is expected based on its biodegradability.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the assessed chemical or analogue chemical 1 are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Freshwater Fish Toxicity	96 h LC50 > 10 mg/L	Not harmful to freshwater fish at 10 mg/L
Marine Fish Toxicity Study 1	96 h LC50 > 0.81 mg/L	Not harmful to marine fish at 0.81 mg/L
Marine Fish Toxicity Study 2	96 h LC50 > 4.8 mg/L	Not harmful to marine fish at 4.8 mg/L
Freshwater Daphnia Toxicity*	48 h EC50 = 9.3 mg/L	Toxic to freshwater invertebrates
Marine Copepod Toxicity	48 h EC50 = 20 mg/L	Harmful to marine copepods
Marine Algal Toxicity	72 h EC50 = 5.1 mg/L NOEC = 3 mg/L	Toxic to marine algae
Inhibition of Bacterial Respiration*	3 h IC50 = 915 mg/L	Not inhibitory to microorganisms at STPs
Sediment Amphipods Toxicity	10 d LC 50 = 4,409 mg/kg dry sediment	Practically non-toxic to sediment amphipods

* Studies conducted on analogue chemical 1

The ecotoxicological endpoints above shows that the assessed chemical is toxic to freshwater invertebrates and marine algae. Therefore, the assessed chemical is classified under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) as “Acute Category 2; Toxic to aquatic life” (United Nations, 2009). As the assessed chemical is rapidly degraded, no chronic classification is made. The assessed chemical is practically not harmful to marine sediment amphipods.

7.2.1. Predicted No-Effect Concentration

As the fish toxicity tests were conducted as limit test with no effect observed at the test concentration, the predicted no-effects concentration (PNEC) has been calculated based on the most sensitive acute endpoint for algae as shown in the table below. An assessment factor of 100 was used given the acute endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
72 h EC50 for algae	5.1	mg/L
Assessment Factor	100	
Mitigation Factor	1	
PNEC	51	µg/L

7.3. Environmental Risk Assessment

Based on the above predicted PEC and PNEC, the following Risk Quotient (Q = PEC/PNEC) has been calculated.

Risk Assessment	PEC (µg/L)	PNEC (µg/L)	Q
Q – River	21.9	51	**0.430**
Q – Ocean	2.19	51	**0.043**

The conservative risk quotient for discharge of effluents containing the assessed chemical to the aquatic environment indicates that the assessed chemical is unlikely to reach ecotoxicologically significant concentrations based on its annual importation quantity. Based on its biodegradability and low log Pow, the assessed chemical is not expected to bioaccumulate. Furthermore, the assessed chemical is practically not toxic to marine sediment dwellers. Therefore, on the basis of the predicted PEC/PNEC ratio, the assessed chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 55.0 °C

Method	OECD TG 102 Melting Point/Melting Range EC Council Regulation No 440/2008 A.1 Melting Temperature
Remarks	Differential scanning calorimetry method.
Test Facility	Defitraces (2012a)

Boiling Point 311.6 °C at 101.3 kPa

Method	OECD TG 103 Boiling Point EC Council Regulation No 440/2008 A.2 Boiling Temperature
Remarks	Differential scanning calorimetry method.
Test Facility	Defitraces (2012a)

Density 1,304 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	A gas comparison pycnometer was used.
Test Facility	Defitraces (2012a)

Partition Coefficient (n-octanol/water) log Pow = 2.1 at 25 °C

Method	OECD TG 123 Partition Coefficient (n-octanol/water).
Remarks	Slow Stirring Method, the test chemical was analysed by liquid chromatography using an UV detector.
Test Facility	Defitraces (2013a)

Surface Tension 38.5 mN/m at 20 °C

Method	OECD TG 115 Surface Tension of Aqueous Solutions EC Council Regulation No 440/2008 A.5 Surface Tension
Remarks	Test substance: 36.3% assessed chemical in aqueous solution. Concentration: 1g/L of test substance in demineralised water.
Test Facility	Defitraces (2012b)

Flash Point > 120 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point
Remarks	Test substance: 36.3% assessed chemical in aqueous solution. Closed cup method. No flash point was observed up to 120 °C. At 120 °C the test substance boiled and overflowed the small dish just before presentation of the flame. The test was stopped.
Test Facility	Defitraces (2012b)

Flammability Not pyrophoric

Method	EC Council Regulation No 440/2008 A.13 Pyrophoric Properties of Solids and Liquids
Remarks	Test substance: 36.3% assessed chemical in aqueous solution.
Test Facility	Defitraces (2012b)

Autoignition Temperature 396 °C

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases)
Test Facility	Defitraces (2012b)

Oxidizing Properties Non-oxidising

Method	Manual of tests and criteria (fifth edition), United nations (2009) O.2 Oxidizing Properties (Liquids)
Remarks	A pressure vessel was used.
Test Facility	Defitraces (2013b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE: Aqueous product containing 42% of analogue chemical 1

METHOD: OECD TG 401 Acute Oral Toxicity (1987)
Species/Strain: Rat/Wistar/Crl:WI (BR)
Vehicle: None
Remarks – Method: No protocol deviation.

A preliminary range finding test was conducted in 2 females at a dose of 2,000 mg/kg which led to a death of 1 animal within 24 hours of treatment.

Dose was adjusted for concentration of analogue chemical 1 in aqueous product.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5M/5F	1,000	0/10
2	5M/5F	2,000	2F/10
3	5M/5F	3,000	3M, 2F/10

LD50: 2,950 mg/kg bw

Signs of Toxicity: No mortalities and signs of systemic toxicity were observed in the animals in Group 1.

In Group 2 and 3, 2 animals (females) and 5 animals (3 males and 2 females) were killed *in extremis,* respectively, within 24 hours of the exposure period.

Common clinical signs of toxicity observed in all groups was diarrhoea. In addition, the animals in Group 3 showed decreased in activity and squatting positon, reduced skin turgor, cyanosis and piloerection during days 0-2. All surviving animals appeared normal on day 2-3 post exposure.

Effects in Organs: Animals in Group 1 showed petechial haemorrhage of lung (1 female) and pelvis dilatation of kidney (2 females and 2 males) which was attributed by the study authors to the strain and age of the rats.

Macroscopic examination of the animals that were killed *in extremis* in Group 2 and 3 revealed discolouration in lung and kidney (1 female), haemorrhagic content in enlarged stomach (2 females), yellow mucous content in enlarged intestine (1 female) and reddish pelvis in kidney (3 males, 2 females), congestion in liver (3 males and 2 females) and spleen (1 male and 1 female), lysed mucous membrane in enlarged stomach (3 males and 2 females) and yellow mucous content in enlarged intestine (3 males and 2 females), respectively.

At 14 day necroscopy, petechial haemorrhage in lungs (2 males and 2 females), retractions and renal calculi (1 female), hydrometra of genital system (1 female) were observed in animals in Group 2. Similarly, in Group 3, marbled kidney was evident in 1 male.

The study authors considered these adverse findings in Group 2 and 3 to be treatment related.

Remarks – Results: All surviving animals showed expected body weight gains during the observation period.

CONCLUSION	Analogue chemical 1 is of low acute toxicity via the oral route.
TEST FACILITY	IBR (1990)

B.2. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Aqueous product containing 41.5% of analogue chemical 1
METHOD	EC Directive 92/69/CEE B.1 bis Acute toxicity (oral) fixed dose method.
Species/Strain	Rat/Wistar/Crl:WI (BR)
Vehicle	Bidistilled water
Remarks – Method	No protocol deviation. A preliminary test was conducted in 1 female at a dose of 2,000 mg/kg prior to the main study. Soft faeces were noted in this animal post treatment; however, no other signs of toxicity and organ abnormalities were noted.

RESULTS

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

LD50	> 2,000 mg/kg bw (equivalent to 830 mg/kg bw for analogue chemical 1)
Signs of Toxicity	There were no mortalities. No signs of systemic toxicity observed except soft faeces in animals post treatment.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks – Results	All surviving animals showed expected body weight gains during the observation period.
CONCLUSION	The test substance is of low acute toxicity via the oral route.
TEST FACILITY	CIDA (1995)

B.3. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Aqueous product containing 36.2% of analogue chemical 1
METHOD	OECD TG 402 Acute Dermal Toxicity (1987)
Species/Strain	Rat/ Sprague Dawley
Vehicle	None
Type of dressing	Semi-occlusive
Remarks – Method	No significant deviations from the study protocol. Dose was adjusted for concentration of analogue chemical 1 in aqueous product.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Very slight or well defined erythema was observed at the test site of two females in group 2, only on day 2 after dosing.
Signs of Toxicity – Systemic	There were no deaths or test-substance related clinical signs.
Effects in Organs	Enlargement of spleen was observed in all animals but was considered by the study authors to be a normal background finding in rats of this strain and age. There were no other macroscopic pathological findings in the animals sacrificed at the end of the observation period.

Remarks – Results	All females in group 2 showed a slight decrease in the mean body weight when compared to historical control data (33% decrease than control) during the study period. This mean decrease in body weight (42% decrease than control) was notably higher during the first week of the treatment. All males (Group 1) showed expected body weight gain over the observation period.
CONCLUSION	Analogue chemical 1 is of low acute toxicity via the dermal route.
TEST FACILITY	Citroxlab (2012a)

B.4. Skin Irritation – Rabbit

TEST SUBSTANCE	Aqueous product containing 41.5% of analogue chemical 1
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion (1992)
Species/Strain	Rabbit/New Zealand White Male
Number of Animals	3
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive
Remarks – Method	No deviation from the study protocol.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	0	0.67	1	< 72 h	0
Oedema	0	0	0	0	-	-

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

Remarks – Results	Very slight erythema (grade 1) was observed in all animals 1 hr after treatment. While 2 animals recovered within 24 hours, the symptom persisted in one animal up to the 48 hour observation period. All signs of irritation were resolved at the 72-hour observation. No mortalities or other significant clinical signs of toxicity were observed.
CONCLUSION	The test substance is slightly irritating to the skin.
TEST FACILITY	CIDA (1995b)

B.5. Eye Irritation – *In Vitro* HET-CAM Test

TEST SUBSTANCE	Aqueous product containing 42% of the assessed chemical
METHOD	The Hen's Egg Test – Utilizing the Chorioallantoic Membrane (HET-CAM) Test. Modification of that described by Kemper and Luepke (1986).
Species/Strain	White Leghorn chicken eggs
Vehicle	None
Remarks – Method	Embryonic hens eggs (HETs) were incubated for 8 days at 37±2 °C and rotated to prevent an attachment of the embryo to one side of the egg. On the 10th day, the chorioallantoic membrane (CAM) was exposed by removing the outer shell and shell membrane. Test substance was tested on 4 eggs. Each CAM was exposed to 300 µL of test substance at 1.25% concentration (equivalent to 0.5% concentration of the assessed chemical) and observed for 5 minutes.

A dilution of Johnson's baby shampoo and Head & Shoulders at 50% concentration was used as reference item because of their known moderately and severely eye irritating properties, respectively.

RESULTS

Test substance	*Total scores of quadruplicate samples*			Mean Irritation Score
	0.5 min	2 min	5 min	
*Test substance (1.25%)**	48	0	0	12
Johnson's baby shampoo (50%)	36	8	0	11
Head & Shoulders shampoo (50%)	48	7	29	21

*Equivalent to the assessed chemical at 0.5% concentration

Remarks – Results

With a mean irritation score of 12, the test substance is considered to be moderately irritating. The reference products performed as expected.

The study authors state that the hen's egg is more sensitive to liquid irritants than the rabbit eye. As such the study authors stated in the report that the *in vitro* test results indicated that the test substance would have a moderate eye irritation potential *in vivo* at 2.5% concentration (equivalent to the assessed chemical at 1% concentration).

CONCLUSION

The assessed chemical at 0.5% concentration was considered to be moderately irritating under the conditions of the test.

TEST FACILITY

CPT (2009)

B.6. Eye Irritation – Rabbit

TEST SUBSTANCE

Aqueous product containing 41.5% of analogue chemical 1

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion (1987)

Species/Strain: Rabbit/New Zealand White

Number of Animals: 3

Observation Period: 21 days

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	2.7	3.0	2.7	3	< 21 days	0
Conjunctiva – Chemosis	1.3	2.3	2.0	3	21 days	1
Corneal Opacity	1.0	1.0	1.0	1	< 21 days	0
Iridial Inflammation	1.0	1.0	1.0	1	< 14 days	0

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

Remarks – Results

Conjunctival irritation (grade 2 to 3), corneal opacity (grade 1) and iridial inflammation (grade 1) was observed in all animals at the 24, 48 and 72 hour observation periods. Some corneal opacity and conjunctival irritation persisted at up to the day 14 observation. All signs of irritation were resolved at the end of the study period (day 21), except for conjunctival chemosis (grade 1) in one animal.

	Based on the results of this study, analogue chemical 1, and by inference the assessed chemical, warrants classification as a Category 1 eye irritant according to the GHS.
CONCLUSION	The test substance is severely irritating to the eye.
TEST FACILITY	CIDA (1995c)

B.7. Skin Sensitisation – Guinea Pig Maximisation Test

TEST SUBSTANCE	Aqueous product containing 42% of analogue chemical 1
METHOD	OECD TG 406 Skin Sensitisation – Maximisation Test (1981)
Species/Strain	Guinea pig/Pirbright-Hartley
PRELIMINARY STUDY	Maximum non-irritating concentration: Topical: 100%
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 20
Vehicle	Deionised water
Positive Control	Not conducted in parallel with the test
INDUCTION PHASE	Induction concentration: Intradermal: 10% Topical: 100%
Signs of Irritation	No signs of irritation
CHALLENGE PHASE	
1st Challenge	Topical: 100%
Remarks – Method	No protocol deviation.
Remarks – Results	No signs of irritation were observed at 24 and 48 hours after challenge in any treated animals.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the test substance under the conditions of the test.
TEST FACILITY	IBR (1988)

B.8. Repeat Dose 90-day oral toxicity – Rat

TEST SUBSTANCE	Aqueous product containing 35.4% of analogue chemical 1
METHOD	OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents (1998)
Species/Strain	Rats/Crl:WI Wistar
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week
Vehicle	Distilled water
Remarks – Method	No significant protocol deviation. Dose was adjusted for concentration of analogue chemical 1 in aqueous product. Dose levels were selected in agreement with the sponsor and based on results of a 14-day gavage study, in which dosing at 600 mg/kg bw/day caused multifocal thickening of the non-glandular stomach mucosa (3/4 F) and a red single focus in the glandular stomach mucosa (1/4 F). No further details on this study were provided by the applicant.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10M/10F	0	0
Low Dose	10M/10F	66.7	0

Mid Dose	10M/10F	200	0
High Dose	10M/10F	600	2

Mortality and Time to Death

Two high dose animals, one male and one female were found dead on day 32 and day 57, respectively. Agonal pulmonary congestion was observed in both animals. Severe bronchoalveolar pneumonia was considered the cause of death in the male rat. In the female rat, the cause of death was likely due to aspiration or respiratory tract irritation (see below). Microscopic findings such as slight multifocal hepatocellular vacuolation in the male and moderate congestion/haemorrhage in the thymus of the female were considered either not to reflect a treatment-related systemic effect or to be incidental by study authors.

Clinical Observations

Noisy respiration was recorded at all doses in both sexes on several occasions. This effect was considered related to pulmonary irritation that could be due to reflux of the strong surfactant substance that enters the upper respiratory tract. Other clinical observations such as several episodes of whole body tonic convulsion at ≥ 200 mg/kg bw/day, (1/10 F each dose from day 50 or 68 onwards), and piloerection (1/10 M, 3/10 F), hunched back (1/10 F) and red discharge from the nose/eyes (1/10 M, 1/10 F) at 600 mg/kg bw/day were considered incidental or background effects.

Mean bodyweight gain was statistically significantly lower in high dose males on one occasion (day 57) but overall they were comparable with the corresponding control means. Differences in food consumption were noted in both sexes in the treatment groups compared with the control group, but were statistically significant in males only. The study authors concluded that these changes were non-adverse.

Statistically significant decrease (23.4% compared to the control group) in the grip strength of the hind limb of the high dose female rats was considered treatment related but non-adverse by study authors. Other animal behaviour, general physical and neurobehavioral assessments were unaffected by the treatment.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Increases in mean cell volume (4.7%) and mean cell haemoglobin (5.7%) in high dose males were within the historical ranges. Decreases in urea (20.7%) and albumin (5.3%) in high dose males and decreases in albumin/globulin ratio (13.6%) in high dose females were within the historical control ranges. Although the creatinine decreases in mid and high dose females (up to 15 % compared to the control group) were slightly out of historical range, they were not considered to be toxicologically significant.

Effects in Organs

Statistically significant increases in the absolute and relative kidney weights were observed in both sexes (M-F: 12.6–9.2% and 20–13.6%, respectively, compared to the control group) at high dose. In female rats, an increased incidence and severity of the kidney tubular cell vacuolation was also reported at high dose (2/9 slight and 7/9 moderate) and mid dose (3/10 minimal). However, these histopathological changes were not observed in the male rats; they were not associated with any degenerative or inflammatory responses and hence were considered non-adverse by the study authors.

Increases in absolute and relative weights of pituitary in males (12.8% and 20.4% compared with the control mean, respectively) and of ovary in females (20.2% and 26.1% compared with the control mean, respectively) were reported at high dose. However, there was no microscopic correlation and hence they were not considered treatment related.

Gastric irritation with multifocal hyperkeratosis of the non-glandular mucosa (not accompanied with cell hyperplasia) was observed in the high dose groups of both sexes (6/9 M, 9/9 F) and some mid dose animals (2/10 M). In high dose female rats, multifocal erosion or ulcers (2/7) and a dark red depressed area (1/10) were also reported.

Histopathological findings such as kidney tubular basophilia and casts, slight multifocal hepatocellular vacuolation, inflammatory prostate cell infiltrate, testis tubule dilatation, congestion/haemorrhage in the thymus, and bronchoalveolar pneumonia were reported in various dosed animals. However, due to low incidence and lack of significant differences between treatment and control groups, they were considered by the study authors as incidental or to be of no toxicological significance.

Remarks – Results

Irritant effects (including pulmonary irritation at all doses and gastric irritation at mid and high doses) were considered to reflect irritant effects of the surfactant properties of the test substance and not to be a direct systemic effect in the context of this study. In the most severe cases, mortality occurred as seen in the 2 high dose animals.

CONCLUSION
A No Observed Adverse Effect Level (NOAEL) was established as 66.7 mg/kg bw/day for analogue chemical 1 in this study, based on gastric irritation, multifocal hyperkeratosis and increased kidney weights observed at higher doses.

TEST FACILITY Citoxlab (2018)

B.9. Genotoxicity – Bacteria

TEST SUBSTANCE Aqueous product containing 50% of analogue chemical 1

METHOD OECD TG 471 Bacterial Reverse Mutation Test (1983)
Plate incorporation procedure

Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA1538, TA98, TA100
Metabolic Activation System	S9 mix from Aroclor 1254-induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0.02 – 2 µL/plate b) Without metabolic activation: 0.02 – 2 µL/plate
Vehicle	Dimethyl sulfoxide (DMSO)
Remarks – Method	The dose selection for the main test (Test 2) was based on the toxicity observed in a preliminary test (Test 1) carried out at 0.002 – 20 µL/plate. Positive control: With metabolic activation: 2-aminoanthracene (TA1535, TA1537, TA1538) and 2-aminofluorene (TA 98, TA100) Without metabolic activation: sodium azide (TA1535, TA100), 9-aminoacridine (TA1537) and 2-nitrofluorene (TA1538, TA98).

RESULTS

Metabolic Activation	*Test Substance Concentration (µL/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 2	-	> 20	negative
Test 2	-	≥ 0.6	> 2	negative
Present				
Test 1	≥ 2	-	> 20	negative
Test 2	-	≥ 0.6	> 2	negative

Remarks – Results	No significant increases in the frequency of revertant colonies were observed for any of the bacterial strains, with any concentration of the test substance, either with or without metabolic activation. The positive and negative controls gave a satisfactory response confirming the validity of the test system.
CONCLUSION	The test substance was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Cosmital SA (1997)

B.10. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE Aqueous product containing 36.2% of analogue chemical 1

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (1997)

	EC Directive No 440/2008/ EC B.10 Mutagenicity – *In vitro* Mammalian Chromosome Aberration Test
Species/Strain	Human
Cell Type/Cell Line	Lymphocyte
Metabolic Activation System	S9 mix from Aroclor 1254-induced rat liver
Vehicle for test item	Water for injection
Vehicle for cell culture	RPMI medium
Remarks – Method	GLP certificate. Dose was adjusted for concentration of analogue chemical 1 in aqueous product. No significant deviations from the protocol. Vehicle and positive controls: without metabolic activation – mitomycin C; with metabolic activation – cyclophosphamide, were run concurrently with the analogue chemical.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 39.06, 78.13*, 156.3*, 312.5*, 625, 1250, 2500, 5000	3 h	20 h
Test 2	0*, 9.38, 18.8, 37.5, 75*, 150*, 300*, 600	20 h	20 h
Test 3	0*, 9.38, 18.8, 37.5, 75, 150, 300*, 600	44 h	44 h
Present			
Test 1	0*, 39.06*, 78.13*, 156.3*, 312.5, 625, 1250, 2500, 5000	3 h	20 h
Test 2	0*, 9.4*, 18.8*, 37.5*, 75, 150, 300	3 h	20 h
Test 3	0*, 9.4, 18.8, 37.5, 75*, 150, 300	3 h	44 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	-	≥ 39.06	> 5000	negative
Test 2	-	≥ 150	> 600	negative
Test 3		≥ 150	> 600	negative
Present				
Test 1	-	≥ 78.13	> 5000	negative
Test 2	-	≥ 9.4	> 300	negative
Test 3		≥ 18.8	> 300	negative

Remarks – Results	The test substance did not induce any statistically significant or dose related increases in the frequency of cells with chromosome aberrations either in the absence or presence of metabolic activation when compared with the vehicle control at any of the concentrations tested. No precipitate was observed at the end of the treatment periods at any dose-levels. The positive and vehicle controls provided a satisfactory response confirming the validity of the test system.
CONCLUSION	Analogue chemical 1 was not clastogenic to cultured human lymphocytes treated *in vitro* under the conditions of the test.
TEST FACILITY	Citroxlab (2012b)

B.11. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE	Aqueous product containing 36.2% of analogue chemical 1

METHOD	OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test (1997) EC Directive No 440/2008; B.17 Mutagenicity – *In vitro* Mammalian Cell Gene Mutation Test
Species/Strain	Mouse
Cell Type/Cell Line	Lymphoma cells/L5178Y $TK^{+/-}$
Metabolic Activation System	S9 mix from Aroclor 1254-induced rat liver
Vehicle for test item	Water for injection
Vehicle for cell culture	RPMI medium
Remarks – Method	GLP certificate. Dose was adjusted for concentration of analogue chemical 1 in aqueous product. A preliminary toxicity test was conducted at a concentration range of 10, 100, 500, 1000, 2500 and 5000 µg/mL for 3 hours, with and without metabolic activation (Test 1). An additional study without metabolic activation was also conducted for 24 hours (Test 2). The choice of the highest dose-level for the main experiments was adjusted based on the level of toxicity found in the preliminary test. Positive control: Without S9: methylmethane sulfonate With S9: cyclophosphamide

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	6.25*, 12.5*, 25*, 50*, 75*, 100*, 200*	3 h	48 h	11-12 days
Test 2	3.13*, 6.25*, 12.5*, 25*, 50*	24 h	48 h	11-12 days
Present				
Test 1	12.5*, 25*, 50*, 100*, 200*, 300*, 400*	3 h	48 h	11-12 days
Test 2	6.25*, 12.5*, 25*, 50*, 100*, 200*	3 h	48 h	11-12 days

*Cultures selected for mutation frequency 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	≥ 100	≥ 25	> 200	negative
Test 2	≥ 100	≥ 3.13	> 50	negative
Present				
Test 1	≥ 100	≥ 50	> 400	negative
Test 2	-	≥ 25	> 200	negative

Remarks – Results	No biologically relevant increase in the number of mutant colonies was observed at any concentration, with and without metabolic activation. The positive and vehicle controls gave a satisfactory response, confirming the validity of the test system
CONCLUSION	Analogue chemical 1 was not clastogenic to mouse lymphoma L5178Y $TK^{+/-}$ cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Citroxlab (2012c)

B.12. Genotoxicity – *in vivo* Mammalian Erythrocyte Micronucleus Test

TEST SUBSTANCE	Aqueous product containing 36.2% of analogue chemical 1

METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test
Species/Strain	Rat/ Sprague-Dawley, Crl CD® (SD)
Route of Administration	Oral –gavage
Vehicle	Drinking water
Remarks - Method	No significant protocol deviation. Dose was adjusted for concentration of analogue chemical 1 in aqueous product. The dose levels were selected based on results of the OECD TG 422 screening study.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	5M, 5F	-	24
II (low dose)	5M, 5F	30	24
III (mid dose)	5M, 5F	100	24
IV (high dose)	5M, 5F	300	24
V (positive control, CP)	5M, 5F	30	24

CP=cyclophosphamide

RESULTS	
Doses Producing Toxicity	The dose related toxicity was similar to observed in the OECD TG 422 study. For details, see B.13.
Genotoxic Effects	None
Remarks - Results	Since there were no treatment related differences noted in both the sexes, micronucleus analysis was performed on males only. The test substance induced no statistically significant increases in the mean values of the polychromatic erythrocytes/normochromatic erythrocytes (PE/NE) ratios and mean frequencies of micronucleated polychromatic erythrocytes (MPE) at any dose. The positive control performed as expected, confirming the validity of the test system.
CONCLUSION	Analogue chemical 1 was not clastogenic under the conditions of this *in vivo* bone marrow micronucleus test.
TEST FACILITY	CIT (2012d)

B.13. Combined Repeated Dose Toxicity with Reproductive/Developmental Toxicity – Rat

TEST SUBSTANCE	Aqueous product containing 36.2% of analogue chemical 1
METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (1996)
Species/Strain	Rat/ Sprague-Dawley, Crl CD® (SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: Males (M): at least 5 weeks (2-week premating and 3-week mating) Females (F): at least 6 weeks (2-week premating, during mating, gestation days (GD) 0–20 and lactation days (LD) 1–5 Dose regimen: 7 days per week
Vehicle	Drinking water
Remarks – Method	Some protocol deviations without compromising the integrity of the study. Dose was adjusted for concentration of analogue chemical 1 in aqueous product. Dose levels were selected in agreement with the sponsor and based on results of a 14-day gavage study at 0, 250, 500 or 1000 mg/kg/day. Dosing at 1,000 led to unscheduled deaths (2/3 M, 2/3 F). At ≥ 5 00 mg/kg bw/day, discolouration and thickened forestomach of the animals were observed. At 250 mg/kg/day, ptyalism or salivation (1/3 M, 3/3 F) was observed.

There were marked dose-dependent decreases in food consumption during 1st week (M-F: 9.9%, 24.2–16.8%, 61.4–43.2%) and body weight on day 14 (M: 8.3%, 13.2%, 22.2%) at all doses, compared with the control mean, respectively. Therefore, 300, 100 and 30 mg/kg bw/day were selected for the main study.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10M / 10F	0	0
Low Dose	10M / 10F	30	0
Mid Dose	10M / 10F	100	0
High Dose	10M / 10F	300	1M

Mortality and Time to Death
In high dose group, one male rat found dead on day 34. The cause of death was moderate pulmonary bronchioalveolar inflammation that was considered secondary to aspiration of the test substance after regurgitation. At necropsy, the animal showed enlargement and red discoloration of lungs and irregular surface and white discoloration of stomach wall. Microscopic examination of the lungs revealed multiple areas of acute necrosis, interstitial fibrosis, inflammatory foci with basophilic material, bronchial ulcer with underlying fibrosis, slight alveolar oedema, agonal congestion and haemorrhage. Microscopic findings correlated with the macroscopic findings.

Clinical Observations
Loud breathing (or noisy respiration) was recorded at low and high dose in several animals. Ptyalism (excess salivation) were frequently noted in high dose animals.

A trend towards slower horizontal movements was reported in female rats; however, such findings in functional observation battery and motor activity were not evident in male animals and in absence of associated clinical signs, they were not considered to have obvious toxicological significance by the study authors.

Dose-related reductions were noted in mean body weight gain in female rats during premating (10%, 27%, 37%) and lactation period (7%, 14%, 29%), and in mean body weight on GD 0 (3%, 5%, 7%) and LD 5 (0%, 4%, 8%) at all doses, respectively, compared with the control group, although the differences were statistically significant at high dose only. There were no effects on mean food consumption during the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
No changes in clinical chemistry, haematology and urinalyses parameters were considered to be of toxicological significance as the differences were observed in males only (decreased protein concentration in mid and high dose males and increased alanine aminotransferase activity in high dose group males) and without physiological or histopathological correlation.

Effects in Parental Organs
Although the following effects were statistically significantly different from the controls, they were not considered to be toxicologically significant by the study author due to low magnitude or occurrence in one sex only.

- 13% increase in mean relative kidney weight in high dose females. There were no differences in absolute kidney weights in either sex of animals or relative kidney weights in male animals.
- A trend towards lower mean absolute heart and liver weight in all treated females.
- 12% decrease in the mean absolute heart weight in high dose males
- 27% increase in the mean relative thymus weight in mid dose males
- A trend towards lower mean absolute kidney, liver and testes weights in all treated males.

Histopathological findings were found in the forestomach, stomach, lungs, trachea and kidneys at 300 mg/kg bw/day as follows:

- Slight to moderate squamous cell hyperplasia in the forestomach (3/5 M, 1/5 F)
- Focal minimal degeneration/necrosis of stomach mucosa (1/5 M)
- Subacute to chronic pulmonary bronchioalveolar inflammation (1/5 M, 3/5 F)

- Tracheal epithelial alteration (3/5 M, 2/5 F), including horizontal orientation of epithelial cells (1/5 F). Minimal epithelial alteration in the trachea in a single male rat at 100 mg/kg bw/day was not considered adverse due to its low incidence and magnitude
- Minimal to slight degeneration or hypertrophy of the kidney tubular epithelium (4/5 M, 1/5 F) and minimal vacuolation of tubular cells (3/5 F).

Reproductive/developmental findings
Fertility and developmental parameters (e.g. number of corpora lutea, number of pre- and post-implantation loss, mating index, fertility index, gestation index, live birth index, viability index, sex-ratio, and pup body weight) were not affected at any dose.

A dose-related trend towards increased pre-implantation loss was noted; however, the values remained within the historical control data. The mean body weight changes in both male and female pups were also dose-dependent towards high dose, but not statistically significant. The reported pup weight changes over days 1–5 postpartum were 4.1–3.9, 4.0–3.8, 3.9–3.9, 3.5–3.3 (M–F) grams at 0, 30, 100, 300 mg/kg bw/day, respectively.

Remarks – Results
Histopathological findings such as forestomach cell hyperplasia, pulmonary bronchioalveolar inflammation and tracheal epithelial alteration at 300 mg/kg bw/day were considered to be related to aspiration or irritant properties of the test substance.

CONCLUSION
A No Observed Adverse Effect Level (NOAEL) for systemic toxicity was established as 100 mg/kg bw/day for analogue chemical 1 in this study, based on the histopathological findings on the lungs, trachea and kidneys at 300 mg/kg bw/day.

A NOAEL was established as 300 mg/kg bw/day for analogue chemical 1, the highest dose tested in this screening study for reproductive/developmental effects.

TEST FACILITY CIT (2012d)

B.14. Prenatal developmental toxicity – Rat

TEST SUBSTANCE	Aqueous product containing 36.2% of analogue chemical 1
METHOD	OECD TG 414 Prenatal Developmental Toxicity Study (2001)
Species/Strain	Rats/Hannover Wistar (Crl:WI(Han))
Route of Administration	Oral – gavage
Exposure Information	Exposure period – gestation days (GD) 6–19
Vehicle	Distilled water
Remarks – Method	No significant protocol deviations. Dose was adjusted for concentration of analogue chemical 1 in aqueous product. The dose levels were selected based on results of the OECD TG 408 screening study above.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	24F	0	0/24
Low dose	26F	66.7	0/26
Mid dose	26F	200	0/26
High dose	27F	600	2/27

Mortality and Time to Death
Two high dose animals were found dead on days 12 and 16. One animal showed hunched back, piloerection, moderately noisy respiration. Another animal showed white skin on the limbs and pinna, extremely decreased activity, prostration and tonic convulsion. Microscopic examination revealed dark red discoloration in lung lobes (multifocal), glandular stomach mucosa and thymus in one animal, and multifocal thickening of non-glandular stomach mucosa in another animal.

Effects on Dams

Noisy respiration was observed at ≥ 66.7 mg/kg bw/day (3/23, 12/24 and 20/25 dams, respectively), and piloerection at ≥ 200 mg/kg bw/day (10/24 and 25/25 dams, respectively). In one high dose animal, slight to moderate laboured respiration, hunched back, decreased activity and red discharge around the vulva and the nose was also noted. The findings were considered to be caused by surfactant properties of the chemical.

The following effects were reported:

At 600 mg/kg bw/day:

- Decreases in mean body weight (13%) on GD 20 and mean body weight gain (39%) on GD 0–20
- Microscopically, diffuse or multifocal thickening of the non-glandular stomach mucosa (12/25 animals).
-

At ≥ 200 mg/kg bw/day:

- Large decreases in mean body weight gain (16–45%) and mean net body weight gain (36–100%) on GD 6–20 (i.e. treatment period)
- Large decreases in food consumption (9–30%) on GD 6–20.

Fertility parameters were not affected at any dose.

Effects on Foetus

Pup viability and sex ratio were comparable with the control. However, incidences of retarded foetuses (runts) and retarded ossification were statistically significantly increased at high dose were considered associated with maternal toxicity.

Foetal variations or malformations were considered to be incidental by the study author as they showed no dose-response relationship or not statistically significant or within the historical control range.

Remarks – Results

Maternal toxicity reported at ≥ 200 mg/kg bw/day was considered to be related to irritant properties of the test substance.

CONCLUSION

A NOAEL for maternal systemic effects was established as 66.7 mg/kg bw/day for analogue chemical 1 in this study, based on significant maternal toxicity and thickened stomach mucosa at ≥ 200 mg/kg/day.

A NOAEL for prenatal developmental toxicity was established as 600 mg/kg bw/day for analogue chemical 1, the highest dose tested.

TEST FACILITY Citoxlab (2019)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability in fresh water

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 310 A Ready Biodegradability: CO_2 in sealed vessels
Inoculum	Activated sludge from a domestic sewage treatment plant
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Carbon dioxide
Remarks – Method	No major deviations from the test guidelines were reported. A toxicity control was run.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
4	25	4	78
7	40	7	93
14	52	14	97
21	59	21	97
28	62	28	97

Remarks – Results	All validity criteria for the test were satisfied. The toxicity control exceeded 25% biodegradation after 14 days showing that toxicity was not a factor inhibiting the biodegradability of the test substance. The degree of degradation of the test substance after 28 days was 62%. The 10 day window criterion was not met.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	Rhodia (2010)

C.1.2. Biodegradability in seawater

TEST SUBSTANCE	Assessed chemical
METHOD	OECD TG 306 Biodegradability in seawater – Closed Bottle Method
Inoculum	Seawater
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen consumption
Remarks – Method	No major deviations from the test guidelines were reported. A toxicity control was run.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	48	4	70
14	51	14	79
21	56	21	85
28	61	28	95

Remarks – Results	All validity criteria for the test were satisfied. The blank respiration did not exceed 30% of the oxygen in the test bottles. The test substance showed an inhibition of 22% to seawater bacteria. The Dissolved Oxygen (DO) in the

test bottles was ≥ 2.4 mg/L during the test. The degree of degradation of the test substance after 28 days was 61%.

CONCLUSION The test substance is biodegradable in seawater.

TEST FACILITY Opus (2009a)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Freshwater Fish

TEST SUBSTANCE Aqueous product containing 30-50% of the assessed chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi-static
EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - Semi-static

Species	*Danio rerio*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	125 mg $CaCO_3$/L
Analytical Monitoring	High performance liquid chromatography with UV detector
Remarks – Method	A limit test was conducted to demonstrate that the test item at 28.1 mg/L has no toxic effect on the test organisms (the threshold approach as agreed with the study sponsor). No major deviations from the test guidelines were reported. The test media was renewed daily. Water samples were taken at the start and the end of the first and the last renewal periods for analysis of the test substance.

RESULTS

Nominal concentration (mg/L)	*Number of Fish*	*Mortality (96 h)*
Control	7	0
28.1	7	0

LC50	> 28.1 mg/L of test substance (> 10 mg/L of assessed chemical) at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The DO concentration was > 90% saturation during the test. The measured test substance concentration ranged between 94 and 118% of the nominal value.

CONCLUSION The assessed chemical is not harmful to fish at 10 mg/L.

TEST FACILITY Harlan (2013a)

C.2.2. Acute Toxicity to Marine Fish Study 1

TEST SUBSTANCE Aqueous product containing 30-50% of the assessed chemical

METHOD OSPAR Commission (1995), Protocol for a fish acute-toxicity test

Species	*Scophthalmus maximus*
Exposure Period	96 hours
Auxiliary Solvent	None
Salinity	36-38 ‰
Analytical Monitoring	None
Remarks – Method	A limit test was conducted to demonstrate that the test item at 1.69 mg/L has no toxic effect on the test organisms (the threshold approach as agreed with the study sponsor). No major deviations from the test guidelines were reported. The test media was renewed after 48 hours.

RESULTS

Nominal concentration (mg/L)	*Number of Fish*	*Mortality (96 h)*
Control	7	0
1.69	7	0

LC50	> 1.69 mg/L of test substance (> 0.81 mg/L of assessed chemical) at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The DO concentration was > 89% saturation during the test.

CONCLUSION	The assessed chemical is not harmful to marine fish at 0.81 mg/L.
TEST FACILITY	Opus (2009b)

C.2.3. Acute Toxicity to Marine Fish Study 2

TEST SUBSTANCE	Aqueous product containing 30-50% of the assessed chemical
METHOD	OSPAR Commission (1995), Protocol for a fish acute-toxicity test
Species	*Scophthalmus maximus*
Exposure Period	96 hours
Auxiliary Solvent	None
Salinity	32-34 ‰
Analytical Monitoring	None
Remarks – Method	A limit test was conducted to demonstrate that the test item at 10 mg/L has no toxic effect on the test organisms (the threshold approach as agreed with the study sponsor). No major deviations from the test guidelines were reported. The test media was renewed after 48 hours.

RESULTS

Nominal concentration (mg/L)	*Number of Fish*	*Mortality (96 h)*
Control	7	0
10	7	0

LC50	> 10 mg/L of test substance (> 4.8 mg/L of assessed chemical as calculated by the applicant) at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. The DO concentration was > 92% saturation during the test.

CONCLUSION	The assessed chemical is not harmful to marine fish at 4.8 mg/L.
TEST FACILITY	Chemex (2009)

C.2.4. Acute Toxicity to Freshwater Invertebrates

TEST SUBSTANCE	Analogue chemical 1
METHOD	Official Journal of EC December 1992 Acute Toxicity for Daphnia – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not provided
Analytical Monitoring	None
Remarks – Method	A definitive test was conducted based on a preliminary test result with no major deviations from the test guidelines. A reference test with potassium dichromate was run concurrently with the definitive study.

RESULTS

Nominal Concentration (mg/L)	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
Control	20	0	0
2.2	20	0	0
3.4	20	0	1
5.0	20	0	1
7.5	20	0	0
11	20	8	19
15	20	19	20
22	20	12	20
34	20	13	20
50	20	15	20

EC50 9.3 mg/L (95% CL of 7.5 – 11 mg/L) at 48 hours

Remarks – Results All validity criteria for the test were satisfied. The DO concentration was > 97% saturation during the test. The 24 h EC50 for *D.magna* exposed to potassium dichromate was 1.3 mg/L which was within the range of expected responses. The EC50 was determined by the binominal method.

CONCLUSION The test substance is toxic to freshwater invertebrates.

TEST FACILITY Rhone-Poulenc (1994)

C.2.5. Acute Toxicity to Marine Copepods

TEST SUBSTANCE Aqueous product containing 30-50% of the assessed chemical

METHOD ISO 14669 (1999) Acute lethal toxicity to marine adult copepods

Species *Acartia tonsa*

Exposure Period 48 hours

Auxiliary Solvent None

Salinity 35 ‰

Analytical Monitoring None

Remarks – Method A definitive test was conducted based on a preliminary test result with no major deviations from the test guidelines. A reference test with 3,5 dichlorophenol was run concurrently with the definitive study.

RESULTS

Nominal Concentration (mg/L)	*Number of copepods*	*Number Immobilised*	
		24 h	*48 h*
Control	20	0	0
10	20	0	1
32	23	1	3
100	22	8	9
320	21	11	11
1001	21	11	11

EC50 66.75 mg/L of test substance (20 mg/L of assessed chemical as calculated by the applicant) at 48 hours

Remarks – Results All validity criteria for the test were satisfied. The DO concentration was > 96% saturation during the test. The 48 h EC50 for *Acartia tonsa* exposed to 3,5 dichlorophenol was 0.96 mg/L which was within the range of expected responses.

CONCLUSION The test substance is harmful to marine copepods.

TEST FACILITY Opus (2009c)

C.2.6. Marine Algal Growth Inhibition Test

TEST SUBSTANCE	Aqueous product containing 30-50% of the assessed chemical
METHOD	ISO 10253 (2006) Marine Algal Growth Inhibition Toxicity
Species	*Skeletonema costatum*
Exposure Period	72 hours
Nominal Concentration	10, 18, 32, 56, 100 mg/L
Auxiliary Solvent	None
Salinity	36 ‰
Analytical Monitoring	None
Remarks – Method	A definitive test was conducted based on a preliminary test result with no major deviations from the test guidelines. A reference test with 3,5 dichlorophenol was run concurrently with the definitive study.

RESULTS

Growth	
ErC50 (mg/L at 72h)	*NOErC (mg/L)*
16.89 mg/l of test substance (5.1 mg/L of assessed chemical as calculated by the applicant)	10 mg/L of test substance (3 mg/L of assessed chemical as calculated by the applicant)

Remarks – Results	All validity criteria for the test were satisfied. The 72 h EC50 for algae exposed to 3,5 dichlorophenol was 2.19 mg/L which was within the range of expected responses.
CONCLUSION	The test substance is toxic to marine algae.
TEST FACILITY	Opus (2009d)

C.2.7. Acute Toxicity to Sediment Amphipods

TEST SUBSTANCE	Aqueous product containing 30-50% of the assessed chemical
METHOD	OSPARCOM (2005) Sediment bioassay using an amphipod *Corophium* sp.
Species	*Corophium volutator*
Exposure Period	10 days
Nominal concentration	14.78, 146.77, 470.49, 1468.73, 14692.04 mg/kg dry sediment
Auxiliary Solvent	None
Salinity	34-39 ‰
Analytical Monitoring	None
Remarks – Method	A definitive test was conducted with no major deviations from the test guidelines.
RESULTS	
LC50	> 14,695 mg/kg dry sediment of test substance (> 4,409 mg/kg dry sediment of assessed chemical) at 10 days
NOEC	14,695 dry sediment mg/kg of test substance (> 4,409 mg/kg dry sediment of assessed chemical)
Remarks – Results	There are no formal validity criteria, but all guideline criteria, including control mortality and dissolved oxygen, were met.
CONCLUSION	The assessed chemical is practically non-toxic to sediment amphipods.
TEST FACILITY	Opus (2009e)

C.2.8. Inhibition of Microbial Activity

TEST SUBSTANCE	Analogue chemical 1
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test
Inoculum	Activated sludge from a domestic sewage treatment plant
Exposure Period	3 hours
Concentration Range	Nominal: 25.6, 64, 169, 400 and 1000 mg/L
Remarks – Method	A definitive test was conducted based on a preliminary test result with no major deviations from the test guidelines. A reference test with 3,5 dichlorophenol was run.
RESULTS	
IC50	915 (95% CL: 831-1026) mg/L at 3 hours
Remarks – Results	All validity criteria for the test were satisfied. The DO concentration was > 60% saturation during the test. The 3 h EC50 for algae exposed to 3,5 dichlorophenol was 3.5 mg/L which was within the range of expected responses.
CONCLUSION	The test substance is not inhibitory to microorganisms at sewage treatment plant.
TEST FACILITY	Harlan (2013b)

BIBLIOGRAPHY

ACI (2010) Consumer Product Ingredient Safety, Exposure and risk screening methods for consumer product ingredients, 2nd Edition, American Cleaning Institute, Washington DC.

Chemex (2009) The acute toxicity of Mackam LSB-50 to Turbot (*Scophthalmus maximus)* over a 96 hour exposure period (Study No. ENV 8702/060901, June, 2009). Cambridge, UK, Chemex Environmental International Limited (Unpublished report submitted by the applicant)

CIDA (1995a) Acute oral toxicity test in rats. Fixed dose method (Study No. CD-95/4391T, July, 1995). Barcelona, Spain, Centro de Investigación y Desarrollo Aplicado S.A.L. (Unpublished report submitted by the applicant)

CIDA (1995b) Primary skin irritation in rabbits (Study No. CD-95/4390T, July, 1995). Barcelona, Spain, Centro de Investigación y Desarrollo Aplicado S.A.L. (Unpublished report submitted by the applicant)

CIDA (1995c) Primary eye irritation in rabbits (Study No. CD-95/4389T, July, 1995). Barcelona, Spain, Centro de Investigación y Desarrollo Aplicado S.A.L. (Unpublished report submitted by the applicant)

Citoxlab (2012a) Acute dermal toxicity study in rats (Study No. 38718 TAR, August, 2012). Evreux, France, CiToxLab. (Unpublished report submitted by the applicant)

Citoxlab (2012b) *In vitro* mammalian chromosome aberration test in cultured human lymphocytes (Study No. 38716 MLH, November, 2012). Evreux, France, CiToxLab (Unpublished report submitted by the applicant)

Citoxlab (2012c) *In vitro* mammalian cell gene mutation test in L5178Y $TK^{+/-}$ mouse lymphoma cells (Study No. 38717 MLY, December, 2012). Evreux, France, CiToxLab (Unpublished report submitted by the applicant)

Citoxlab (2012d) Combined repeated dose toxicity study with the reproduction/development toxicity screening test by oral route (gavage) with a micronucleus assessment in rats (Study No. 38720 RSR, December, 2012). Evreux, France, CiToxLab (Unpublished report submitted by the applicant)

Citoxlab (2018) [Analogue chemical 1]: A 90-day oral (gavage) toxicity study in Wistar rats (Study No. 17/133-101P, February, 2018). Szabadságpuszta, Hungary, Citoxlab Hungary Ltd (Unpublished report submitted by the applicant)

Citoxlab (2018) [Analogue chemical 1]: Prenatal developmental toxicity study by oral (gavage) administration in Hannover Wistar rats (Study No. 17/133-105P, March, 2019). Szabadságpuszta, Hungary, Citoxlab Hungary Ltd (Unpublished report submitted by the applicant)

Cosmital SA (1997) Assessment of the potential mutagenicity of [Analogue chemical 1] in the Ames reversion assay with *Salmonella typhimurium* (Study No. AT 547, January, 1997). Marly, France, Cosmital SA/TOX (Unpublished report submitted by the applicant)

CPT (2009) The Hen's egg test- Utilising the chorioallantoic membrane (HET-CAM) (Study No. V08-5607-6, May, 2009). New Jersey, USA, Consumer Product Testing Co. (Unpublished report submitted by the applicant)

Defitraces (2012a) Melting point, boiling point, relative density on [assessed chemical] (on dried substance) (Study No. 12-918032-001, August, 2012). Brindas, France, Defitraces (Unpublished report submitted by the applicant)

Defitraces (2012b) Surface tension, flash point, viscosity, pyrophoric properties, self-ignition temperature, corrosion for metals on aqueous solution of [assessed chemical] (Study No. 12-918032-002, August, 2012). Brindas, France, Defitraces (Unpublished report submitted by the applicant)

Defitraces (2013a) 1-Octanol/water partition coefficient of [Analogue chemical 1] slow stirring method (Study No. 12-918032-005, January, 2013). Brindas, France, Defitraces (Unpublished report submitted by the applicant)

Defitraces (2013b) Oxidizing properties of liquids test on aqueous solution of [Analogue chemical 1] (Study No. 12-918032-006, February, 2013). Brindas, France, Defitraces (Unpublished report submitted by the applicant)

ECHA (2017) Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance, June 2017, version 3.0. European Chemicals Agency, https://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf

enHealth (2012) Australian Exposure Factor Guide, companion document to: Environmental Health Risk Assessment: Guidelines for assessing human health risks from environmental hazards, EnHealth, Commonwealth of Australia.

Olkowska *et al*., (2014) Review Article – Occurrence of surface active agents in the environment, Journal of Analytical Methods in Chemistry.

Harlan (2013a) [Assessed chemical]: Acute toxicity to Zebra fish (*Danio rerio*) in a 96-hour test (Study No. D67832, March, 2013). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the applicant)

Harlan (2013b) [Analogue chemical 1]: Toxicity to activated sludge in a respiration inhibition test (Study No. D67865, February, 2013). Itingen, Switzerland, Harlan Laboratories Ltd (Unpublished report submitted by the applicant)

IBR (1988) Skin sensitisation test in rats (Study No. 2-5-1194-87, February, 1988). Hanover, Germany, Bioanalytisches Zentrum KG, IBR Forschungs GmbH & Co. (Unpublished report submitted by the applicant)

IBR (1990) Acute oral toxicity test of [aqueous product containing analogue chemical 1] in rats (Study No. 10-04-0337-90, May, 1990). Hanover, Germany, Bioanalytisches Zentrum KG, IBR Forschungs GmbH & Co. (Unpublished report submitted by the applicant)

Opus (2009a) Assessment of aerobic biodegradability of Mackam LSB-50 in seawater (Study No. 1146b-9, February, 2009). Flotta, Scotland, Opus Plus Limited (Unpublished report submitted by the applicant)

Opus (2009b) Assessment of the aquatic-phase (96 h limit test) toxicity of Mackam LSB-50 to the marine fish *Scophthalmus maximus* (Study No. 1146b-5, March, 2009). Flotta, Scotland, Opus Plus Limited (Unpublished report submitted by the applicant)

Opus (2009c) Assessment of the toxicity (48 h EC50) of Mackam LSB-50 to the marine copepod *Acartia tonsa* (Study No. 1146b-1, January, 2009). Flotta, Scotland, Opus Plus Limited (Unpublished report submitted by the applicant)

Opus (2009d) Assessment of the toxicity (10 d LC50) of Mackam LSB-50 to the marine crustacean *Corophium volutator* (Study No. 1146b-2, January, 2009). Flotta, Scotland, Opus Plus Limited (Unpublished report submitted by the applicant)

Opus (2009e) Assessment of the toxicity (72h EC50) of Mackam LSB-50 to the marine unicellular algae *Skeletonema costatum* (Study No. 1146b-3, January, 2009). Flotta, Scotland, Opus Plus Limited (Unpublished report submitted by the applicant)

Rhodia (2010) Evaluation of ultimate aerobic biodegradability of Mackam LSB-50 (Study No. MA-Biodeg-003/10, April, 2010). Paulinia, Brazil, Rhodia Poliamida and Especialidades Ltd (Unpublished report submitted by the applicant)

Rhone-Poulenc (1994) Determination of the acute ecotoxicity after 24 hours and 48 hours of exposure of [Analogue chemical 1] with respect to *Daphnia magna* (Study No. D 218, March, 1994). Decines, France, Rhone-Poulenc Industrialsation Research Centre, Engineering, Technology (Unpublished report submitted by the applicant)

SWA (2012) Code of Practice: Managing Risks of Hazardous Chemicals in the Workplace, Safe Work Australia, https://www.safeworkaustralia.gov.au/doc/model-code-practice-managing-risks-hazardous-chemicals-workplace

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), <http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html >