File No: STD/1473 and STD/1474

February 2014

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

STD/1473: Component 1 in O3270
STD/1474: Component 2 in O3270

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and 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/1473 and STD/1474	Ecolab Pty Ltd	Component 1 in O3270 and Component 2 in O3270	No	STD/1473: ≤125 tonnes per annum STD/1474: ≤125 tonnes per annum	Component of a scale inhibitor

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemicals are 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).

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

Hazard classification	*Hazard statement*
Acute Category 3	H402 - Harmful to aquatic life
Chronic Category 3	H411 - Harmful to aquatic life with long lasting effects

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

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

Environmental risk assessment
On the basis of the marine PEC/PNEC ratio and the assessed use pattern, the notified chemicals are not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- If products and mixtures containing the notified chemicals 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.

Environment

Disposal

- The notified chemicals should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if

- the chemicals are proposed to be used in on-shore drilling operations involving hydraulic fracturing;
- the chemicals for onshore applications are proposed to be used at sites where there are no on-site Effluent Treatment Plant (ETP) or Produced Water Re-Injection (PWRI) facilities.

or

(2) Under Section 64(2) of the Act; if

- the function or use of the chemicals have changed from a component of a scale inhibitor, or is likely to change significantly;
- the amount of chemicals being introduced has increased, or is likely to increase, significantly;
- the chemicals have begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemicals on occupational health and safety, public health, or the environment.

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

(Material) Safety Data Sheet
The (M)SDS of the notified chemicals 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)
Ecolab Pty Ltd (ABN 59 000 449 990)
2 Drake Avenue
Macquarie Park NSW 2113

NOTIFICATION CATEGORY
STD/1473: Standard – Chemical other than polymer (more than 1 tonne per year)

STD/1474: Standard (Reduced fee notification) – Chemical other than polymer (more than 1 tonne per year) – Chemical is being notified at the same time as a similar chemical

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, use details, import volume and identity of recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Water solubility, adsorption/desorption and dissociation constant.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
O3270 (>90% mixture of the notified chemicals: typically contains approximately 50% Component 1 [STD/1473] and approximately 50% Component 2 [STD/1474])
NALCO EC6662A (product containing the inseparable mixture at up to 20% concentration)

MOLECULAR WEIGHT
STD/1473: < 500 Da
STD/1474: < 500 Da

ANALYTICAL DATA
Reference UV, IR, ^{31}P-NMR, ^{1}H-NMR, ^{13}C-NMR spectra were provided.

3. COMPOSITION

Inseparable mixture of the notified chemicals.

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Pale yellow translucent solid*

Property	Value	Data Source/Justification
Melting Point/Freezing Point	129 °C	Measured*. Decomposition occurred just above melting
Density	1740 kg/m^3 at 21 °C	Measured*
Vapour Pressure	< 3.8 × 10^{-6} kPa at 25 °C	Measured*
Water Solubility	757 – 772 g/L at 20 °C	Measured
Hydrolysis as a Function of pH	Not determined	The notified chemicals may undergo reversible hydrolysis: equilibrium

		varies depending on pH.
Partition Coefficient (n-octanol/water)	log P_{ow} = < 0 – 0.8 at 35 °C	Measured
Surface Tension	69.5 mN/m at 21 ± 0.5°C	Measured
Adsorption/Desorption	log K_{oc} = 0.35-1.48	Estimated using KOCWIN Program (v2.00). The notified chemicals are expected to adsorb to soil and sediment given the potential cationicity and chelating anionic functional groups.
Dissociation Constant	Not determined	Expected to be ionised in the environmental pH range (4-9) as they contain multiple dissociating groups
Flash Point	Not determined	Not expected to flash based on low flammability
Flammability	Not highly flammable	Measured*
Autoignition Temperature	>400 °C	Measured*
Explosive Properties	Predicted negative	Contain no functional groups that would infer explosive properties
Oxidising Properties	Predicted negative	Contain no functional groups that would infer oxidising properties

*Inseparable mixture of the notified chemicals.

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

Reactivity
The notified chemicals are 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 chemicals are 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 chemicals will be imported in formulated scale inhibitor products (up to 20% combined concentration).

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

Year	*1*	*2*	*3*	*4*	*5*
STD/1473	5-25	5-25	25-75	25-75	75-125
STD/1474	5-25	5-25	25-75	25-75	75-125
Tonnes	10-50	10-50	50-150	50-150	150-250

PORT OF ENTRY
Perth, Fremantle and Sydney.

TRANSPORTATION AND PACKAGING
Formulated products containing the notified chemicals (up to 20% combined concentration) will be transported in 1000 L or 4000 L totes or in 200 L drums. The containers will be transported by road, rail or boat.

USE
The notified chemicals will be used as components (up to 20% combined concentration) of a scale inhibitor in the oil and gas industry, for onshore (35%) and offshore (65%) applications.

OPERATION DESCRIPTION
Manufacturing will not occur in Australia.

Repackaging
The notified chemicals (up to 20% combined concentration) will be transferred by a metering line and repackaged into 4000 L offshore totes.

End-use
The notified chemicals (up to 20% combined concentration) will be transferred into the dosing system via umbilicals for use as a scale inhibitor. Workers will couple the dose tank to the closed system.

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/week)*
Repackaging	1	1
End-users	0.25	1

EXPOSURE DETAILS
Transport and storage workers
Transport and storage workers will only come into contact with the notified chemicals (up to 20% combined concentration) in the unlikely event of an accident.

Repackaging and end-use
Dermal and ocular exposure to the notified chemicals (up to 20% combined concentration) may occur to workers when connecting or disconnecting transfer hoses, or during cleaning and maintenance operations. Inhalation exposures are not expected based on the low vapour pressure of the notified chemicals (3.8×10^{-6} kPa) and because aerosols are not expected during repackaging or end-use operations. The remainder of the oil and gas mining process is expected to be automated and enclosed.

6.1.2. Public Exposure
The notified chemicals will only be used by the oil and gas industry and public exposure is not expected.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the inseparable mixture of the notified chemicals are summarised in the following table. The bacterial reverse mutation assay was conducted with an analogue chemical. This analogue is considered to be similar to the chemical notified as STD/1473. 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)	non-corrosive
Rabbit, skin irritation	non-irritating
Eye irritation (in vitro)	non-corrosive
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity with reproduction/developmental toxicity screening	NOAEL (systemic and reproductive) = 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation*	non-mutagenic
Genotoxicity – *in vitro* chromosome aberration	non-clastogenic
Genotoxicity – *in vitro* forward mutation assay	non-mutagenic

*Conducted with an analogue chemical

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemicals were submitted. Absorption across biological membranes is supported by the low molecular weight (<500 Da) and the partition coefficient of the notified chemicals.

Acute toxicity
The notified chemicals were of low acute oral (LD50 >2000 mg/kg bw) and dermal (LD50 >2000 mg/kg bw) toxicity to rats.

Irritation and sensitisation
The notified chemicals were non-corrosive to the skin and eye in *in vitro* studies. The notified chemicals were non-irritating to the skin but were a slight eye irritant, to rabbits. The notified chemicals were not skin sensitisers in an LLNA study when tested up to 10% concentration. It is also noted that the notified chemicals do not contain any structural alerts for sensitisation.

Repeated Dose/Reproductive Toxicity
In a combined repeat dose toxicity study with a reproduction/developmental screening test, rats were administered the notified chemicals by gavage at 0, 100, 350 or 1000 mg/kg bw/day. Rats were paired and mated after 14 days dosing and allowed to produce offspring. Adults were sacrificed following approximately 6 weeks dosing. Offsprings were sacrificed on post-partum day 5. The NOAEL for systemic toxicity and reproductive toxicity was established as 1000 mg/kg bw/day, based on the lack of adverse effects. Therefore, the notified chemicals are of low repeated dose toxicity. There was no evidence of reproductive or developmental effects in this screening study.

Mutagenicity/Genotoxicity
The notified chemicals were not clastogenic in an *in vitro* chromosome aberration assay or mutagenic in an *in vitro* forward mutation assay. The analogue chemical was not mutagenic in a bacterial reverse mutation assay. Overall, the available evidence indicates that the notified chemicals are unlikely to be mutagenic or genotoxic.

Health hazard classification
Based on the available information, the notified chemicals are 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
The inseparable mixture of the notified chemicals will be imported at up to 20% combined concentration. There are no toxicological effects of concern at this concentration, based on the results of toxicology studies above, which were all indicative of low hazard. Slight eye irritation resulting from ocular exposures to the notified chemicals is not expected at 20% combined concentration.

Oil and gas plant workers are also expected to wear PPE, including overalls, safety glasses and gloves, thus further minimising exposure. Exposure is also expected to be minimised because the dosing system is automated and enclosed.

Overall, based on the low hazard and the expected low exposure, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health
As the public is not expected to be exposed to the notified chemicals, the risk to public health 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 chemicals, as an inseparable mixture, will be imported into Australia as components of scale inhibitor products in 1000 L totes or in 200 L drums. The products will be used for onshore (35% of the total import volume) and offshore (65%) application in the oil and gas industry.

No further reformulation of the imported products is required in Australia. However, the products containing the notified chemicals will be transferred to 4000 L Swire Tote containers and be transported from shore to off-shore platforms. The containers will be returned to the repackaging plant for cleaning and refilling. No significant release of the notified chemicals to the aquatic compartment is expected at this stage.

RELEASE OF CHEMICAL FROM USE

The notified chemicals will be used as scale inhibitors in oil/gas wells to stop or interfere with inorganic scale nucleation, precipitation and adherence to the production conduit, completion system or processing facilities. In the oilfield, the formulation containing up to 20% of an inseparable mixture of the notified chemicals will be injected to wells via an umbilical line at a dose rate of 100 mg/L. Inside wells, some of notified chemicals are expected to adsorb onto the rock matrix and remain for up to 18 months. During this time, the notified chemicals will be slowly desorbed from the rock matrix and released into the oil and produced water with the majority in the water phase, based on its water solubility and octanol/water partition coefficient. The majority of the notified chemicals will then flow back with the produced fluids to the processing facilities. Notified chemicals contained in the produced water are expected to be either reinjected into wells or further treated before disposed of as waste water.

For offshore application, some of the wells pipe the oil and produced water back onshore for treatment and disposal. However, the majority of wells discharge the treated produced water directly to the ocean (Cobby, 2002). Therefore, up to 65% of the notified chemicals may be discharged to the ocean.

For onshore applications, the notifier indicates that there are two planned onshore application sites. One has systems that will ensure there is complete Produced Water Re-Injection (PWRI). Thus, when PWRI is the disposal method, there is not expected to be significant release of the notified chemicals to the aquatic environment. At the other site, the notifier indicates that the water phase containing the notified chemicals will be treated via an on-site effluent treatment plant (ETP) before discharging to sewers for further treatment at the sewage treatment plants (STPs). Therefore, the notified chemicals are not expected to be released significantly to surface waters from onshore applications.

Notified chemicals within the oil phase will either share the same end-use fate as the oil or be removed during oil refining, in which case it will remain in the distillation residues/tar fraction. Environmental release of the notified chemicals in oil pipelines is expected to be limited.

The principal application for the formulated product is for scale control in the gas projects running continuous monoethylene glycol (MEG) application for hydrate control, with the MEG regenerated on-site. The notified chemicals associated with MEG stream would ultimately be collected in the solids recovery process and be disposed with the solids.

RELEASE OF CHEMICAL FROM DISPOSAL

Spillage of small amounts of the notified chemicals will be soaked up with absorbent material which is likely to be disposed of to landfill. The empty 1000 L import containers are expected to be recycled or disposed of to landfill. Swire Totes of formulation(s) containing residual notified chemicals will be returned to the repackaging plant for cleaning and refilling. The residues are expected to be less than 1% and may be washed with water during recycling/cleaning and disposed of to sewer, or to landfill with the import containers.

7.1.2. Environmental Fate

Off shore fate

For offshore application, most of the notified chemicals are expected to be either re-injected into wells or released into ocean after use. The worst case scenario is that ~65% of the total import volume of the notified chemicals will be discharged to the ocean directly. In the marine environment, the notified chemicals have potential to chelate with metal ions, due to the anionic functionality of the chemicals, and/or will adsorb to sediment and any suspended particulate matter, based on their cationic properties. The notified chemicals are not easily biodegradable in seawater based on the results of a laboratory test (12% over 28 days). However, based on their high water solubility and the presence of potential anionic and cationic functional groups, the notified chemicals are not expected to bioaccumulate in aquatic organisms.

On shore fate

For onshore application, the notified chemicals will share the fate of the produced water to be either re-injected into wells or be treated by onsite ETPs. The ETP provides primary, secondary and tertiary treatment of produced water prior to reuse or disposal. The notified chemicals in the produced water are expected to be efficiently removed by adsorbing to sludge sediment during ETP processes. The treated produced water is expected to be further treated at STPs before being released as treated effluent to the aquatic environment. At STPs, notified chemicals are expected to be removed from the water column by adsorbing to sludge based on their ionic properties. Notified chemicals that partitioned to sludge are expected to be disposed to landfill or used for soil remediation. Therefore, very little, if any, of the notified chemicals are expected to be released to surface waters from onshore applications.

In water, landfill and soil, the notified chemicals are expected to degrade via abiotic or biotic pathways forming water and oxides of carbon, phosphorus and nitrogen.

Small amounts of the notified chemicals contained in the oil phase are expected to be sent to oil refineries in the oil phase. They may either be removed during oil refining and remain in the distillation residues/tar fraction that will be most likely used as road base, or share the fate of the oil product. The oil products are expected to be eventually thermally decomposed during use to form water and oxides of carbon, nitrogen and phosphorus.

7.1.3. Predicted Environmental Concentration (PEC)

Offshore release

The calculations of the Predicted Environmental Concentrations (PECs) of the notified chemicals in sea water from offshore applications have been calculated based on CHARM modelling (Thatcher et al, 2005). For the key parameters, the concentration of the notified chemicals in the total produced fluid was reported by the notifier to be 100 mg/L. For the worst case scenario, it is assumed that all the produced water will be directly discharged into the ocean. The worst case PEC was determined to be 1700 µg/L on the assumption of 1000 fold dilution by the sea water after the discharge of the water based on the CHARM model. For details of the calculation, please refer to Appendix C.

Onshore release

It is indicated that 35% of the total import volume of the notified chemicals will be used for onshore applications on two sites.

Of the two planned onshore application sites, one has systems that will ensure there is complete Produced Water Re-Injection (PWRI). Therefore, there will be no environmental exposure to controlled or surface waters from the proposed use of the formulation containing the notified chemicals.

The other site has an ETP in place. The ETP treats all continuously oil contaminated, accidentally contaminated and produced water. The ETP provides primary, secondary and tertiary treatment of contaminated water prior to reuse or marine discharge. These treatment systems are designed to provide complicated abiotic and biotic treatment processes. Therefore, the notified chemicals remaining in produced water are expected to be efficiently removed by adsorbing to sludge sediment and/or biodegradation. The treated produced water is expected to be further treated at STPs and therefore, the release of the notified chemicals to aquatic environment from onshore application is not expected to reach ecotoxicologically significant concentrations. Hence, the PEC for the notified chemicals released from the onshore applications was not calculated.

7.2. Environmental Effects Assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LC50 > 1000 mg/L	Not harmful to fish
Daphnia Toxicity	48 h EC50 > 100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity		
Pseudokirchneriella subcapitata	72 h EC50 = 46 mg/L 72 h NOEC = 10 mg/L	Harmful to fresh water algae
Skeletonema costatum	72 h EC50 = 672 mg/L 72 h NOEC = 250 mg/L	Not harmful to marine algae
Inhibition of Bacterial Respiration	3 h EC50 > 1000 mg/L	Not inhibitory to microbial activity

Under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) the notified chemicals are considered to be not harmful to fish and aquatic invertebrates, but are considered to be harmful to algae. Based on the toxicity to algae the notified chemicals are formally classified under the GHS as "Acute category 3; Harmful to aquatic life". The notified chemicals were not demonstrated to be readily biodegradable. Thus based on the test result for algae, the notified chemicals are formally classified as "Chronic category 3; Harmful to aquatic life with long lasting effects" under the GHS.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) has been calculated based on the endpoint for marine algae (72 h EC50 = 672 mg/L) as it reflects a more realistic situation. Although the most sensitive aquatic species are freshwater algae (72 h EC50 = 46 mg/L), the majority of the notified chemicals is expected to be released to ocean based on their use pattern. A safety factor of 100 is used since ecotoxicity data for three trophical levels of aquatic organisms are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
ErC50 (algae)	672	mg/L
Assessment Factor	100	
PNEC:	6720	µg/L

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) for the offshore application has been calculated using the highest PEC value of 1700 µg/L (refer to the Appendix C for PEC calculation).

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - Ocean	1700	6720	**0.25**

The risk quotient (RQ = PEC/PNEC) for this release scenario is ≤ 0.25, indicating that the use of the notified chemicals as proposed is not expected to cause unreasonable risk to the aquatic environment. Based on their high water solubility and the presence of potential anionic and cationic functional groups, the notified chemicals are not expected to bioaccumulate in aquatic organisms. The risk quotient (RQ = PEC/PNEC) for onshore application was not calculated as the PEC for this release scenario was not calculated.

Therefore, on the basis of the marine PEC/PNEC ratio and the assessed use pattern, the notified chemicals are not considered to pose an unreasonable risk to the environment for both offshore and onshore oil and gas applications.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 129 ± 0.5 °C

Method OECD TG 102 Melting Point/Melting Range
Remarks Determined (for inseparable mixture of the notified chemicals) by differential scanning calorimetry. Thermograms indicate that decomposition occurred soon after melting.
Test Facility Harlan (2012a)

Density 1740 kg/m^3 at 21 °C

Method OECD TG 109 Density of Liquids and Solids
Remarks Density (of inseparable mixture of the notified chemicals) determined using a pycnometer.
Test Facility Harlan (2012a)

Vapour Pressure < 3.8×10^{-6} kPa at 25 °C

Method OECD TG 104 Vapour Pressure
Remarks Determined (for the inseparable mixture of the notified chemicals) by thermogravimetric analysis. A vapour pressure balance was used at several temperatures between 30-40°C. Extrapolation to 25°C gave a vapour pressure of 3.76 10^{-6} kPa, which was taken as the maximum value.
Test Facility Harlan (2012b)

Water Solubility 757-772 g/L at 20°C

Method OECD TG 105 Water Solubility.
Remarks Flask Method. The determination was carried out on the inseparable mixture of the notified chemicals by visual assessment using a procedure based on the flask method. At the nominal concentration of 757 g/L, the solution is clear and viscous. No undissolved test item was observed. At the nominal concentration of 772 g/L, undissolved test item was observed. The pH of the test solutions was determined to be 4.2-4.3.
Test Facility Harlan (2012a)

Partition Coefficient (n-octanol/water) log P_{ow} = < 0 - 0.8 at 35°C

Method OECD TG 117 Partition Coefficient (n-octanol/water).
Remarks HPLC Method. The test substance is the inseparable mixture of the notified chemicals. Two peaks, corresponding to two compounds in the test substance, were observed in the chromatogram. The values of log P_{ow} were determined to be 0.8 (28% peak area) and < 0 (72% peak area), respectively.
Test Facility Aquateam (1997a)

Surface Tension 69.5 mN/m at 21.0 ± 0.5°C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Council Regulation No 440/2008 A.5 Surface Tension.
Remarks The test was conducted on the inseparable mixture of the notified chemicals using the ring method at a nominal concentration of 1.0 and 1.1 g/L and 21.0 ± 0.5°C. The values of surface tension for two duplicate samples were determined to be 69.2 and 69.7 mN/m.
Test Facility Harlan (2012a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Wistar(RccHan)
Vehicle	None
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	6F	2000	0/6

LD50	>2000 mg/kg bw
Signs of Toxicity	None
Effects in Organs	None
CONCLUSION	The inseparable mixture of the notified chemicals is of low toxicity via the oral route.
TEST FACILITY	Harlan (2012d)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test
Species/Strain	Rat/Wistar
Vehicle	None
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	None
Signs of Toxicity - Systemic	None
Effects in Organs	None
CONCLUSION	The inseparable mixture of the notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Harlan (2012e)

B.3. Irritation – skin (in vitro)

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 430 In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)
Species/Strain	Rat/Wistar (RccHan)
Vehicle	None

Remarks - Method	The rats were 21-23 days old when sacrificed for skin collection. Negative and positive controls were conducted (water and 36% hydrochloric acid, respectively).

RESULTS

Test Item	*Mean Electrical Resistance (standard deviation)*
Negative control	18.0 kΩ (7.0)
Test substance	21.6 kΩ (6.5)
Positive control*	0.6 kΩ

*Based on one result only due to perforation of two other replicates.

CONCLUSION	The inseparable mixture of the notified chemicals was non-corrosive to the skin under the conditions of the test.
TEST FACILITY	Harlan (2012f)

B.4. Irritation – skin

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion
Species/Strain	Rabbit/New Zealand White (Hsdlf)
Number of Animals	3
Vehicle	None
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations.

RESULTS

Remarks - Results	Scores of zero were observed for erythema and oedema formation at all observations points.
CONCLUSION	The inseparable mixture of the notified chemicals is non-irritating to the skin.
TEST FACILITY	Harlan (2012g)

B.5. Irritation – eye (in vitro)

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
METHOD	OECD TG 437 Bovine Corneal Opacity and Permeability Test Method
Vehicle	None
Remarks - Method	No significant protocol deviations. A 0.9% sodium chloride solution was used for the negative control and undiluted ethanol for the positive control.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	2.3 (0.6)	0.03 (0.01)	2.8 (0.5)
*Test substance**	0.6 (0.1)	0.01 (0.009)	0.7 (1.1)
*Positive control**	24.7 (3.6)	0.5 (0.2)	32.8 (0.5)

Data presented as mean ± standard deviation
IVIS, in vitro irritancy score
*Corrected for background values

CONCLUSION The inseparable mixture of the notified chemicals was not corrosive or a severe eye irritant under the conditions of the test.

TEST FACILITY Harlan (2012h)

B.6. Irritation – eye

TEST SUBSTANCE Inseparable mixture of the notified chemicals

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

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.0	0.3	0.3	2	<72 hours	0
Conjunctiva: chemosis	0.3	0	0.3	2	<48 hours	0
Conjunctiva: discharge	0	0	0	1	<24 hours	0
Corneal opacity	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	-	0

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

CONCLUSION The inseparable mixture of the notified chemicals is slightly irritating to the eye.

TEST FACILITY Harlan (2012i)

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

TEST SUBSTANCE Inseparable mixture of the notified chemicals

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/CBA/Ca
Vehicle 1% pluronic L92 in distilled water
Remarks - Method The highest tested concentration of 10% was noted as being the maximum attainable concentration that was suitable for dosing, although it was not stated whether this was due to solubility or homogeneity. This concentration did not produce systemic toxicity or excessive local irritation in a preliminary screening test.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/animal)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	1177 ± 443	1.00
2.5	1811 ± 487	1.54
5	1179 ± 457	1.00
10	1194 ± 308	1.01
Positive Control (HCA)		
85	10957 ± 2949	9.31

Data presented as mean ± standard deviation
HCA, hexylcinnamaldehyde

CONCLUSION There was no evidence of induction of a lymphocyte proliferative

response indicative of skin sensitisation to the inseparable mixture of the notified chemicals under the conditions of the test and at the tested concentrations.

TEST FACILITY Harlan (2012j)

B.8. Repeat dose toxicity, with reproduction/developmental toxicity screening

TEST SUBSTANCE Inseparable mixture of the notified chemicals

METHOD OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

Species/Strain Rat/Wistar (Han:RccHan:WIST)

Route of Administration Oral – gavage

Exposure Information Total exposure: 42 days (males), up to 42-53 days (females)
Dose regimen: 7 days per week

Vehicle Water

Remarks - Method In a range finding study, rats (3/sex/dose) were administered the test substance by gavage for 7 days, then sacrificed on day 8 (doses of 100, 500, and 1000 mg/kg bw/day). There were no treatment related findings (mortality, clinical signs of toxicity, body weight, food or water consumption, or macroscopic abnormalities) in any treatment group (Harlan, 2012k).

Rats were treated with the test substance by gavage for 14 days then paired for mating on day 15 for a maximum of 14 days. Animals were separated when evidence of mating was noted. Males and females (5/sex/dose) were evaluated for functional, behavioural and sensory responses during week 6 and day 4 post-partum, respectively. Males were sacrificed on day 43. Females and offspring were sacrificed on post-partum day 5 (days 42-53).

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	10M + 10F	0	0/20
low dose	10M + 10F	100	0/20
mid dose	10M + 10F	350	0/20
high dose	10M + 10F	1000	0/20

Clinical Observations

There were no treatment related clinical signs of toxicity, or functional, behavioural or sensory observations. Absolute body weights and body weight gains were similar in treated and controls groups, for males and females. There were statistically significant increases in food consumption in females treated at 350 and 1000 mg/kg bw/day from gestation days 14 to 20. All other food consumption and food efficiency measurements were similar between treated and control groups, for males and females.

Laboratory Findings – Clinical Chemistry and Haematology

There were statistically significant decreases in total leucocyte count and neutrophil count in females treated at 1000 mg/kg bw/day, but the changes were within historical control values and were considered to be unrelated to treatment. All other measured haematology parameters were similar in treated and control groups.

There were statistically significant decreases in cholesterol (up to ↓22%) and inorganic phosphorous (up to ↓15%) and a statistically significant increase in chloride (up to ↑3%) in all groups of treated males. These changes were within expected limits of biological variability and there was no dose response, thus are considered to be of low toxicological importance. There was a statistically significant increase in bile acids in males treated at 1000 mg/kg bw/day (↑298%), which may indicate hepatic activity. There was a statistically significant decrease in calcium concentration in females treated at 1000 mg/kg bw/day (↓11%). There was a statistically significant decrease in creatinine in the 350 and 1000 mg/kg bw/day females (up to ↓16%).

Effects in Organs

There were no treatment related macroscopic findings in adults. There was a statistically significant increase in absolute and relative pituitary weights in males treated at 1000 mg/kg bw/day. There were statistically significant decreases in absolute and relative thyroid/parathyroid weights and absolute and relative adrenal weight in females treated at 1000 mg/kg bw/day. Overall, these organ weight changes were not accompanied by associated histopathological findings and were therefore considered to be of low toxicological concern.

Effects on reproduction and offspring

There were no treatment related effects on mating performance, fertility, gestation length, litter size, sex ratio, viability, or the number of corpora lutea or implantation sites.

There were no treatment related effects in offspring body weight, surface righting reflex or clinical signs of toxicity.

CONCLUSION

The NOAEL for systemic and reproductive toxicity was established as 1000 mg/kg bw/day under the conditions of the study, based on the lack of adverse effects.

TEST FACILITY Harlan (2013a)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Analogue chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test – Plate incorporation 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: 50-5000 µg/plate b) Without metabolic activation: 50-5000 µg/plate
Vehicle	Water
Remarks - Method	No significant protocol deviations.

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

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

TEST FACILITY SafePharm (2003)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE	Inseparable mixture of the notified chemicals
36METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test
Cell Type	Human peripheral blood lymphocytes
Metabolic Activation System	S9 fraction from Phenobarbitone/β-naphthoflavone induced rat liver
Vehicle	Water

Remarks - Method A 2% concentration of S9 metabolic activation was used for test 1, whereas a 1% concentration was used for test 2.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 150, 300, 600*, 1200*, 2401*, 4801*, 0.4 MMC*	4 hours	20 hours
Test 2	0*, 85, 170, 340, 680*, 1360*, 2720*, 0.2 MMC*	24 hours	24 hours
Present			
Test 1	0*, 150, 300, 600*, 1200*, 2401*, 4801*, 5 CP*	4 hours	20 hours
Test 2	0, 85, 170, 340, 680*, 1360*, 2720*, 5 CP*	4 hours	20 hours

*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	>4801	>4801	>4801	negative
Test 2	≥4801	>2720	>2720	negative
Present				
Test 1	>4801	>4801	>4801	negative
Test 2	-	>2720	>2720	negative

Remarks - Results There were no statistically significant increases in the frequency of cells with chromosomal aberrations in either test, with or without S9.

The plates tested at 4800 µg/mL were tested above the recommended 10 mM concentration. The plates tested at 2401 µg/mL were slightly below the recommended 10mM concentration. The concentration was adjusted to 2720 µg/mL to reach the maximum recommended concentration.

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

TEST FACILITY Harlan (2013b)

B.11. Genotoxicity – in vitro

TEST SUBSTANCE Inseparable mixture of the notified chemicals

36METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test
Cell Type/Cell Line Chinese Hamster Ovary (CHO) cells
Metabolic Activation System S9 fraction from Phenobarbitone/β-naphthoflavone induced rat liver
Vehicle Water
Remarks - Method No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 85, 170, 340, 680, 1360, 2720, 500 EMS, 750 EMS	4 hours	7 days	14 days
Test 2	0, 85, 170, 340, 680, 1360, 2720, 200 EMS, 300 EMS	24 hours	7 days	14 days

Present				
Test 1	0, 85, 170, 340, 680, 1360, 2720, 0.5 DMBA, 1 DMBA	4 hours	7 days	14 days
Test 2	0, 85, 170, 340, 680, 1360, 2720, 0.5 DMBA, 1 DMBA	4 hours	7 days	14 days

EMS, Ethyl methane sulfonate. DMBA, Dimethyl benzanthracene.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>2720	>2720	>2720	negative
Test 2	>2720	>2720	>2720	negative
Present				
Test 1	>2720	>2720	>2720	negative
Test 2	>2720	>2720	>2720	negative

CONCLUSION The inseparable mixture of the notified chemicals was not clastogenic to CHO cells treated *in vitro* under the conditions of the test.

TEST FACILITY Harlan (2012l)

APPENDIX C: PREDICTED ENVIRONMENTAL CONCENTRATIONS (PECS)

Assuming for the worst case scenario that all the produced water will be directly discharged into the ocean, the Predicted Environmental Concentrations (PECs) of the notified chemicals in sea water have been calculated based on the CHARM method (Thatcher M. et al., 2005).

The concentration of the chemicals in the total produced fluid has been calculated by equation 1:

$$C_t = \frac{F_{flow} \times C_{flow}}{F_t}$$

In which:

C_t = concentration of the chemicals in the total produced fluid (mg/L),

F_{flow} = volume of flow in terms of which the dosage is expressed (m^3/day),

C_{flow} = concentration of the chemicals in that flow (mg/L),

F_t = total fluid production (m^3/day),

The concentration of the chemicals in produced water has been calculated by equation 2:

$$C_{pw} = \frac{C_t F_t}{10^{logPow} \times F_{o/c} + F_{pw}}$$

in which:

C_{pw} = concentration of the chemicals in produced water (mg/L),

C_t = concentration of the chemicals in the total produced fluid (mg/L),

F_t = total fluid production (m^3/day),

P_{ow} = partition coefficient between octanol and water,

$F_{o/c}$ = total oil or condensate production (m^3/day),

F_{pw} = volume of produced water discharged per day (m^3/day).

Equation 2 considers the partitioning behaviour of the notified chemicals between octanol and water, which is determined by the log Pow. The notified chemicals were determined to have log Pow < 0 - 0.8. For a worst case scenario, it is assumed that log Pow < 0 and that the notified chemicals do not partition to the oil phase. Based on this assumption, the safe concentration of the notified chemicals in the produced water is adjusted to be 100% discharge and is determined using equation 3:

$$C_{pws} = \frac{C_t \times F_t}{F_{pw}}$$

in which:

C_{pws} = concentration of the chemicals in the produced water including a safety factor (mg/L).

The PEC can be further calculated using equation 4:

$$PEC = C_{pws} \times D_{distance\ x}$$

in which:
PEC = Predicted Environmental Concentration of a chemical at a certain distance from the platform (mg/L),

$D_{distance\ x}$ = dilution factor at distance x from the platform (0-1).

The dilution factor at a distance of x = 500 m is set to a realistic worst case default value of 0.001.

The notifier has provided raw data required by the CHARM modelling for the off-shore application. The Predicted Environment Concentration (PEC) has been calculated based on these data. Some examples for the calculation are shown in the following table, including the worst cases having the maximum PECs.

Examples for the PEC calculation including worst case situations

F_{flow} (m^3/day)	$F_{o/c}$ (m^3/day)	F_t (m^3/day)	C_{flow} (mg/L)	C_t (mg/L)	F_{pw} (m^3/day)	C_{pw} (mg/L)	C_{pws} (mg/L)	PEC (mg/L)
Oil production (80 m depth): Standard: Produced water								
4000	1600	5600	100	71.4	4000	71.4	100	0.1
Oil production (80 m depth): Standard: Total fluids								
5600	1600	5600	100	100	4000	69.9	140	0.14
Gas production (40 m depth): Standard: Produced water								
500	4500	5000	100	10	500	10	100	0.1
Gas production (40 m depth): Standard: Total fluids								
5000	4500	5000	100	100	500	100	1000	1
Gas production (125 m depth): Standard: Produced water								
500	4500	5000	100	10	500	10	100	0.1
Gas production (125 m depth): Standard: Total fluids								
5000	4500	5000	100	100	500	100	1000	1
Gas production (125 m depth): Standard: Produced water								
500	8000	8500	100	5.88	500	5.88	100	0.1
Gas production (125 m depth): Standard: Total fluids								
8500	8000	8500	100	100	500	100	1700	1.7
Gas production (125 m depth): Standard: Produced water								
1000	4500	5500	100	18.2	1000	18.2	100	0.1
Gas production (125 m depth): Standard: Total fluids								
5500	4500	5500	100	100	1000	100	550	0.55

APPENDIX D: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

D.1. Environmental Fate

D.1.1. Biodegradability

TEST SUBSTANCE	The inseparable mixture of the notified chemicals
METHOD	OECD TG 306 Biodegradability in Seawater - Closed bottle test.
Inoculum	Microorganisms present in aged seawater treated with aeration.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Chemical oxygen demand (COD)
Remarks - Method	The test substance was directly added to the test medium at a concentration of 2-10 mg/L. The test was conducted according to the test guidelines above with no significant deviation from the protocol.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	12	5	78
15	7	15	86
28	12	28	86

Remarks - Results	All validity criteria for the test were satisfied. The oxygen consumption by the nitrification of ammonium processes was subtracted from the total oxygen consumption to accurately assess the biochemical oxygen demand (BOD). The toxicity control obtained 53% degradation over 28 days, less than the sum of the BOD of the separate solutions of the test substance and reference. Therefore, the test substance is considered to be inhibitory to bacteria in the seawater.
CONCLUSION	The notified chemicals were not easily biodegraded under the conditions of this test.
TEST FACILITY	Aquateam (1997b)

D.2. Ecotoxicological Investigations

D.2.1. Acute toxicity to fish

TEST SUBSTANCE	The inseparable mixture of the notified chemicals
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static, modified to marine conditions
Species	Sheepshead minnow (*Cyprinodon variegates)*
Exposure Period	96 hours
Auxiliary Solvent	None
Salinity	33 ± 3 ‰
Analytical Monitoring	N/A
Remarks – Method	Fish were exposed in the test media prepared by dissolving of the test substance directly in seawater. The test was carried out under semi-static conditions with the change of the test media after 48 hours. The test fish is not a species recommended by OECD test guideline. However, it is suitable for the seawater test. The study was conducted in

compliance with the good laboratory practice (GLP) standards without other significant deviations from the protocol above.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality (%)*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	Not determined	10	0	0	0	0
100	Not determined	10	0	0	0	0
300	Not determined	10	0	0	0	0
1000	Not determined	10	0	0	0	0

LC50 > 1000 mg/L at 96 hours.
NOEC 1000 mg/L at 96 hours.
Remarks – Results All validity criteria for the test were satisfied. Given no abnormal behaviour for fish was reported at the test concentrations up to 1000 mg/L in the test, the test outcome is considered reliable. The results reported above were based on nominal concentrations.

CONCLUSION The notified chemicals are not harmful to fish

TEST FACILITY AnalyCen (2004a)

D.2.2. Acute toxicity to fish

TEST SUBSTANCE The inseparable mixture of the notified chemicals

METHOD OECD TG 203 Fish, Acute Toxicity Test – Semi-static, modified to marine conditions
Species Turbot (*Scophthalmus maximus*)
Exposure Period 96 hours
Auxiliary Solvent None
Salinity 34-36g/L sodium chloride
Analytical Monitoring N/A
Remarks – Method Fish were exposed in the test media prepared by dissolving of the test substance in artificial seawater. The test was carried out under semi-static conditions with the change of the test media at 48 hours.

The test fish is not a species recommended by OECD test guideline. However, it is suitable for the seawater test. The study was conducted in compliance with the good laboratory practice (GLP) standards without other significant deviations from the protocol above.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality(%)*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
Control	Not determined	7	0	0	0	0
180	Not determined	7	0	0	0	0
320	Not determined	7	0	0	0	0
560	Not determined	7	0	0	0	0
1000	Not determined	7	0	0	0	0
1800	Not determined	7	0	0	0	0

LC50 > 1800 mg/L at 96 hours.
NOEC Not reported
Remarks – Results All validity criteria for the test were satisfied.

No abnormal behaviour for fish was observed for the test concentrations during the test. The actual concentration was not determined and the results reported above were based on the nominal concentrations. The

	results should be treated with caution as the purity of the test substance was not available.
CONCLUSION	The notified chemicals are not harmful to fish
TEST FACILITY	Chemex (2001)

D.2.3. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	The inseparable mixture of the notified chemicals
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	The test concentration was analysed at 0 and 48 hours of the test.
Remarks - Method	GLP standards followed. Following a range finding test at 0.09-89 mg/L, the test was conducted at a nominal concentration of 100 mg/L. The test was conducted according to the test guidelines above with no significant deviation from the protocol.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*
Nominal	*Actual*		*48 h*
Control	0	20	0
100	104	20	0

EC50	> 100 mg/L at 48 hours (nominal)
NOEC	100 mg/L at 48 hours
Remarks - Results	All the test guideline validity criteria were met. All the test concentrations were corrected to account for purity of the test substance. The measured concentration was 104% of the nominal concentration. The notified chemicals are considered not harmful to daphnids based on the test result.
CONCLUSION	The notified chemicals are not harmful to daphnids
TEST FACILITY	Harlan (2013c)

D.2.4. Algal growth inhibition test

TEST SUBSTANCE	The inseparable mixture of the notified chemicals
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	96 hours
Concentration Range	Nominal: 10, 20, 40, 80, and 160 mg /L Actual: 9.11, 18.6, 39.4, 77.8 and 160 mg active ingredient/L at 0 hour 7.41, 16.8, 39.5, 85.2 and 167 mg active ingredient/L at 96 hours
Auxiliary Solvent	None
Water Hardness	Not provided
Analytical Monitoring	Test concentrations were analysed using phosphorous analysis by inductively coupled plasma with mass spectrometric detection (ICP-MS) using external standard.
Remarks - Method	Following a preliminary range-finding test, the definitive test was conducted by exposing algae in the test media prepared in freshwater.

Samples of the algal population were removed daily and cell concentration was determined for each control and treatment group. The test was conducted according to the test guidelines above with no significant deviation from the protocol. GLP standards were followed.

RESULTS

Growth		*Biomass*	
E_rC50 *mg/L at 72 h*	*NOEC* *mg/L*	*E_bC50* *mg/L at 72 h*	*NOEC* *mg/L*
46 (95% CL 40 – 52)	10	21 (95% CL 18-24)	10

Remarks - Results

Chemical analysis of the test preparations at 0 and 96 hours indicated that the test concentration was close to nominal concentration with the mean measured concentration of 83% nominal concentration. Therefore, it is considered reasonable to base the results on nominal test concentrations only.

A re-growth test was performed after the algae were exposed in culture medium for 96 hours. The old culture medium was removed from each control and test preparations. Fresh sterile culture medium was added to each sample to ensure that the test concentration of the test substance was reduced to below the inhibiting level. The result from the re-growth test indicated that the test substance was algistatic in effect. All the test guideline validity criteria were met.

CONCLUSION The notified chemicals are harmful to freshwater alga

TEST FACILITY Harlan (2013d)

D.2.5. Algal growth inhibition test

TEST SUBSTANCE The inseparable mixture of the notified chemicals

METHOD ISO 1995 – Water Quality Marine Algal Growth Inhibition Test With *Skeletonema costatum* and *Phaeodactylum tricornutum,* ISO 10253 standard method

Species *Skeletonema costatum*
Exposure Period 72 hours
Concentration Range Nominal: Control, 10, 50, 250 and 1000 mg/L
Actual: Not determined
Auxiliary Solvent None
Water Hardness Not provided
Analytical Monitoring N/A
Remarks - Method Following a preliminary range-finding test, the definitive test was conducted by exposing algae in the test medium at the above nominal concentrations. GLP standards were followed.

RESULTS

Growth		*Biomass*	
E_rC50 *mg/L at 72 h*	*NOEC* *mg/L*	*E_bC50* *mg/L at 72 h*	*NOEC* *mg/L*
672	250	N/A	N/A

Remarks - Results All the test guideline validity criteria were met.

CONCLUSION The notified chemicals are not harmful to marine algae

TEST FACILITY AnalyCen (2009)

D.2.6. Inhibition of microbial activity

TEST SUBSTANCE	The inseparable mixture of the notified chemicals
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.
Inoculum	Activated sewage sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10, 100, 1000 mg/L Actual: Not determined
Remarks – Method	The test was conducted at 10 – 1000 mg/L. Three replicates were established for the top test concentration of 1000 mg/L. 3,5-Dichlorophenol was used for the reference test. The test was conducted according to the test guidelines above with no significant deviation from the protocol. GLP standards were followed.
RESULTS	
IC50	> 1000 mg/L
NOEC	1000 mg/L
Remarks – Results	All the test guideline validity criteria were met. The test concentrations were not determined during the test. All nominal test concentrations were corrected to account for the purity of the test substance. No inhibitory effects on microbial activity were reported at the top test concentration of 1000 mg/L.
CONCLUSION	The notified chemicals are not inhibitory to microbial activity
TEST FACILITY	Harlan (2013e)

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