File No: STD/1546

March 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in NOVAMUL EH

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.

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

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

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Director
NICNAS

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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1546	M-I Australia Pty Ltd	Chemical in NOVAMUL EH	ND*	≤ 100 tonne/s per annum	Additive in drilling fluid

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Human health risk assessment
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

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical:
  - use of enclosed automated processes, where possible

- 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:
  - Impervious gloves
  - Goggles
  - Protective clothing

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures

consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Disposal

- Where reuse or recycling are not available or 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, 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(2) of the Act; if
- the function or use of the chemical has changed from additive in drilling fluid, 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.

No additional secondary notification conditions are stipulated.

(Material) Safety Data Sheet
The (M)SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
M-I Australia Pty Ltd (ABN: 67 009 214 162)
Level 5, 256 St Georges Terrace
Perth WA 6000

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: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, use details and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: All toxicological endpoints apart from bacterial reverse mutation and acute fish toxicity and inhibition of bacterial respiration

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
Commercial Evaluation Permit (2014) and Section 30 Permit (2014)

NOTIFICATION IN OTHER COUNTRIES
Europe (2014)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Chemical in NOVAMUL EH

MOLECULAR WEIGHT
> 500 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Dark amber colour paste

Property	Value	Data Source/Justification
Melting Point/Freezing Point	33 ± 3 °C	Measured
Boiling Point	> 184 °C at 101.6 kPa	Measured
Density	0.984 kg/m^3 at 20 °C	Measured
Viscosity	8.46×10^5 mPa·s at 50 °C 5.96×10^4 mPa·s at 70°C	Measured[1]

Vapour Pressure	2.4×10^{-6} kPa at 25 °C	Measured
Water Solubility	$< 2 \times 10^{-4}$ g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable functionalities but not expected to significantly hydrolyse in the environmental pH range of 4-9
Partition Coefficient (n-octanol/water)	Log KOW > 6.5	Measured
Adsorption/Desorption	Not determined	The notified chemical is expected to adsorb to soil, sediment and sludge based on the presence of potentially cationic groups and low water solubility.
Dissociation Constant	Not determined	The notified chemical contains potentially dissociable functionalities and may be ionised in the environment pH range of 4 – 9.
Flash Point	239 ± 2 °C at 101.3 kPa	Measured
Autoignition Temperature	408 ± 5 °C at 101.3 kPa	Measured
Explosive Properties	Predicted negative	Estimated
Oxidising Properties	Predicted negative	Estimated

[1] Test conducted on Analogue 1.

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia in the liquid formulation NOVAMUL EH at a 30-60% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 100	< 100	< 100	< 100	< 100

PORT OF ENTRY
Fremantle, Dampier and Broome

TRANSPORTATION AND PACKAGING
The liquid formulation NOVAMUL EH (30 – 60% notified chemical) will be imported by sea in 208 L drums and will be transported by road to a warehouse and/or the on-shore mud plant. The mud containing the notified chemical will be transported by supply boat to the offshore ENSCO 5006 drilling rig at the Ichthys gas-condensate field in the north-west shelf of Western Australia.

USE
The notified chemical will be used as a drilling fluid additive in the oil and gas industry to be used in offshore drilling applications at a concentration of approximately 2.4%.

OPERATION DESCRIPTION
The notified chemical will be imported into Australia in liquid formulation at a concentration of 30-60% in 208 L drums, and will not be reformulated or repackaged. At the mud plant the drums will be unloaded and decanted manually into a large tank containing other mud products for mixing. The final mud mixture containing 2.4% of the notified chemical will be transported in bulk by supply boat to the rig site for use. At the rig site, the mixture will be pumped via an enclosed system to mud pits and then down the well to the drill bit for drilling.

The drill cuttings will return to the surface where the rock will be filtered out by shale shakers and the mud which contains the notified chemical (2.4% concentration) will be returned to mud pits before being pumped back down the well for reuse.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	1	30-60
Blending operators	1-2	50

EXPOSURE DETAILS

Transport and storage workers are not expected to be exposed to the notified chemical (at 30 – 60 % concentration) except in the unlikely event of an accidental release due to container breach or spill. Potential route of exposure are dermal and ocular. Due to the low vapour pressure (2.4×10^{-6} kPa at 25 °C) inhalation exposure is not expected.

Blending operators may be exposed to the notified chemical at up 60% concentration via the dermal and ocular route during transfer, mixing and equipment cleaning and maintenance. Worker exposure to drilling mud containing up to 2.4% of the notified chemicals may also occur after drilling, when the mud is returned to the surface for removal of the rock. Exposure is expected to be minimized by using personal protective equipment (PPE) including safety helmets, impervious gloves, coveralls, boots and safety goggles as anticipated by the notifier in the application dossier

6.1.2. Public Exposure

The notified chemical is intended only for use in the oil and gas industry. Public exposure to the notified chemical is not expected except in the unlikely event of an accident occurring during road transport.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and two analogues 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*	LD_{50} > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity#	LD_{50} > 2,000 mg/kg bw; low toxicity
Skin Corrosion (in vitro)	non-corrosive
Skin irritation (in vitro)	non-irritating
Rabbit, skin irritation#	slightly irritating
Eye irritation (in vitro)	non-irritating
Rabbit, eye irritation#	slightly irritating
Mouse, skin sensitisation – Local lymph node assay*	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 – 54 days#[1]	LOAEL ≤ 300 mg/kg bw
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosome aberration test 1*	non genotoxic
Genotoxicity – in vitro mammalian chromosome aberration test 2*	non genotoxic
Rat, reproductive and developmental toxicity#[1]	NOAEL 1,000 mg/kg bw

'*' – study conducted on analogue 1
'#' – study conducted on analogue 2
'1' – studies conducted together as combined repeated dose oral toxicity study with the reproduction/developmental toxicity screening test according to the OECD guideline No. 422.

Toxicokinetics, metabolism and distribution.

No toxicokinetics, metabolism and distribution studies were submitted for the notified chemical. However, based on the high molecular weight (> 500 Da), low water solubility (< 0.2 mg/L) and high partition coefficient (Log Kow > 6.5) dermal absorption of the notified chemical is expected to be very low (ECHA 2012).

Acute toxicity.
No acute toxicity information was provided for the notified chemical. Acute oral and dermal toxicity studies conducted on 2 different analogue chemicals suggest the notified chemical to be of low toxicity via the oral and dermal routes.

Irritation and sensitisation.
In vitro skin corrosion and skin and eye irritation studies done on the notified chemical suggest the chemical is non-irritating to the skin and the eye. Analogue 2 was found to be slightly irritating to the skin and the eyes of rats.

No information on skin sensitization potential of the notified chemical was provided. Analogue 1 was found to be a skin sensitizer in a local lymph node assay (LLNA) in mice, with an EC3 value of 29%.

Repeated dose toxicity.
No repeated dose toxicity information was provided for the notified chemical. A combined repeated dose oral toxicity and reproduction/developmental toxicity screening test was conducted on rats using analogue 2. Adverse effects on the lung and brain were observed at the lowest dose tested and subsequently the NOAEL could not be determined for general toxicity. Based on the adverse effects observed, a Low Observed Adverse Effect Level (LOAEL) of ≤ 300 mg/kg bw was established by the study author. No adverse treatment related effects were reported on the reproduction and foetal development parameters and subsequently the NOAEL for reproductive/developmental effects was > 1,000 mg/kg bw/day.

Mutagenicity/Genotoxicity.
The notified chemical was non-mutagenic when tested using a bacterial reverse mutation assay (Ames Test). In vitro mammalian cell gene mutation tests carried out on mouse L5178Y lymphoma and Chinese hamster V79 cells using analogue 2 concluded that the analogue chemical was not genotoxic.

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the studies conducted on 2 analogue chemicals, the notified chemical may be slightly irritating to the skin and eyes and have adverse effects following repeated exposure. There is a potential for dermal, ocular and perhaps inhalation exposure of workers to the notified chemical at up to 60% concentration, during blending or addition of the notified chemical to the drilling fluid, equipment cleaning and maintenance. The notifier anticipates that the use of PPE such safety helmets, goggles, coveralls, impervious gloves and boots along with good general ventilation will minimize the exposure.

Overall, provided that control measures are in place to minimize worker exposure to the notified chemical, including the use of PPE and well ventilated environments, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
The public is not expected to be exposed to the notified chemical except in an event of an accident during road transport; hence the risk to the public is not considered unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported from overseas as a component of a formulation for end-use in Australia and will not be reformulated in Australia. Therefore, no environmental release is expected from the manufacture or reformulation of the notified chemical in Australia.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used in drilling mud during off-shore oil well drilling operations. Up to 6 tonnes of the notified chemical will be added to drilling mud for each well that is drilled. All drilling will be off-shore and a single drilling operation is expected to last between 1 - 6 weeks. During oil well drilling operations, drilling mud containing up to 2.4 % of the notified chemical will be pumped down the drill shaft during drilling of deep wells. The drilling mud will eventually be pushed out of the well and transferred to the surface for solids processing. This involves a low speed centrifugation in order to remove the drill cuttings. This drilling mud containing the notified chemical will be recovered and then replenished with additional mud containing more notified chemical and then transferred back into the well. Drill cuttings representing about 5-10% (assuming a common standard application scenario) of the material is transferred to the surface for separation. After separation, the drill cuttings will contain approximately 5% entrained drilling mud. Although it is possible for cuttings to be re-injected into the well or collected for on-shore disposal or re-use as general fill, it would appear that this is not generally practiced in Australia. Consequently, in the case of off-shore drilling, the cuttings (and the entrained mud) will be discharged into the ocean. Thus, 5% of the notified chemical that is used in drilling mud for each well (300 kg) will be released into the ocean with drill cuttings during drilling operations off-shore.

RELEASE OF CHEMICAL FROM DISPOSAL

The empty 200 L import containers are expected to be recycled or disposed of to landfill. Release from residue in import drums will be minimal (< 1% loss) as the product is a liquid and residues are expected to be either disposed of to landfill with empty containers or treated when drums are recycled. Accidental spills of the product are expected to be absorbed with inert absorbent material, swept up and placed into containers and disposed of to landfill.

7.1.2. Environmental Fate

The notified chemical is not expected to biodegrade rapidly based on to the provided study. For the details of the environmental fate studies please refer to Appendix C. Based on its low water solubility and limited bioavailability, the notified chemical is not expected to bioaccumulate.

In most circumstances, the notified chemical will be incorporated into the bulk drilling fluid and share the fate of the drilling mud. The mud systems will be pumped out of the wells for disposal, recycling or reuse after the completion of drilling operations. For off-shore application, the water-based mud solids may be disposed of to the ocean after use.

In landfill, the notified chemical is not expected to be mobile based on its low water solubility. In the ocean, the notified chemical is expected to disperse and biodegrade in the aqueous compartment, it is not expected to reach ecotoxicologically significant concentrations in the sediment compartment. In each case, the notified chemical is expected to ultimately degrade via biotic or abiotic pathways to form water and oxides of carbon and nitrogen.

7.1.3. Predicted Environmental Concentration (PEC)

Off-shore application

$PEC_{water,batch:}$

As direct discharge of the notified chemical into seawater is likely from off-shore use, the Predicted Environmental Concentration in seawater (PEC_{water}) has been calculated based on the CHARM model (Thatcher et al., 2005). Given that batchwise discharges are preferred, the conservative PEC water associated with batchwise discharges has been calculated as follows:

$$PEC_{water,batch} = \frac{M}{V_m} \times D_{batch}$$

where: $PEC_{water, batch} = PEC_{water}$ for batchwise discharges

M = 6,000 kg (mass of product discharged; upper range used as worst-case scenario);

V_m = 375 m^3 (volume mud discharged from a specific section; default value from Thatcher *et al*. 2005; p. 46) ; and

D_{batch} = 7.7 x 10^{-5} (batchwise discharge dilution factor; default value from Thatcher *et al*. 2005; p. 69).

The above equation represents the $PEC_{water,batch}$ for the notified chemical in water-based muds during batchwise discharges. Thus, the output of this equation (1.23 mg/L) was multiplied by 2.4% (*i.e.*, highest concentration of notified chemical) to determine the $PEC_{water,batch}$ for the notified chemical, 0.029 mg/L.

The $PEC_{sediment}$ for a batch-wise discharge scenario is not calculated in the CHARM model because there is assumed to be insufficient time to allow the establishment of equilibrium between the short-term levels of the notified chemical in the water column arising from batch-wise release of mud and the levels of the notified chemical in sediments near the discharge point. Furthermore, as the notified chemical is expected to disperse and biodegrade in the aqueous compartment, it is not expected to reach ecotoxicologically significant concentrations in the sediment compartment. Therefore, the $PEC_{sediment}$ has not been calculated for this assessment.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 100 mg/L (WAF)	Not harmful
Daphnia Toxicity	48 h EC50 > 100 mg/L (WAF)	Not harmful
Algal Toxicity	72 h EC50 > 100 mg/L (WAF)	Not harmful
Inhibition of bacterial respiration	10 d LC50 > 10,00 mg/kg soil	Not harmful

Based on the endpoints for toxicity of the notified chemical to aquatic organisms, the notified chemical is not considered to be harmful to aquatic organisms under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). Therefore, the notified chemical is not formally classified under the GHS.

Based on its measured acute toxicity, biodegradability and expected low bioaccumulation potential, the notified chemical is not formally classified under the GHS for chronic hazard.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) for the notified chemical in the water phase has been calculated and is presented in the table below. The PNEC is calculated based on the lower limits of the endpoints for the notified chemical (fish 96 h LC50 > 100 mg/L, daphnia > 100 mg/L or alga 72 h EC50 > 100 mg/L) and an assessment factor of 100. An assessment factor of 100 has been used as acute toxicity endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
LC50/EC50 (fish, daphnia or alga)	> 100	mg/L
Assessment Factor	100	
PNEC:	> 1	mg/L

7.3. Environmental Risk Assessment

Risk Assessment	PEC mg/L	PNEC mg/L	Q
Q - Ocean	0.029	> 1	**< 0.029**

The risk quotient (Q = PEC/PNEC) for the ocean environment is calculated to be < 0.01 for the water column.

The ratio suggests that the notified chemical is not expected to have a potential concern to aquatic organisms. Based on its low water solubility, the notified chemical is not expected to bioaccumulate in aquatic organisms.

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

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 33 ± 3 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Pour point method
Test Facility	Harlan (2013a)

Boiling Point > 184 °C at 101.6 kPa

Method	OECD TG 103 Boiling Point.
Remarks	Differential scanning calorimetric method. The notified chemical started decomposing from approximately 184 °C.
Test Facility	Harlan (2013a)

Density 984 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Pycnometer method.
Test Facility	Harlan (2013a)

Viscosity 8.46×10^5 mPa·s at 50.0 ± 0.5 °C and 5.96×10^4 mPa·s at 70 ± 0.5 °C

Method	OECD TG 114 Viscosity of Liquids.
Remarks	The test substance was Analogue 1. Rotational viscometer method
Test Facility	Harlan (2013b)

Vapour Pressure 2.4×10^{-6} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Vapour pressure balance method
Test Facility	Harlan (2013c)

Water Solubility < 2.0 10^{-4} g/L at 20 °C

Method	OECD TG 105 Water Solubility. EC Council Regulation No 440/2008 A.6 Water Solubility.
Remarks	Flask Method, analysed by HPLC.
Test Facility	Harlan (2013a)

Partition Coefficient (n-octanol/water) log Pow > 6.5 at 20 °C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Council Regulation No 440/2008 A.8 Partition Coefficient.
Remarks	HPLC Method
Test Facility	Harlan (2013a)

Flash Point 239 ± 2 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup method
Test Facility	Harlan (2013d)

Autoignition Temperature 408 ± 5 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.15 Auto-Ignition Temperature (Liquids and Gases).
Remarks	A carbolite flask heater was used.
Test Facility	Harlan (2013d)

Explosive Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	There are no structural alerts within the chemical structure of the notified chemical
Test Facility	Harlan (2013d)

Oxidizing Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	There are no structural alerts within the chemical structure of the notified chemical
Test Facility	Harlan (2013d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure.
- Species/Strain: Rat/RccHan™:WIST
- Vehicle: Dimethyl sulphoxide
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	1 Female	2,000	0/1
II	4 Female	2,000	0/4

- LD50: > 2,000 mg/kg bw
- Signs of Toxicity: Hunched posture and lethargy were noted during the day of dosing in one animal.
- Effects in Organs: No adverse macroscopic findings were recorded at necropsy.
- Remarks - Results: No mortalities were recorded. The gain in body weight during the study period was as expected.

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

TEST FACILITY: Harlan (2013e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Analogue 2

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test.
- Species/Strain: Rat/Crl:WI(Han)
- Vehicle: Cotton seed oil
- Type of dressing: Semi-occlusive.
- Remarks - Method: No significant protocol deviations. The test substance was heated up to 80 °C in order to ensure good skin contact, it was moistened with cotton seed oil on the patch. The test substance was held in contact with the skin for 24 hours.

RESULTS

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

- LD50: > 2,000 mg/kg bw
- Signs of Toxicity - Local: Mild erythema was observed in 3 female and 2 male rats. Desquamation was observed in all the rats. Eschar was seen in 1 female and 3 male rats. All the signs of irritation were reversible within the observation period.
- Signs of Toxicity - Systemic: Slight nasal discharge was observed in 1 female rat immediately after application of test substance and at 4 h after application in another female. This was attributed to stress during handling of animals by the study author. No other treatment related effects were observed.
- Effects in Organs: No adverse macroscopic findings were recorded at necropsy.
- Remarks - Results: No change in weight gains was observed during the 14-day period of study.

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

TEST FACILITY BSL (2010a)

B.3. Corrosion – skin (in vitro)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 431 In vitro Skin Corrosion - Human Skin Model Test
EpiSkin™ Reconstituted Human Epidermis Model

Vehicle None

Remarks - Method No significant protocol deviations. The test reports quality assurance statement and study director statement of GLP compliance were not signed. 50 µL of undiluted test substance was applied to the tissues in duplicate. Following exposure periods of 3, 60 and 240 minutes at room temperature, the tissues were rinsed, treated with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) (0.3 mg/mL) and incubated at 37 °C, 5% CO_2 for 3 hours. Following extraction, the optical densities were determined at 540 nm.
0.9% w/v sodium chloride and glacial acetic acid were used as negative and positive controls respectively. The controls were shared with another study and the exposure period was 240 minutes.
A preliminary test was conducted to assess the potential of the test substance to reduce MTT.

RESULTS

Test material	*3 minute exposure*		*60 minute exposure*		*240 minute exposure*	
	Mean OD_{540} of duplicate tissues	*Relative mean viability (%)*	*Mean OD_{540} of duplicate tissues*	*Relative mean viability (%)*	*Mean OD_{540} of duplicate tissues*	*Relative mean viability (%)*
Negative control	N.D.	N.D	N.D.	N.D	0.934	100
Test substance	1.030	110.3	0.979	104.8	0.974	104.3
Positive control	N.D.	N.D.	N.D.	N.D.	0.038	4.1

OD = optical density; N.D. = Not determined

Remarks - Results The test substance was not able to directly reduce MTT. The Relative mean viability (%) stayed above 35%, which was listed as the cut-off for considering the test substance as corrosive in the study report. The positive and negative controls gave satisfactory results, confirming the validity of the test system.

CONCLUSION The notified chemical was non-corrosive to the skin under the conditions of the test.

TEST FACILITY Harlan (2013f)

B.4. Irritation – skin (in vitro)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method.
EpiSkin™ Reconstituted Human Epidermis Model

Vehicle None

Remarks - Method No significant protocol deviations. The test reports quality assurance statement and study director statement of GLP compliance were not

signed. 10 µL of undiluted test substance was applied to the tissues in triplicate. Following an exposure period of 15 minutes at room temperature, the tissues were rinsed and incubated at 37 °C, 5% CO_2 for 42 hours. The tissues were then treated with MTT and incubated at 37 °C for 3 hours. Following extraction, the optical densities were determined at 562 nm.
Phosphate Buffered Saline with Calcium and Magnesium and Sodium Dodecyl Sulphate (5% w/v) were used as negative and positive controls respectively.
A preliminary test was conducted to assess the potential of the test substance to reduce MMT.

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.946	100	12.6
Test substance	0.706	74.6	4.5
Positive control	0.100	10.6	1.5

OD = optical density; SD = standard deviation

Remarks - Results
The test substance was not able to directly reduce MTT.
The test substance did not reduce the Relative mean Viability (%) of the tissues to < 50%, which was listed in the test report as being the cut-off for predicting the test substance was irritating.
The positive and negative controls gave satisfactory results, confirming the validity of the test system.

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

TEST FACILITY
Harlan (2013g)

B.5. Irritation – skin

TEST SUBSTANCE
Analogue 2

METHOD
OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 (male)
Vehicle: None
Observation Period: 72 hours
Type of Dressing: Occlusive
Remarks - Method: The test substance was applied with an occlusive dressing.

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	0.33	0.33	0.67	1	< 72 h	0
Oedema	0	0.33	0	1	< 48 h	0

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

Remarks - Results
Slight erythema was observed in all animals at the 24 h observation, this had cleared in two animals by the 48 h observation with the remaining animal being clear of irritation at the 72 h observation. One animal had slight oedema present at the 24 h observation, but was free of signs of irritation at the 48 h observation. No irritation was seen at 3 min or 1 h observations.

CONCLUSION The test substance is slightly irritating to the skin.

TEST FACILITY RBM (1999a)

B.6. Irritation – eye (in vitro)

TEST SUBSTANCE Notified chemical

METHOD Determination of Ocular Irritation Potential Using the SkinEthic Reconstituted Human Corneal Epithelium Model

Vehicle None

Remarks - Method The test reports quality assurance statement and study director statement of GLP compliance were not signed. 30 µL of test substance was applied to the tissues in triplicate. Following at 10 min exposure period at 37 °C, 5% CO_2, the tissues were rinsed and treated with MTT for 3 hours at 37 °C, 5% CO_2. Following extraction, the optical densities were determined at 562 nm.
Solution A containing Na_2HPO_4, glucose, HEPES, KCl and NaCl by SkinEthic and Sodium Dodecyl Sulphate (2% w/v) were used as negative and positive controls respectively.
A preliminary test was conducted to assess the potential of the test substance to reduce MMT.

RESULTS

Test material	*Mean OD_{562} of triplicate tissues*	*Relative mean viability (%)*
Negative control	0.940	100
Test substance	0.960	102.1
Positive control	0.063	6.7

OD = optical density

Remarks - Results The test substance was not able to directly reduce MTT. The relative mean tissue viability (5) was greater than 60%, which was listed in the study report as the cut-off for predicting the test substance was irritating. The positive and negative controls gave satisfactory results, confirming the validity of the test system.

CONCLUSION The notified chemical was considered to be non-irritating to the eye under the conditions of the test.

TEST FACILITY Harlan (2013h)

B.7. Irritation – eye

TEST SUBSTANCE Analogue 2

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.

Species/Strain Rabbit/New Zealand White

Number of Animals 3 (male)

Observation Period 72 hours

Remarks - Method No significant protocol deviations. Fluorescein staining was performed on test eyes 24 hour after test substance application.

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	0.33	1	2	< 72 h	0
Conjunctiva: chemosis	0.33	0	0	1	< 48 h	0

Conjunctiva: discharge	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
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.
N.D. = Not Determined

Remarks - Results: Slight to moderate conjunctival redness was observed in all rabbits at the 24 h observation, which had resolved in one rabbit by the 48 h observation and in the remaining two rabbits by the 72 h observation. Slight chemosis was observed in one rabbit at the 24 h observation but had cleared by the 48 h observation.

There were no deaths or test substance-related clinical signs during the study period.

CONCLUSION: The test substance is slightly irritating to the eye.

TEST FACILITY: RBM (1999b)

B.8. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE: Analogue 1

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain: Mouse/CBA/Ca (CBA/CaOlaHsd)
Vehicle: Acetone/olive oil (4:1 v/v)
Remarks - Method: No significant protocol deviations. A preliminary screening test to assess the irritancy potential of test substance at 50% concentration in vehicle was conducted.
α-Hexylcinnamaldehyde was used as a positive control. The control was not run in parallel and the stimulation index for 25% of control in acetone/olive oil (4:1) was reported to be 5.76.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1,858.37	-
10	1,897.51	1.02
25	4,893.45	2.63
50	8,827.08	4.75

Remarks - Results: Mild erythema was noted on days 3 to 6 in all animals treated with test substance at a concentration of 50%. There were no deaths and no signs of systemic toxicity were observed during the duration the study. Based on these results, the EC3 value was calculated to be 29%.

CONCLUSION: There was evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.

TEST FACILITY: Harlan (2013i)

B.9. Repeat dose toxicity

TEST SUBSTANCE: Analogue 2

METHOD: OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.
Species/Strain: Rat/Wistar Crl:WI

Route of Administration	Oral – gavage
Exposure Information	Total exposure days: male – 28-29 days and female – ≤ 54 days Dose regimen: 7 days per week
Vehicle	Corn oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10 F & 10 M	0	0/20
low dose	10 F & 10 M	300	0/20
mid dose	10 F & 10 M	600	2/20
high dose	10 F & 10 M	1000	0/20

Mortality and Time to Death

One male and 1 female rat from mid dose group died. The deaths were reported to be not treatment related by the study authors.

Clinical Observations

Clinical observations noted were salivation, nibbling of fur, nasal discharge, piloerection, vocalization, regurgitation and pushing of the bedding. There was a statistically significant reduction in weight gain in female animals in the mid dose group during premating days 7-14. The clinical signs were random and seen in control group also.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No statistically significant effects on haematology, clinical chemistry and urinalysis parameters were observed at any test doses.

Effects in Organs

Lung discoloration and hardening was observed in rats from all dose groups. Increase in relative brain weight was observed in female rats from low and high dose groups.

Effects on 1st Filial Generation (F1)

Slight but statistically significant ($p < 0.05$) decreases (14% and 13%) were seen in the group mean litter weights in the mid and high dose groups respectively at post natal day 4. Statistically significant decreases in body weight were not seen at post natal day 0. Statistically significant decreases were also observed in the % of pre implantation loss in the mid and high dose groups. The study authors considered the changes to be incidental and not treatment related. No other statistically significant changes were seen in the litters.

Remarks – Results

Due to the histopathological changes seen in the lungs at all the doses, a No Observed Adverse Effect Level (NOAEL) for general toxicity could not be determined.

CONCLUSION

A Lowest Observed Adverse Effect Level (LOAEL) was established at ≤ 300 mg/kg bw/day due to the adverse effects seen on lungs with the lowest dose tested.

A No Observed Adverse Effect Level (NOAEL) for reproductive/developmental toxicity was established at > 1000 mg/kg bw/day due to an absence of observed effects at any dose levels on reproductive/developmental parameters.

TEST FACILITY BSL (2010b)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure and Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98 & TA100 *E. coli*: WP2uvrA

Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 50 – 5,000 µg/plate b) Without metabolic activation: 50 – 5,000 µg/plate
Vehicle	Acetone
Remarks - Method	No significant protocol deviations. N-ethyl-N'-nitrosoguanidine, 9-Aminoacridin and 4-Nitroquinoline-1-oxide were used as positive controls for tests without metabolic activation and 2-Aminoanthracine and Benzo(a)pyrene were used as positive controls for test with metabolic activation. Test 1 used the plate incorporation method and test 2 the pre-incubation method.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 5,000	≥ 5,000	Negative
Test 2	> 5,000	≥ 1,500	Positive
Present			
Test 1	> 5,000	≥ 5,000	Negative
Test 2	> 5,000	≥ 1,500	Positive

Remarks - Results	Slight but significant increase in the frequency of revertant colonies was observed in test 2 with and without metabolic activation with bacterial strains TA1537 and TA98. According to the study author, the positive results were deemed to be false positive due to the lack of a dose response and reproducibility and the fact that the number of revertant colonies at statistically significant dose levels were within the in-house historical untreated/vehicle controls. The positive controls produced satisfactory responses, thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.
CONCLUSION	The notified chemical was considered not mutagenic to bacteria under the conditions of the test by the study author.
TEST FACILITY	Harlan (2013j)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE	Analogue 2
METHOD	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain	Mouse
Cell Type/Cell Line	L5178Y Lymphoma
Metabolic Activation System	S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Vehicle	None
Remarks - Method	No significant protocol deviations. Ethylmethanesulfonate and methylmethansulfonate were used as positive controls in absence of metabolic activation and benzo[a]pyrene was as positive control in presence of metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	20, 50, 100, 200, 400, 500, 600, 700	4 h	24 h
Test 2	2, 10, 40, 70, 100, 140, 180, 220	24 h	24 h
Present			
Test 1	200, 400, 600, 800, 1000, 1300, 1500, 1700	4 h	24 h
Test 2	10, 30, 75, 150, 300, 500, 700, 1000	4 h	24 h

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 625	≥ 500	≥ 20	Negative
Test 2		≥ 100	≥ 2	Negative
Present				
Test 1	≥ 1250	≥ 1300	≥ 200	Negative
Test 2		≥ 1000	≥ 75	Negative

Remarks - Results: No significant increases in mutant cells were seen in any of the test groups. The positive controls produced satisfactory response, thus confirming the activity of the S9-mix and the validity of the assay.

CONCLUSION: The test substance was not mutagenic to mouse L5178Y lymphoma cells treated in vitro under the conditions of the test.

TEST FACILITY: BSL (2010c)

B.12. Genotoxicity – in vitro

TEST SUBSTANCE: Analogue 2

METHOD: OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain: Chinese Hamster
Cell Type/Cell Line: V79
Metabolic Activation System: S9 fraction from phenobarbitone/β-naphthoflavone induced rat liver
Vehicle: None
Remarks - Method: No significant protocol deviations. Ethylmethanesulfonate and cyclophosphamide were used as positive controls in absence and presence of metabolic activator respectively.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	125, 250, 500, 1000*, 2500*, 5000*	4 h	24 h
Test 2	31.3, 62.5, 125, 250*, 500*, 1000*, 2500, 5000	20 h	20 h
Present			
Test 1	125, 250, 500, 1000*, 2500*, 5000*	4 h	24 h
Test 2	500, 1000, 2000, 3000*, 4000*, 5000*	4 h	24 h

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	> 5000	≥ 125	Negative
Test 2	> 5000	≥ 31.3	Negative
Present			
Test 1	> 5000	≥ 125	Negative
Test 2	> 5000	≥ 500	Negative

Remarks - Results: The preliminary toxicity study conducted showed no reduction in cell density at any test concentrations up to 5,000 μg of test substance.
In experiment 2 a reduction of 53% in mitotic index was observed at 1000 μg test substance concentration when compared to control.
Test substance precipitation was noted at all test concentrations.

	No statistically or biologically significant increases in the percentage of aberrant cells, above the vehicle control values, were recorded for any cultures treated with the test substance in either the presence or absence of metabolic activation. Positive controls confirmed the sensitivity of the test system.
CONCLUSION	The test substance was not clastogenic to V79 treated in vitro under the conditions of the test.
TEST FACILITY	BSL (2010d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test.
Inoculum	Activated sludge
Exposure Period	29 days
Auxiliary Solvent	None
Analytical Monitoring	Inorganic Carbon Analysis (ICA)
Remarks - Method	Tested in accordance with the test guideline without significant deviation from the protocol. Good Laboratory Practices (GLP) was followed.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	1	2	52
6	15	6	55
8	22	8	62
10	27	10	61
14	45	14	64
21	43	21	70
28	53	28	92
29*	57	29	94

* Day 29 values corrected to include any carry-over of CO_2

REMARKS-RESULTS	The validity criteria for the test were satisfied. The test substance attained 57% degradation after 29 days, however, the 10-day window criteria was not satisfied. The test substance was not considered to be readily biodegradable. The degradation of the toxicity control was 74% over 29 days, implying that the test substance was not toxic to the sewage treatment microorganisms used in the study.
CONCLUSION	The notified chemical is not considered to be readily biodegradable.
TEST FACILITY	Harlan (2013k)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static
Species	Zebra fish (*Danio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	10-250 mg $CaCO_3$/L
Analytical Monitoring	High Performance Liquid Chromatography (HPLC0
Remarks – Method	Tested in accordance with the test guideline without significant deviation from the protocol. Good Laboratory Practices (GLP) was followed.

RESULTS

Concentration mg/L *Nominal*	*Number of Fish*	*Mortality* *24 h*	*48 h*	*72 h*	*96 h*
Control	20	0	0	0	0
100	20	0	0	0	0

LC50	> 100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. The highest test concentration resulting in 0% mortality was determined to be 100 mg/L. The results were based on nominal concentration as the actual concentrations for the test substance were not determined. There was neither mortality nor abnormal behaviour fish observed for either the control or the test groups.
CONCLUSION	The notified chemical is not harmful to fish
TEST FACILITY	ChemService (2010a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – Static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	High performance Liquid Chromatography Mass Spectrum (HPLC-MS)
Remarks - Method	The test was carried out according to the test guideline above without significant deviation from the protocol. Good Laboratory Practices (GLP) was followed. Due to the low aqueous solubility and complex nature-of the test substance, for the purposes of the study the test medium was prepared as a Water Accommodated Fraction (WAF) of the test substance.

RESULTS

Concentration mg/L *Nominal*	*Number of D. magna*	*Number Immobilised* *24 h*	*48 h 21 d*
Control	10	0	0
100	10	0	0

Remarks – Results	The highest test concentration was determined to be 100 mg/L. The result was based on nominal concentration as the actual concentrations for the test substance were not determined.
EL50	> 100 mg/L at 48 hours (WAF)
NOEL	100 mg/L at 48 hours (WAF)
CONCLUSION	The notified chemical is not harmful to aquatic invertebrates.
TEST FACILITY	Harlan (2013l)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical

METHOD	OECD TG 201 Alga, Growth Inhibition Test. EC Council Regulation No 440/2008 C.3 Algal Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	High performance Liquid Chromatography Mass Spectrum (HPLC-MS)
Remarks - Method	The test was carried out according to the test guideline above without significant deviation from the protocol. Good Laboratory Practices (GLP) was followed. Due to the low aqueous solubility and complex nature-of the test substance, for the purposes of the study the test medium was prepared as a Water Accommodated Fraction (WAF) of the test substance.

RESULTS

Biomass(WAF)		*Growth(WAF)*	
EbL50	*NOEL*	*ErL50*	*NOEL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 100	100	>100	100

Remarks – Results	All validity criteria for the test were satisfied. The highest test concentration was determined to be 100 mg/L. The result was based on the nominal concentration as the actual concentrations for the test substance were not determined.
CONCLUSION	The notified chemical is not harmful to algae.
TEST FACILITY	Harlan (2013m)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10.0, 17.8, 31.6, 56.2 and 100 mg/L
Remarks – Method	The test was conducted according to the guidelines above and good laboratory practice (GLP) principles. No significant deviations from the test guidelines were reported.
RESULTS	
EC50	> 100 mg/L
Remarks – Results	All validity criteria for the test were satisfied. The EC50 was out of the tested concentration range (> 100 mg/L).
CONCLUSION	The notified chemical is not expected to inhibit microbial respiration.
TEST FACILITY	ChemService (2010b)

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