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March 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in Emul S50

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/1548	Newpark Drilling Fluids (Australia) Limited	Chemical in Emul S50	Yes	≤ 80 tonnes per annum	Drilling fluid additive

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Skin Irritation (Category 2)	H315 – Causes skin irritation
Skin Sensitisation (Category 1)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrases:

R38: Irritating to skin
R43: May cause sensitisation by skin contact

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

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

Environmental risk assessment
Based on the low hazard and reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Skin Irritation (Category 2): H315 – Causes skin irritation
  - Skin Sensitisation (Category 1): H317 – May cause an allergic skin reaction

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

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following isolation and engineering controls to minimise occupational exposure to the notified chemical:
  - 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

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:
  - Coveralls
  - Impervious gloves

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

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

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

Disposal

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

Storage

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

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent 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 a drilling fluid additive, 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 products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Newpark Drilling Fluids (Australia) Limited (ABN: 11 099 949 452)
11 Alacrity Place
Henderson WA 6166

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, impurities, additives/adjuvants, use details and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Emul S50 (< 80% notified chemical)

MOLECULAR WEIGHT
> 500 Da

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

3. COMPOSITION

DEGREE OF PURITY
> 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: amber viscous liquid

Property	Value	Data Source/Justification
Pour Point	30 ± 3 °C	Measured
Boiling Point	> 400 °C at 102.2-102.3 kPa	Measured
Density	1,010 kg/m^3 at 20 °C	Measured
Viscosity	1.81 x 10^6 mPa.s at 20 ± 0.5 °C 1.31 x 10^5 mPa.s at 40 ± 0.5 °C	Measured
Vapour Pressure	2.3 x 10^{-6} kPa at 25 °C	Measured
Water Solubility	3.2 x 10^{-8} g/L at 20 °C	QSAR KSKOWWIN, Version 1.41a
Hydrolysis as a Function of pH	Not determined	Not expected as the notified chemical does not contain readily hydrolysable functionalities.
Partition Coefficient (n-octanol/water)	Log K_{ow} = 11 at 20 °C	QSAR KOWWIN, Version 1.67a
Adsorption/Desorption	Log K_{oc} = 6.9-9.5	QSAR KOCWIN, Version 2.00

Dissociation Constant	Not determined	Contains ionisable functionalities. Therefore, the notified chemical is expected to be ionised at the environmental pH range of 4 – 9.
Flash Point	120 ± 2 °C at 101.3 kPa	Measured
Autoignition Temperature	416 ± 5 °C	Measured
Explosive Properties	Predicted negative	Contains no functional groups that would imply explosive properties
Oxidising Properties	Predicted negative	Contains no functional groups that would imply oxidative properties

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported into Australia as a component of Emul S50 at < 80% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 80	≤ 80	≤ 80	≤ 80	≤ 80

PORT OF ENTRY
Perth, Fremantle, Sydney and Melbourne

IDENTITY OF MANUFACTURER/RECIPIENTS
Newpark Drilling Fluids (Australia) Limited

TRANSPORTATION AND PACKAGING
The product containing the notified chemical (at < 80% concentration) will be imported by sea in 1000 L Tote tanks or in steel/plastic drums (200 L). The imported product will be transferred to stainless steel tanks (~ 1000 L) for transport offshore. The products containing the notified chemical are expected to be transported within Australia by road or rail.

USE
The notified chemical will be used as a drilling fluid additive (at < 3% concentration) in the oil and gas industry for offshore drilling applications.

OPERATION DESCRIPTION
The imported product (< 80% notified chemical) will be transferred (via a liquid 'drum pump') into stainless steel tanks prior to transport offshore. At offshore sites, the imported product containing the notified chemical will be transferred into the drilling fluid. The drilling fluid (< 3% notified chemical) will then be pumped via a largely 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 and the mud containing the notified chemical 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

Transport and storage workers are unlikely to be exposed to the notified chemical, except in the unlikely event of an accident.

Workers may have dermal and ocular exposure to the notified chemical (at < 80% concentration) during transfer processes (including connecting and disconnecting of hoses/pipes) and during cleaning and maintenance tasks (e.g. changing drill bits). Inhalation exposure is not expected. Exposure is expected to be limited by the use of enclosed and/or automated processes, where possible, and by the use of personal protective equipment (PPE), such as gloves, protective clothing and eye protection.

6.1.2. Public Exposure

The notified chemical is intended to be used in industrial settings only. Therefore, exposure of the public to the notified chemical is not expected.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Skin irritation (in vitro)	irritating
Skin corrosion (in vitro)	not corrosive
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 – 14 days	NOAEL = 1000 mg/kg bw/day
Rat, combined repeat dose oral toxicity with reproduction/developmental toxicity screen	NOAEL = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non-mutagenic
Genotoxicity – in vitro chromosome aberration	non-clastogenic
Genotoxicity – in vitro gene mutation assay	non-mutagenic

Toxicokinetics.

No data on the toxicokinetics of the notified chemical were provided. Absorption of the notified chemical across the gastrointestinal tract and skin is expected to occur, but the extent may be limited by the predicted partition coefficient (log K_{ow} = 11), water solubility (3.2 x 10^{-8} g/L) and the molecular weight (680-760 Da) of the notified chemical. The notified chemical may be absorbed via the respiratory tract.

Acute toxicity.

The notified chemical was of low acute oral (LD50 > 2000 mg/kg bw) and dermal (LD50 > 2000 mg/kg bw) toxicity in rats. No acute inhalation toxicity data were provided for the notified chemical.

Irritation.

Two *in vitro* studies were conducted using the reconstructed human epidermis model to determine the skin irritation/corrosion potential of the notified chemical. The *in vitro* skin irritation study indicated that the notified chemical was an irritant (relative mean viability of 10.2%). The *in vitro* skin corrosion study indicated that the notified chemical was not corrosive to the skin under the conditions of the test, based on the criteria used.

An *in vitro* ocular irritation study using the reconstituted human corneal epithelium model indicated that the notified chemical was non-irritating to the eyes. However, the notified chemical was a slight eye irritant in rabbits

Skin sensitisation.

The notified chemical was a skin sensitiser in a local lymph node assay (LLNA) in mice (EC3 = 3%).

Repeated dose toxicity and reproduction/developmental toxicity.
In a 14-day repeated dose gavage study, rats (3/sex/dose) were treated at 0, 250, 500 or 1000 mg/kg bw/day. The no observed adverse effect level (NOAEL) was established as 1000 mg/kg bw/day, based on the lack of treatment related adverse effects.

In a combined repeat dose and reproduction/developmental toxicity screening gavage study, rats (12/sex/dose) were treated at 0, 30, 300 or 1000 mg/kg bw/day. The NOAEL for systemic and reproductive toxicity was established by the study authors as 1000 mg/kg bw/day, based on the lack of toxicologically significant effects.

Mutagenicity/Genotoxicity.
The notified chemical was not mutagenic in a bacterial reverse mutation study or an *in vitro* mammalian cell gene mutation test. The notified chemical was not clastogenic to human peripheral blood lymphocytes in an *in vitro* chromosome aberration study.

Health hazard classification
Based on the available information, the notified chemical is 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. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Skin Irritation (Category 2)	H315 – Causes skin irritation
Skin Sensitisation (Category 1)	H317 – May cause an allergic skin reaction

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R38: Irritating to skin
R43: May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Workers may be exposed to the notified chemical at < 80% concentration. At such concentrations, the notified chemical presents a concern for skin irritation and sensitisation effects. Dermal exposure is expected to be limited by the use of enclosed and/or automated processes, where possible, and by the use of PPE. Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health
As exposure of the public to the notified chemical is not expected, the risk to the public from use of the chemical is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will not be manufactured or reformulated in Australia. Therefore, release of the notified chemical from these activities is not expected. Release of the notified chemical to the environment during import, storage, and transport is also unlikely. Release from residues in storage and shipping containers is expected to be minimal.

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 with the majority added initially and smaller amounts added throughout the drilling operation. 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

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 will involve a sifting step along with low speed centrifugation in order to remove the drill cuttings. The drilling mud containing the notified chemical will be recovered and then replenished with additional mud containing more notified chemical and then transferred back down into the well. The drill cuttings that represent about 5-10% (assuming a common standard application scenario) of the material transferred to the surface will contain some adhered drilling mud. 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) is expected to be released into the ocean with drill cuttings during drilling operations off-shore.

RELEASE OF CHEMICAL FROM DISPOSAL

The empty 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 properly in the drums recycling process. 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 readily biodegradable based on the results of a laboratory test (0% over 28 days). However, the test results for biodegradability studies in seawater have not been provided by the notifier. While the low molecular weight may suggest potential for bioaccumulation, the presence of potentially cationic ions is expected to significantly reduce the bioaccumulation potential. Given the presence of potentially cationic functional groups and a high adsorption/desorption coefficient (log Koc = 6.9 - 9.5), the notified chemical is expected to bind strongly to soil and/or sediment soon after entering the water/sediment system.

In offshore application, most of the notified chemical is expected to be either re-injected into wells or released into ocean after use. The worst case scenario is that 5% of the total import volume of the notified chemical will be discharged to the ocean directly. Based on its calculated high log Koc and low water solubility, the notified chemical released to the ocean is expected to sorb to particulates and sediment. In all cases, 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)

The notified chemical will be used as a drilling fluid additive (at < 3% concentration) in the oil and gas industry for offshore drilling applications. The standard risk assessment procedure (modelling using CHARM by Thatcher et al., 2005) cannot be used in these cases to derive the predicted environmental concentration (PEC). This is because CHARM does not consider drilling chemicals containing organic phase fluids (oil based and synthetic based fluids).

A predicted environmental concentration (PEC) has not been calculated in this assessment. Based on the assessed used pattern, the amount of the notified chemical expected to be discharged to the sea is potentially significant. The notifier has advised that 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. Once the notified chemical reaches the sea, it is expected to disperse. Since the notified chemical has very low water solubility and potential cationicity, the remaining notified chemical is expected to bind to sediment and be removed from the seawater column. Hence, the notified chemical is not expected to reach ecotoxicologically significant concentrations in the marine environment.

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	LC50 (96 h) > 100 mg/L	Not harmful to fish
Daphnia Toxicity	EC50 (48 h) > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	E_rC50 (72 h) > 100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	EC50 (3 h) > 1,000 mg/L	Not inhibitory to bacterial respiration

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 the chronic hazard.

The notified chemical is expected to be used in offshore oil and gas operations. However, ecotoxicity studies and data for marine (saltwater) fish, marine aquatic invertebrates and marine algae have not been provided.

7.2.1. Predicted No-Effect Concentration

A predicted no effect concentration (PNEC) has not been calculated as the notified chemical is not considered to be harmful to aquatic biota up to the limit of its solubility in water.

7.3. Environmental Risk Assessment

A risk quotient RQ (PEC/PNEC) has not been derived since neither the PEC nor the PNEC is calculated. The notified chemical is expected to degrade in soil/sediment, although it is expected to be neither readily biodegradable, nor be bioaccumulative. Based on the low hazard and the assessed use pattern of the notified chemical, it is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 30 ± 3 °C

Method	OECD TG 102 Melting Point/Melting Range.
Remarks	Pour point method.
Test Facility	Harlan (2012a)

Boiling Point > 400 °C at 102.2-102.3 kPa

Method	OECD TG 103 Boiling Point.
Remarks	Determined by differential scanning calorimetry. No definitive signs of boiling or decomposition were noted.
Test Facility	Harlan (2012a)

Density 1,010 kg/m^3 at 20 °C

Method	OECD TG 109 Density of Liquids and Solids.
Remarks	Pycnometer method. Due to the viscosity of the test substance, glass tubes were used. The test substance was heated to ~50 °C to aid filling the tubes (prior to equilibration at 20 °C).
Test Facility	Harlan (2012a)

Viscosity 1.81×10^6 mPa.s at 20 ± 0.5 °C
1.31×10^5 mPa.s at 40 ± 0.5 °C

Method	OECD TG 114 Viscosity of Liquids.
Remarks	Rotational viscometer method.
Test Facility	Harlan (2013a)

Vapour Pressure 2.3×10^{-6} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure.
Remarks	Vapour pressure balance method.
Test Facility	Harlan (2012b)

Flash Point 120 ± 2 °C at 101.3 kPa

Method	EC Council Regulation No 440/2008 A.9 Flash Point.
Remarks	Closed cup
Test Facility	Harlan (2012c)

Autoignition Temperature 416 ± 5 °C

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

Explosive Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks	Predicted negative based on the chemical structure
Test Facility	Harlan (2012c)

Oxidizing Properties Predicted negative

Method	EC Council Regulation No 440/2008 A.21 Oxidizing Properties (Liquids).
Remarks	Predicted negative based on the chemical structure
Test Facility	Harlan (2012c)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified Chemical

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure.
- Species/Strain: Rat/Wistar
- Vehicle: Arachis oil BP
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1 F	2000	0/1
2	4 F	2000	0/4

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: None observed.
- Effects in Organs: No gross abnormalities observed.

CONCLUSION: The notified chemical is of low toxicity via the oral route.

TEST FACILITY: Harlan (2013b)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity.
- Species/Strain: Rat/Wistar
- Vehicle: Arachis oil BP used to moisten test substance
- Type of dressing: Semi-occlusive.
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 M/5 F	2000	0/10

- LD50: > 2000 mg/kg bw
- Signs of Toxicity - Local: Very slight erythema was observed in all animals but resolved within 7 days. Other local signs were small superficial scattered scabs, crust formation, slight desquamation and scab lifting to reveal glossy skin. A possible clipping abrasion and scratching caused by the animal were observed in one male after dosing. Physical damage and possible animal scratch were observed in another male at all observations.
- Signs of Toxicity - Systemic: Two males had a body weight loss or no body weight gain over the first week of the observation but gained weight over the second week. No other signs of toxicity were observed.
- Effects in Organs: No gross abnormalities observed.

CONCLUSION: The notified chemical is of low toxicity via the dermal route.

TEST FACILITY: Harlan (2013c)

B.3. Irritation – skin (in vitro)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 439 In vitro Skin Irritation: Reconstructed Human Epidermis Test Method.

Remarks - Method EpiSkin model.

To examine whether the test substance interferes with or reduces the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye, a pre-test was conducted where 10 µL was added to 2 mL of 0.3 mg/mL MTT dye solution, incubated in the dark at 37°C in 5% CO_2 in air for 3 hours, with an untreated MTT solution used as a control. There was no evidence of a colour change.

Triplicate tissues were treated in a similar manner to the test substance with positive (5% SDS) and negative (Dulbecco's phosphate-buffered saline [DPBS]) controls. Prior to the post-treatment incubation, tissues were washed with DPBS with Ca^{2+} and Mg^{2+}, although due to its viscosity the test substance could not be completely removed. Optical density (OD_{540}) was measured following formazan extraction.

RESULTS

Test material	*Mean OD_{540} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability (%)*
Negative control	0.883	100	9.8
Test substance	0.090	10.2	5.8
Positive control	0.053	6.0	2.1

OD = optical density; SD = standard deviation

Remarks - Results The positive and negative controls produced results within the acceptance criterion specified in the test guidelines.

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

TEST FACILITY Harlan (2012d)

B.4. Corrosion – skin (in vitro)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 431 In vitro Skin Corrosion – Reconstructed Human Epidermis (RHE) Test Method (2004).

Remarks - Method EpiSkin model.

To examine whether the test substance interferes with or reduces the MTT dye, a pre-test was conducted where 50 µL was added to 2.2 mL of 0.3 mg/mL MTT dye solution, incubated in the dark at room temperature for 3 hours, with an untreated MTT solution used as a control. There was no evidence of a colour change.

Duplicate tissues were treated with 50 µL of positive (glacial acetic acid) or negative (0.9% sodium chloride) controls for 240 minutes. All tissues were washed with DPBS with Ca^{2+} and Mg^{2+} following exposure, although due to its viscosity the test substance could not be completely removed. OD_{540} was measured following formazan extraction (note: the current test guideline, adopted 2014, indicates that optical density should be measured at 545-595 nm).

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	-	-	-	-	0.201	100
Test substance	0.138	68.7	0.195	97.0	0.149	74.1
Positive control	-	--	-	-	0.018	9.0

OD = optical density

Remarks - Results

The positive control gave satisfactory results. The negative control was reported as providing an acceptable optical density measurement (according to the laboratories acceptance criteria, i.e. $OD_{540} \geq 0.115$ and ≤ 0.400), however, the current test guideline (adopted 2014) notes that the acceptability criteria for the negative control are ≥ 0.6 and ≤ 1.5 for the mean OD value of the EpiSkin test method. Therefore, the results of this study should be treated with caution.

CONCLUSION

The notified chemical was not corrosive to the skin under the conditions of the test, based on the criteria used.

TEST FACILITY

Harlan (2013d)

B.5. Irritation – eye (in vitro)

TEST SUBSTANCE

Notified chemical

METHOD

Non-guideline study – Determination of Ocular Irritation Potential Using the SkinEthic Reconstituted Human Corneal Epithelium Model.

Remarks - Method

To examine whether the test substance reduces the MTT dye, a pre-test was conducted where 30 µL was added to 1 mL of 0.5 mg/mL MTT dye solution, incubated at 37 °C in 5% CO_2 in air for 3 hours, with an untreated MTT solution used as a control. There was no evidence of a colour change.

The tissues were incubated overnight at 37 °C in 5% CO_2 in air. In the main study, triplicate tissues were treated with 30 µL of the test item for 10 minutes at 37 °C in 5% CO_2 in air. triplicate tissues were treated identically with negative (0.142 g/L Na_2HPO_4, 1.802 g/L glucose, 7.149 g/L HEPES, 0.224 g/L KCl, 7.597 g/L NaCl) and positive (2% SDS) controls. Tissues were washed with DPBS without Ca^{2+} and Mg^{2+} following exposure, although due to its viscosity the test substance could not be completely removed. Following rinsing, 2 tissues/group were then treated with 300 µL of 0.5 mg/mL MTT solution and incubated at 37 °C for 3 hours, with the remaining tissue retained for histopathology, if necessary. Optical density (OD_{540}) was measured following extraction.

The test substance was considered by the study authors to be an irritant if the relative mean tissue viability was < 60%.

RESULTS

Test material	*Mean OD_{540} of duplicate tissues*	*Relative mean viability (%)*
Negative control	0.792	100
Test substance	0.479	60.5
Positive control	0.202	25.5

OD = optical density

Remarks - Results	The positive control demonstrated a sufficiently positive response.
CONCLUSION	The notified chemical was considered to be non-irritating to the eye under the conditions of the test.
TEST FACILITY	Harlan (2012e)

B.6. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	2 males
Observation Period	72 hours
Remarks - Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*		*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2			
Conjunctiva: redness	0.7	0.7	1	< 72 hours	0
Conjunctiva: chemosis	0.3	0.3	1	< 48 hours	0
Conjunctiva: discharge	0	0	1	< 24 hours	0
Corneal opacity	0	0	0	-	0
Iridial inflammation	0	0	0	-	0

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

Remarks - Results	Minimal conjunctival redness and chemosis were observed in both animals with resolution by 72 hours. Residual test substance was noted around the treated eyes of both animals at all observations.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Harlan (2013e)

B.7. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay.
Species/Strain	Mouse/ CBA/Ca (female)
Vehicle	Acetone:olive oil (4:1)
Remarks - Method	In a preliminary study, 1 mouse per group was treated with 25 µL of test substance at 5, 10, 25 or 50% for three consecutive days. No signs of toxicity were observed. Very slight erythema was observed in the animals treated at 5, 10 and 50%. A greater than 25% increase in the mean ear thickness was observed in animals treated at 10, 25 and 50%. A 5% concentration was selected as the highest concentration for the main study. The main study was conducted using 4 mice/group at 1, 2.5 or 5%. A vehicle control group was conducted using 4 mice/group. A positive control study was conducted in the past four months and confirmed the validity of the test system.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	2980	-
1	6225	2.09
2.5	8575	2.88
5	15358	5.15

Remarks - Results: There were no signs of toxicity observed in the main study. Mild redness on the ears was noted in animals treated at 5%.

The EC3 value was calculated to be 3%.

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

TEST FACILITY: Harlan (2013f)

B.8. Repeat dose toxicity

TEST SUBSTANCE: Notified chemical

METHOD: Non-guideline study.

Species/Strain: Rat/Wistar

Route of Administration: Oral – gavage

Exposure Information: Total exposure days: 14 days

Vehicle: Arachis oil BP

Remarks - Method: In a 14 day repeated dose oral gavage study, rats (3/sex/dose) were treated with the notified chemical at 0, 250, 500 or 1000 mg/kg bw/day. Mortality, clinical signs of toxicity, body weight, and food and water consumption were recorded. All animals were subject to gross necropsy.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	3 M + 3 F	0	0/6
low dose	3 M + 3 F	250	0/6
mid dose	3 M + 3 F	500	0/6
high dose	3 M + 3 F	1000	0/6

Clinical Observations

There were no treatment related clinical signs of toxicity. Body weight, body weight gain and food consumption were similar in treated and control groups. Water consumption was generally increased in males and females treated with the test substance. The relevance of this finding is unclear but is not considered to represent a toxicologically adverse effect.

Effects in Organs

There were no treatment related gross abnormalities noted at necropsy. There was a single observation of increased pelvic space of the right kidney in one female treated at 250 mg/kg bw/day. This finding is considered incidental in the absence of a dose response.

CONCLUSION

The NOAEL was established as 1000 mg/kg bw/day in this study, based on the lack of treatment related adverse effects.

TEST FACILITY: Harlan (2014a)

B.9. Repeat dose toxicity and reproduction/developmental toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.
Species/Strain	Rat/Wistar
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: < 8 weeks
Vehicle	Arachis oil BP
Remarks - Method	In a combined repeated dose and reproduction/developmental toxicity study, rats (12/sex/dose) were treated with the notified chemical at 0, 30, 300 or 1000 mg/kg bw/day. Animals were paired for mating on day 15 for a maximum of two weeks. Females were allowed to maintain their offspring until day 5 post partum. Blood was taken from males on day 42 and from females on day 4 post partum, , with scheduled kill the following day. Females on day 4 post partum and males during week 6 (5/sex/dose) were assessed for functional performance and sensory reactivity. Behavioural assessments were conducted weekly.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	12 M + 12 F	0	0/24
low dose	12 M + 12 F	30	0/24
mid dose	12 M + 12 F	300	0/24
high dose	12 M + 12 F	1000	0/24

Clinical Observations

There were no treatment related clinical signs of toxicity. There were no treatment related changes in absolute body weights, body weight gains, food consumption or food efficiency in males. Females treated at 1000 mg/kg bw/day had statistically significant increases in absolute body weights during gestation, with statistically significant increases in body weight gain in females treated at 30 and 1000 mg/kg bw/day during gestation (↑15% and ↑18%, respectively), with a non-statistically significant increase in females treated at 300 mg/kg bw/day (↑8%). No clear dose response was observed. Furthermore, females treated at 300 and 1000 mg/kg bw/day had statistically significant increases in food consumption during the second (↑11% and ↑12%, respectively) and third (↑28% and ↑27%, respectively) weeks of gestation. Food efficiency was not provided for the gestation period. Whilst these data may demonstrate an effect of the test substance towards increased weight gain in gestating females, it is difficult to attribute these effects to treatment with the test substance due to the lack of a clear dose related trend. There were no treatment related changes in water consumption for either males or females.

There were no changes in weekly behavioural assessments, or on functional performance or sensory reactivity assessments in males or females.

Laboratory Findings – Clinical Chemistry and Haematology

There were statistically significant decreases in haematocrit in all treated males but the changes were small with no dose response and were within expected ranges. There was a statistically significant increase in activated partial thromboplastin time in females treated at 1000 mg/kg bw/day but was not considered to be of toxicological importance by the study authors due to the lack of a dose response. There were no other treatment related haematological changes.

In all treated males, statistically significant increases in chloride concentration and statistically significant decreases in creatinine were observed. There were statistically significant decreases in calcium concentration in all groups of treated females. The changes were not considered by the study authors to be toxicologically significant as they are within the expected range for this strain and age of rat. There were non-statistically significant decreases in glucose concentration in all treated groups of males but this finding is not considered to be toxicologically significant. There were no other treatment related clinical chemistry changes.

Effects in Organs

There were no treatment related macroscopic findings in males or females. Statistically significant increases in absolute ovary (↑16%) and pituitary weights (↑26%) were observed in females treated at 1000 mg/kg bw/day, with associated statistically significant increases in relative weights. Additionally, there were statistically significant decreases in absolute thyroid/parathyroid weights in all treated females (↓25-31%) but there was no dose response. These changes are not considered to be toxicologically significant based on the lack of associated histopathological findings.

There were no treatment related microscopic findings in males or females. Qualitative examination of the stages of spermatogenesis in the testes did not reveal any treatment related abnormalities in the integrity of the various cell types present at different stages of the sperm cycle.

Reproductive/Developmental Performance

There were no treatment related changes in the mating or pregnancy indices, gestation length or parturition index. In the group treated at 1000 mg/kg bw/day, there were statistically significant increases in the number of corpora lutea, number of implantation sites, number of live offspring on days 1 and 4, and litter weight on day 1. The relevance of these findings is unclear but are not considered to represent a toxicologically adverse effect. There were no treatment related changes in litter viability or sex ratio.

There were statistically significant decreases in offspring body weight gain from days 1 to 4 post partum in the group treated at 1000 mg/kg bw/day. The study authors did not consider the reduction in body weight gain to be of toxicological importance, rather they considered it to be a consequence of the larger litter size seen at the highest dose level. There were no treatment related clinical signs of toxicity, changes in surface righting or macroscopic findings at necropsy in the offspring.

CONCLUSION

The NOAEL for systemic toxicity and reproductive toxicity was established by the study authors as 1000 mg/kg bw/day in this study, based on the lack of toxicologically significant effects.

TEST FACILITY Harlan (2014b)

B.10. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test – Plate incorporation procedure/Pre incubation procedure.

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver (S9 homogenate)
Concentration Range in Main Test	a) With metabolic activation: 50, 150, 500, 1500, 5000 µg/plate b) Without metabolic activation: 50, 150, 500, 1500, 5000 µg/plate
Vehicle	Acetone
Remarks - Method	No significant protocol deviations. Test 1 was conducted using the plate incorporation method. Test 2 was conducted using the pre-incubation method.

RESULTS

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

Remarks - Results	There was a statistically significant increase in the number of revertant colonies in *E. coli* strain WP2uvrA in the second test with metabolic activation at 5000 µg/plate. This increase was small and within the historical control range, thus is not considered to indicate a genotoxic effect. No other statistically or biologically significant increases in the frequency of revertant colonies were recorded, either with or without metabolic activation. The positive controls gave satisfactory responses, confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Harlan (2014c)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Cell Type/Cell Line	Human peripheral lymphocytes
Metabolic Activation System	Phenobarbitone/β-naphthoflavone induced rat liver (S9 homogenate)
Vehicle	Dimethyl sulfoxide
Remarks - Method	No significant protocol deviations. The doses selected for the study were based on the outcomes of a preliminary study (cytotoxicity and/or the presence of precipitate).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 78.13, 156.25, 312.5*, 625*, 1250*, 2500, MMC 0.4*	4 h	24 h
Test 2	0*, 19.53, 39.06*, 78.13, 156.25, 312.5*, 625*, MMC 0.2*	24 h	24 h
Present			
Test 1	0*, 78.13*, 156.25, 312.5*, 625*, 1250, 2500, CP 5*	4 h	24 h
Test 2	0*, 19.53, 39.06*, 78.13*, 156.25*, 312.5, 625, CP 5*	4 h	24 h

*Cultures selected for metaphase analysis.
MMC, Mitomycin C. CP, Cyclophosphamide.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 2500	> 1250	≥ 78.13	negative
Test 2	≥ 625	≥ 625	≥ 78.13	negative
Present				
Test 1	> 5000	> 1250	≥ 78.13	negative
Test 2	-	> 156.25	≥ 156.25	negative

*Reduction in mitotic index of ≥50%.

Remarks - Results	Under all experimental conditions, there was no evidence of an increase in the proportion of cells with chromosomal aberrations. No statistically significant increases in polyploidy cells were observed. The positive and vehicle controls gave satisfactory responses confirming

the validity of the test system.

CONCLUSION The notified chemical was not clastogenic to human peripheral blood lymphocytes treated in vitro under the conditions of the test.

TEST FACILITY Harlan (2014d)

B.12. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Cell Line L5178Y TK +/- 3.7.2c mouse lymphoma cells
Metabolic Activation System Phenobarbital/β-naphthoflavone induced rat liver (S9 homogenate)
Vehicle Dimethyl sulfoxide
Remarks - Method No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0*, 19.53, 39.06, 78.13*, 156.25*, 234.38*, 312.5*, 468.75*, 625*, EMS 400*	4 h	2 d	10-14 d
Test 2	0*, 9.77*, 19.53*, 39.06*, 78.13*, 156.25*, 234.37, 312.5, 625, EMS 150*	24 h	2 d	10-14 d
Present				
Test 1	0*, 39.06, 78.13, 156.25*, 312.5*, 468.75*, 625*, 937.5*, 1250*, CP 2*	4 h	2 d	10-14 d
Test 2	0*, 39.06*, 78.13*, 156.25*, 312.5*, 625*, 937.5*, 1250, 1875, CP 2*	4 h	2 d	10-14 d

*Cultures assessed for mutant phenotype
EMS, ethylmethanesulfonate. CP, cyclophosphamide.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test*	*Cytotoxicity* in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥ 312.5	≥ 625	≥ 156.25	negative
Test 2	≥ 625	≥ 156.25	≥ 156.25	negative
Present				
Test 1	≥ 1250	≥ 1250	≥ 156.25	negative
Test 2	-	≥ 625	≥ 39.06	negative

*less than 10% relative suspension growth

Remarks - Results There were no toxicologically or statistically significant increases or dose response relationships in mutant frequencies or number, with or without metabolic activation.

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

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

TEST FACILITY Harlan (2013g)

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 sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None Reported
Analytical Monitoring	Respirometry: CO_2 evolution
Remarks - Method	The test was conducted according to the guidelines above using good laboratory practice (GLP). No significant deviations from the test guidelines were reported.

RESULTS

Test substance		*< Sodium benzoate >*	
Day	*% Degradation*	*Day*	*% Degradation*
6	0	6	52
10	0	10	74
14	0	14	63
21	3	21	70
28	3	28	62

Remarks - Results	After 28 days, the percent degradation for the notified chemical was 0%. The percent degradation calculated in the reference item replicate (procedure control) up to day 28 was 62%. In the toxicity control, more than 25% degradation was observed up to day 14.
CONCLUSION	The notified chemical is not readily biodegradable.
TEST FACILITY	Harlan (2012f)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-Static.
Species	Rainbow Trout (*Oncorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None Reported
Water Hardness	140 mg $CaCO_3$/L
Analytical Monitoring	High performance liquid chromatography mass spectrometry (HPLC-MS)
Remarks – Method	Due to the low aqueous solubility and complex nature of the notified chemical, the test medium was prepared as a Water Accommodated Fraction (WAF) of the notified chemical. The test was conducted in accordance with the test guideline without significant deviations. GLP was followed.

RESULTS

Concentration mg/L	*Number of Fish*	*Mortality*				
Nominal		*3 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	7	0	0	1*	2*	5*+

100	7	0	0	0	0	0

* Mortalities caused by aggressive behaviour in the control group.
+ At the 78-Hour time point, 1 out of the remaining 5 fish was observed to be moribund so was humanely killed due to the approach of the substantial severity limit, and at the 96-Hour time point, l out of the remaining 3fish was observed to be moribund so was humanely killed due to the approach of the substantial severity limit.

LC50	> 100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	The acute toxicity of the notified chemical to the freshwater fish rainbow trout (Oncorhynchus myldss) has been investigated using the threshold approach and gave a 96-Hour LL_{50} value of greater than 100 mg/L loading rate WAF. The No Observed Effect Loading rate was 100 mg/L loading rate WAF.

CONCLUSION The notified chemical is not harmful to fish

TEST FACILITY Harlan (2014e)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified Chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – Limited Test.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None Reported
Water Hardness (Theoretical)	250 mg $CaCO_3$/L
Analytical Monitoring	High performance liquid chromatography mass spectrometry (HPLC-MS)
Remarks - Method	Due to the low aqueous solubility and complex nature of the test item, for the purposes of the range-finding test the test item was prepared as a WAF. The test was conducted in accordance with the test guideline without significant deviations. GLP was followed.

RESULTS

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

LC50	> 100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	Analysis of the test preparations at 0 and 48 hours showed measured test concentrations of less than the limit of quantification (LOQ) of the analytical method employed were obtained which was determined to be 0.054 mg/L. This does not infer that no notified chemical was in solution, just that which was, was at a concentration of less than the LOQ.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates.

TEST FACILITY Harlan (2012g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified Chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L
Auxiliary Solvent	None Reported
Water Hardness	None Reported
Analytical Monitoring	High performance liquid chromatography mass spectrometry (HPLC-MS)
Remarks - Method	Due to the low aqueous solubility and complex nature of the test item, for the purposes of the range-finding test the notified chemical was prepared as a WAF. The test was conducted in accordance with the test guideline without significant deviations. GLP was followed.

RESULTS

Biomass		*Growth*	
E_yL50	*NOE_yL*	*E_rL50*	*NOE_rL*
mg/L at 72 h	*72 mg/L*	*mg/L at 72 h*	*72 mg/L*
> 100	100	> 100	100

Remarks - Results	Statistical analysis of the growth rate data was carried out for the control and 100 mg/L loading rate WAF test group using a Student's t-test incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981). There were no statistically significant differences ($P \geq 0.05$), between the control and 100 mg/L loading rate WAF test group and therefore the "No Observed Effect Loading Rate" (NOEL) based on growth rate was 100 mg/L loading rate WAF.
CONCLUSION	The notified chemical is not harmful to algae
TEST FACILITY	Harlan (2012h)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test.
Inoculum	Activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 100, 1000 mg/L
Remarks – Method	Activated sewage sludge was exposed to an aqueous dispersion of the notified chemical at a temperature of approximately 20 °C with the addition of a synthetic sewage as a respiratory substrate. The rate of respiration was determined after 3 hours contact time and compared to data for the control and a reference item, 3,5-dichlorophenol. The test was conducted in accordance with the test guideline without significant deviations. GLP was followed.
RESULTS	
EC50	> 1000 mg/L at 3 hours
NOEC	1000 mg/L at 3 hours
Remarks – Results	The dissolved oxygen content was not above 60% to 70% of the dissolved oxygen saturation level of 8.9 mg O_2/L in several of the vessels. This deviation was considered to have no adverse effect on the study as all

	oxygen consumption values were measured over the linear portion of the trace. Further, the preparation of the samples varied slightly from that stated in the general study plan. This deviation was considered to have no adverse effect on the study as the overall final concentration of the samples was the same.
CONCLUSION	The notified chemical is not inhibitory to bacterial respiration.
TEST FACILITY	Harlan (2013h)

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