File No: STD/1358

July 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

7838

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 Ageing, 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, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

This Full 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:

Street Address:	334 - 336 Illawarra Road MARRICKVILLE NSW 2204, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

TABLE OF CONTENTS

FULL PUBLIC REPORT 3
1. APPLICANT AND NOTIFICATION DETAILS 3
2. IDENTITY OF CHEMICAL 3
3. COMPOSITION 3
4. PHYSICAL AND CHEMICAL PROPERTIES 4
5. INTRODUCTION AND USE INFORMATION 4
6. HUMAN HEALTH IMPLICATIONS 5
7. ENVIRONMENTAL IMPLICATIONS 7
8. CONCLUSIONS AND REGULATORY OBLIGATIONS 9
APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES 11
APPENDIX B: TOXICOLOGICAL INVESTIGATIONS 13
APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS 20
BIBLIOGRAPHY 31

FULL PUBLIC REPORT

7838

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Robert Wee & Associates (ABN 71 899 891 347)
6 Taylor Ave
LOCKLEYS SA 5032

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 name(s), Molecular formula, Structural formula, Molecular Weight, Spectral Data, Degree of purity, Import volume, Details of Use, Identity of Sites.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Hydrolysis as a function of pH, Acute Inhalation Toxicity, Induction of Germ Cell Damage, Acute toxicity to fish and to aquatic invertebrates, and Algal growth inhibition test, Bioaccumulation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
7838 (notified chemical >90%)
Radiagreen MB LC2

CAS NUMBER
Not assigned

MOLECULAR WEIGHT
<500 Da.

ANALYTICAL DATA
Reference UV/VIS, IR and ^{1}H NMR spectra were provided by the notifier.

3. COMPOSITION

DEGREE OF PURITY
>90%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON-HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None
ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Yellow to amber liquid (faint odour)

Property	Value	Data Source/Justification
Freezing Point	-43°C	Measured
Boiling Point	Not determined	Decomposes at >250°C
Density	1100 kg/m^3 at 20°C	Measured
Vapour Pressure	<1.47 x 10^{-6} kPa at 20°C	Measured
Water Solubility	<1 x 10^{-3} g/L at 20°C	Measured. The notified chemical may be dispersible in water given it is a surfactant.
Hydrolysis as a Function of pH	Not determined	The notified chemical contains groups which are susceptible to hydrolysis. However, hydrolysis is expected to be slow in the environmental pH range of 4 – 9.
Partition Coefficient (n-octanol/water)	log P_{OW} ranges between < 0.3 and > 6.5 (mainly > 4.7)	Measured. The notified chemical is expected to partition mainly to octanol from water phase based on the mainly hydrophobic structure.
Adsorption/Desorption	log K_{OC} ranged between < 1.32 and > 5.63.	Measured. The notified chemical is expected to mainly adsorb to the soil sediment from water phase based on the mainly hydrophobic structure.
Dissociation Constant	Not determined	The notified chemical contains no dissociable functional groups at environmental pH range.
Particle Size	Not determined	Notified chemical is a liquid
Flash Point	208°C at 101.3 kPa	Measured
Flammability	Not expected to be highly flammable	Estimated based on structure
Autoignition Temperature	365°C	Measured
Explosive Properties	Not expected to be explosive	Estimated. Notified chemical contains no unstable or highly energetic functional groups.

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 environmental conditions and unreactive in air and in water.

Dangerous Goods classification
Based on the submitted physical-chemical data in the above table, the notified chemical is not classified as dangerous according to the Australian Dangerous Goods Code (NTC, 2007). However, the data above does not address all Dangerous Goods endpoints. Therefore, consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia, but will be imported undiluted.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	<100	<2000	<2000	<2000	<2000

PORT OF ENTRY
Melbourne and Perth

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in 216 L uncoated steel drums and transported to oil drilling sites for use.

USE
The notified chemical will be used as an additive in the oil industry.

OPERATION DESCRIPTION
The notified chemical will be imported at >90% concentration and transported to drilling sites (mainly onshore) where it will be unloaded and manually transferred or sucked out from the import container and injected into crude oil via the wellhead. The crude oil (an oil/water emulsion) containing the notified chemical will be pumped to a tankfarm (or pumped to storage tanks and then transferred to trucks for transport to a tankfarm) where the emulsion is piped into tanks for decanting. The notified chemical is expected to be present in the oil phase which will settle to the bottom of the tank. The oil containing the notified chemical will be pumped to another tank for further refinement. Any notified chemical remaining in the water phase (expected to be minimal) would be sent to water treatment plants.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS
The number of workers expected to be exposed to the notified chemical is likely to be 2-3 at each site plus several transport workers.

EXPOSURE DETAILS
Transport and storage workers are not likely to be exposed to the notified chemical except in the case of an accident involving damage to the import containers.

Workers handling the imported notified chemical at >90% concentration may encounter dermal and possibly ocular exposure during injection via the wellhead. Dermal exposure is the most likely route and may occur during transfer of the notified chemical into the well and connection and disconnection of pipes. However, once the fluid containing the notified chemical has been added to the oil and water emulsion in the well the processes that follow are mostly enclosed and automated and exposure would not be anticipated. In addition, workers on drilling sites are expected to wear personal protective equipment (PPE) such as safety helmets, safety glasses, coveralls and gloves to further minimise dermal and ocular exposure.

Inhalation exposure to the notified chemical is also possible during addition of the imported formulation to the crude oil. However, inhalation exposure is not anticipated to be significant due to its low vapour pressure ($<1.47 \times 10^{-6}$ kPa) and the mostly enclosed systems used to transport the crude oil and the notified chemical throughout the refinement process.

6.1.2. Public exposure
The notified chemical is intended for industrial use on specific sites and therefore public exposure is not anticipated.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 >5000 mg/kg bw low toxicity
Rat, acute dermal toxicity	LD50 >2000 mg/kg bw low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL>1000 mg/kg/bw
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosomal aberration test	non genotoxic

Toxicokinetics, metabolism and distribution.
The notified chemical contains species with molecular weight <500 Da.; and has low water solubility (<1 mg/L) and variable log Pow (<0.3 and >6.5). Despite the low molecular weight, the limited water solubility and variable log Pow suggest it is not readily absorbed. There was no clear evidence of oral absorption in either the acute or repeat dose oral toxicity studies. If oral absorption were to occur, the notified chemical would be expected to undergo hydrolysis, followed by conjugation and readily excreted (NOTOX B.V., 2008f).

The notified chemical is not expected to penetrate the skin based on its low water solubility and log Pow values but its surfactant properties may enhance dermal penetration. The positive results of the skin sensitisation test indicate dermal penetration can occur to some extent (NOTOX B.V., 2008f).

The notified chemical has a very low vapour pressure ($<1.47 \times 10^{-6}$ kPa) which indicates that the availability of the notifeid chemical for inhlatation will be limited.

Acute toxicity
The notified chemical was found to be of low acute oral and dermal toxicity in rats. No acute inhalation toxicity study was provided. The notified chemical is a liquid with a low vapour pressure ($<1.47 \times 10^{-6}$ kPa), which suggests it will not be readily inhaled.

Irritation and Sensitisation.
The notified chemical was found to be slightly irritating to the skin and eyes of rabbits.

The potential for skin sensitisation was evaluated using a local lymph node assay (LLNA). The mean Stimulation Index (SI) for animals treated with the notified chemical at 25, 50 and 100% concentration was determined to be 1.6, 4.4 and 6.3 respectively. Given the SI>3 at 50% and 100% concentration, the notified chemical was considered to be a skin sensitiser.

Repeated Dose Toxicity
In a 28-day repeat dose oral toxicity study on the notified chemical, minor changes were noted in some clinical chemistry and haematology parameters. However, none were considered to be of toxicological significance. Macroscopic examination also revealed incidental abnormalities but no dose-response could be established and therefore these were not considered to be of toxicological significance. The no observed adverse effect level (NOAEL) was determined to be >1000 mg/kg bw/day.

Mutagenicity and genotoxicity
The notified chemical was shown not to be mutagenic in a bacterial reverse mutation study (Ames Test) and did not induce chromosome aberrations in cultured mammalian somatic cells. Based on the results of these tests, the notified chemical is considered not to be mutagenic or clastogenic.

No data were available to assess the potential for carcinogenicity or reproductive toxicity.

Health hazard classification
Based on the lymphocyte proliferative response observed in the LLNA study, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R43 May cause sensitisation by skin contact

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
The main concern for workers using the notified chemical is skin sensitisation as demonstrated in the LLNA study. Workers connecting and disconnecting hoses to drums of the imported formulation containing the notified chemical at >90% concentration may encounter dermal and ocular exposure to spills and splashes. Dermal and ocular exposure is expected to be minimised by the use of PPE and thus, the risk is not considered unacceptable. The use of PPE would also minimise any risk of irritation to the eyes or skin.

The potential for respiratory irritation and sensitisation has not been determined. The notified chemical has a low vapour pressure (<1.47 x 10^{-6} kPa) which suggests that it would not be available for inhalation. However, the mechanical pumping operations may create aerosols of the notified chemical. The use of respiratory protection or exhaust ventilation would reduce the potential for inhalation exposure to the notified chemical.

The notified chemical was reported to have a NOAEL >1000 mg/kg bw/day and therefore repeated exposure to workers is not expected to result in adverse effects.

Overall, the risk of skin sensitisation is not expected to be unacceptable provided that appropriate PPE is used. Measures to reduce inhalation exposure are recommended to reduce any potential risk.

6.3.2. Public health
The public are not likely to be exposed to the notified chemical as it will only be used at a limited number of industrial sites. Therefore, the risk to public health is expected to be negligible.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported for direct use in oil installations (mainly on-shore) in Australia and will not be reformulated in Australia. Therefore, no environmental release is expected from the manufacture or reformulation of the notified chemical in Australia. Release from residues in import drums will be minimal (2000 kg per annum assuming 0.1% loss) as the product is a liquid and residues are expected to be either disposed of to landfill with empty containers or treated according to State/ Territory regulations in the most likely event of drums being recycled. Accidental spills of the product are expected to be absorbed with inert absorbent material, swept up and placed into containers and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE
The notified chemical will be exclusively used as in the oil industry. For application, it will be injected with a pump into the oil production flow via the wellhead. Together with the produced oil and water, the notified chemical will then be transferred into a tankfarm via a pipeline network. At the tankfarm, the oil/water will be put in big tanks and the mixture allowed to settle for decantation. The bottom phase in the tanks (oil phase) will be pumped into another tank to further continue the oil/water separation process. No spills are expected to occur from these processes.

RELEASE OF CHEMICAL FROM DISPOSAL
Most of the notified chemical will remain in the oil phase and be sent to the refinery. During oil refining, the notified chemical is expected to remain in the residues/tar fraction.

The water phase is estimated to contain 0.01% of the injected notified chemical (200 kg per annum). The majority is used onshore and is therefore normally sent via pipeline or trucks to waste water treatment

plants. In the processes of waste water treatment, most of the notified chemical in the waste water is expected to be associated with the sludge sediment due to its low water solubility and hence be removed for disposal to landfill.

7.1.2 Environmental fate

Although the notified chemical is considered not to be readily biodegradable based on the study provided by the notifier, it is considered to be biodegradable since a degree of 65% degradation was achieved. The notified chemical is considered to have potential for bioaccumulation based on its molecular weight range, mainly hydrophobic structure and low water solubility. However, this bioaccumulative potential should not be significant due to the biodegradability of the notified chemical. In addition, no significant release of the notified chemical to the water compartment is expected. Therefore, the notified chemical is not considered to be bioavailable to aquatic organisms.

For the details of the environmental fate studies, refer to Appendix C.

Most of the notified chemical will end up in the residues/tar fraction in the refinery, which will most likely be used as road base material. A minor amount of the notified chemical will be sent to landfill in the form of sediment sludge from waste water treatment plants. The notified chemical will undergo biotic and abiotic degradation processes, forming water and oxides of carbon.

7.1.3 Predicted Environmental Concentration (PEC)

The PEC has not been calculated since no significant release of the notified chemical to the water environment is expected.

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*
Turbot Fish Toxicity, Acute	LL50 = 172 mg/L[a] (WAF)	Not harmful up to the limit of solubility
Acartia tonsa Invertebrate Toxicity, Acute	EL50 = 290 mg/L[a] (WAF)	Not harmful up to the limit of solubility
Algal Toxicity, Acute	E_rL50 = 220 mg/L[a] (WAF)	Not harmful up to the limit of solubility
Inhibition of Bacterial Respiration	IC50 > 100 mg/L	Not harmful
Corophium sp Invertebrate Toxicity, Acute	LC50 > 12208 mg/kg (dry sediment)[b]	Not harmful

[a] Appears to be based on a physical effect and not a toxic effect.
[b] Endpoint for formulation containing 50% notified chemical.

The notified chemical is not harmful to marine aquatic organisms up to its limit of water solubility. It is also assumed not to be harmful to the 3 freshwater trophic levels.

7.2.1 Predicted No-Effect Concentration

A PNEC for the aquatic compartment has not been calculated since the notified chemical is not harmful up to the limit of its solubility in water based on the studies provided by the notifier.

7.3. Environmental risk assessment

The risk quotient (RQ = PEC/PNEC) has not been calculated since no significant release of the notified chemical to the water compartment is expected. No unacceptable risk to the aquatic organisms is expected from the notified chemical based on its reported use pattern and the absence of any significant acute toxicity effects to species from 3 aquatic trophic levels.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the provided data, the notified chemical is classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] with the following risk phrase:

R43 May cause sensitisation by skin contact.

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Skin sensitisation	1	May cause an allergic reaction

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

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

Environmental risk assessment
On the basis of the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemical:
  - R43 May cause sensitisation by skin contact.

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - ≥1%: R43 May cause sensitisation by skin contact.

- 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.

Material Safety Data Sheet

- The MSDS provided by the notifier should be amended as follows:
  - the notified chemical should be identified by its chemical name (as it is a Type 1 ingredient) based on it skin sensitisation potential.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced in the product 7838.
  - Avoid skin and eye contact
  - Use only in well ventilated areas
  - Avoid generation of aerosols during addition to drilling fluids

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced in 7838:
  - Impervious gloves
  - Coveralls
  - Respiratory protection if ventilation is inadequate.

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

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical 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 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 an additive in the oil industry, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 2000 tonnes, or is likely to increase, significantly;
  - the chemical has begun to be manufactured in Australia;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

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

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Tests were performed on the notified chemical at >90% concentration.

Melting Point/Freezing Point -43°C

Method	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Determined by DSC
Test Facility	NOTOX B.V. (2007a)

Boiling Point Decomposes >250°C at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Determined by DSC
Test Facility	NOTOX B.V. (2007a)

Density 1100 kg/m^3 at 20°C

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Test Facility	NOTOX B.V. (2007a)

Vapour Pressure < 1.47 x 10^{-6} kPa at 20°C

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks	Isothermal thermogravimetric effusion method. The validity of the method was verified using a suspension of hexachlorobenzene in chloroform. The measurement was conducted between 80 – 170˚C. The weight loss of the notified chemical at 100 – 140˚C was less than that of the reference substance (known vapour pressure 1.47 x 10^{-6} kPa at 20°C). Therefore, it is concluded that the vapour pressure of the notified chemical at 20°C is < 1.47 x 10^{-6} kPa.
Test Facility	NOTOX B.V. (2007a)

Water Solubility < 1 x10^{-3} g/L at 20°C

Method	Preliminary test OECD TG 105 Water Solubility (1995). EC Directive 92/69/EEC A.6 Water Solubility. Definitive test Modification of Method *A2* of ASTM International E1148 – 02 guideline
Remarks	A preliminary test indicated that the flask method was not suitable for the notified chemical. This is mainly because of the difficulty to separate the undissolved chemical from the aqueous phase due to the surfactant nature of the notified chemical. Therefore, a method based on Method *A2* of ASTM International E1148 – 02 guideline was applied: five suspensions at nominal concentrations of 1, 10, 25, 50 and 100 mg/L in double distilled water were stirred at 20°C for 70 hours. After the stirring period, turbidity was investigated using a spectrophotometer at 300 nm. At 0.999 mg/L (nominal) the turbidity was significantly higher than the turbidity of the blank double distilled water (no turbidity). The increasing tendency in the absorbance of the test solutions with the increase in test concentration was observed through the entire nominal concentration range of 0.999 – 101 mg/L. Based on this, it was concluded that the water solubility of the notified chemical was < 1 mg/L. It is also concluded that the notified chemical is highly dispersible in water, which complies with the molecular structural features.
Test Facility	NOTOX B.V. (2007a)

Partition Coefficient (n-octanol/water) log P_{OW} ranges between < 0.3 and > 6.5 (mainly > 4.7)

Method OECD TG 117 Partition Coefficient (n-octanol/water).

EC Directive 92/69/EEC A.8 Partition Coefficient.

Remarks HPLC Method. The column temperature was set up as 22±1˚C. Twenty three peaks were observed in the LC-MS chromatograms of the notified chemical, corresponding to log P_{OW} values of between < 0.3 and > 6.5. The majority of the peaks corresponded to log P_{OW} values of > 4.7. DEWHA notes that surfactants are difficult substances to test with respect to partition coefficient. Therefore, these results should be treated with caution.

Test Facility NOTOX B.V. (2007a)

Adsorption/Desorption – main test log K_{OC} between < 1.32 and > 5.63

Method Estimated using OECD 121 "Estimation of adsorption Coefficient (K_{OC}) on soil and on sewerage sludge using High Performance Liquid Chromatography (HPLC)"

EC Directive 2001/59/EC C.19 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography

Remarks HPLC Method. The column temperature was set at 35˚C. Thirteen peaks were observed in the LC-MS chromatograms of the notified chemical, corresponding to log K_{OC} values of between < 1.32 and > 5.63. DEWHA notes that surfactants are difficult substances to test with respect to adsorption/desorption. Therefore, these results should be treated with caution.

Test Facility NOTOX B.V. (2007a)

Flash Point 208ºC at 101.3 kPa

Method EC Directive 92/69/EEC A.9 Flash Point.

Test Facility NOTOX B.V. (2007a)

Autoignition Temperature 365ºC

Method EC Directive 92/69/EEC A.15 Auto-Ignition Temperature (Liquids and Gases).

Test Facility NOTOX B.V. (2007a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (>90% concentration)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar strain Crl:WI
Vehicle	None
Remarks - Method	No significant protocol deviations. The notified chemical was administered to two subsequent groups of these female rats at 2000 mg/kg bw.

RESULTS

LD50	>5000 mg/kg bw
Signs of Toxicity	No mortalities were reported. Hunched posture was observed in all animals as well as piloerection in one animal on Day 1.
Effects in Organs	No abnormalities were found at necropsy.
Remarks - Results	All animals displayed normal weight gain over the course of the study. The oral LD50 value of the notified chemical was established to be >2000 mg/kg bw according to the OECD 423 test guidelines for Acute Toxic Class Method. The LD50 cut-off value in this case was considered to be >5000 mg/kg bw.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	NOTOX B.V. (2008a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical (>90% concentration)
METHOD	OECD TG 402 Acute Dermal Toxicity. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).
Species/Strain	Rat/ Wistar strain Crl:WI
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	5 males	2000	0
2	5 females	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	Grade 1 scales and erythema were observed in 3 females resolving by Day 5. One female had grade 2 erythema which had resolved completely by Day 5 and grade 2 scales which had resolved completely by Day 9.
Signs of Toxicity - Systemic	Piloerection (1 male) and chromodachyorrhoea (4 males and 2 females) around the snout were observed in some animals. These symptoms resolved between Day(s) 1 and 7.
Effects in Organs	No abnormalities were noted in any of the animals at necroscopy.
Remarks - Results	Body weight gains in males and females were within the range expected for this type of rats in this study and therefore were not indicative of toxicity.

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

TEST FACILITY NOTOX B.V. (2008b)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 males
Vehicle None
Observation Period 7 days
Type of Dressing Semi-occlusive.
Remarks - Method No significant protocol deviations

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Observed Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1	1.3	0.7	2	72 hrs	0
Oedema	1	0.7	0	2	48 hrs	0

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

Remarks - Results Grade 2 erythema was observed in all 3 animals, resolving within 72 hours in one animal and 7 days in other 2. Grade 2 oedema was also observed in all 3 animals resolving by 72 hours in all 3.

CONCLUSION The notified chemical is slightly irritating to the skin.

TEST FACILITY NOTOX B.V. (2008c)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 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	3			
Conjunctiva: redness	0.7	0.7	0.7	1	48 hrs	0
Conjunctiva: chemosis	0.3	0.3	0.3	1	24 hrs	0
Conjunctiva: discharge	0.3	0.3	0.3	1	24 hrs	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks - Results Conjunctival redness (Grade 1) was observed in all 3 animals, resolving by 72 hours. Grade 1 chemosis and discharge were also observed in all 3

	animals but had resolved within 48 hours.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	NOTOX B.V. (2008d)

B.5. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical (>90% concentration)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Wistar Crl:(WI)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 28 days
Vehicle	Propylene glycol
Remarks - Method	No recovery groups were tested in the study. No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0 (vehicle)	0
low dose	5 per sex	50	0
mid dose	5 per sex	150	0
high dose	5 per sex	1000	0

Mortality and Time to Death

No unscheduled deaths occurred during the study.

Clinical Observations

No clinical signs of toxicity were observed during the observation period in males treated with 50 mg/kg bw/day or animals from either sex treated with 150 mg/kg bw/day. Some incidental findings such as opacity in the right eye (1 male treated with 1000 mg/kg bw/day), alopecia and scabs on the neck (1 female treated with 50 mg/kg bw/day) and abnormal breathing (rales) in 1 female treated with 1000 mg/kg bw/day were observed. However these findings were considered not unusual in rats of this age and strain and of no toxicological significance.

Statistically significant decreased body weight gains in males of the mid and high dose groups were observed in week 2 but these findings were absent in following weeks and therefore these effects were not considered of toxicological significance. Overall, no toxicologically significant changes in body weights, body weight gains or food consumption were noted.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

No toxicologically relevant changes occurred in haematological parameters of treated rats. However, a statistically significant lower partial thromboplastin time (APTT) and lower relative eosinophil counts of males and females at 1000 mg/kg/day respectively was not considered to be abnormal for rats of this strain and age.

No toxicologically relevant changes occurred in clinical biochemistry parameters of treated rats. However, a higher inorganic phosphate level in males at 1000 mg/kg/day did not show a dose-related response and was thus not considered of toxicological significance.

Effects in Organs

No statistically significant differences in organ weights or organ to body weight ratios were reported in treated rats. No toxicologically relevant abnormalities were noted during macroscopic examination. Incidental

findings common to rats of this strain noted during necroscopy included pale foci on the lungs, pelvic dilation of the kidney, red discoloration of the thymus or lungs, enlarged mandibular lymph nodes, cloudy eyes, fluid in the uterus and ectopic splenic tissue. However, there was no dose relationship with the incidence or severity of these findings and these were considered to be of no toxicological significance.

Remarks – Results
No adverse treatment related effects were seen at any dose level in this study.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as >1000 mg/kg bw/day in this study.

TEST FACILITY NOTOX B.V. (2008e)

B.6. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Pre incubation procedure

Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA
Metabolic Activation System	Rat S9 fraction from phenobarbital/β-napthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 33-3330 µg/plate b) Without metabolic activation: 33-3330 µg/plate
Vehicle	DMSO
Remarks - Method	The plates treated with S9 mix in Test 1 contained 5% S9 fraction. This was increased to 10% in Test 2. In preliminary toxicity testing, precipitation was observed at dose levels of 3330 and 5000 µg/plate. In test strain TA100, toxicity was observed at dose levels of 1000 µg/plate and above in the absence of S9 mix and at 5000 µg/plate in the presence of S9 mix. In test strain WP2uvrA, no toxicity was observed

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥3300	3300	3300	Negative
Test 2	Not performed	3330	3300	Negative
Present				
Test 1	≥3300	3300	3300	Negative
Test 2	Not performed	3300	3300	Negative

Remarks - Results

Slight precipitate was observed at concentrations of 3300 µg/plate in several bacterial strains in the presence and absence of metabolic activation. This was not severe enough to prevent scoring.

Reduced background lawn was reported in several strains at 3300 µg/plate in the presence and absence of metabolic activation.

Microcolonies were observed in the TA1537 and TA100 strains in the absence of metabolic activation only.

All the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the

activity of the S9-mix and the sensitivity of the bacterial strains.

No toxicologically significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

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

TEST FACILITY NOTOX B.V. (2007b)

B.7. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line	Peripheral human lymphocytes
Metabolic Activation System	Rat S9 fraction from phenobarbital/β-napthoflavone induced rat liver
Vehicle	DMSO
Remarks - Method	No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	33*, 100*, 333*	3 hrs	24 hrs
Test 2	33*, 100, 333*, 450, 600*, 750, 900	24 hrs	24 hrs
Test 2 (48 hrs)	33*, 100, 333*, 450, 600*, 750, 900	48 hrs	48 hrs
Present			
Test 1	33*, 100*, 333*	3 hrs	24 hrs
Test 2	33*, 100*, 333*	3 hrs	48 hrs

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>333	-	>100	negative
Test 2	-	>450	>100	negative
Test 2 (48 hrs)	-	>450	>100	negative
Present				
Test 1	-	-	>100	negative
Test 2	-	>333	>100	negative

Remarks - Results A dose range test was conducted at concentrations ranging from 3 to 3300 μg/mL. Precipitation was observed at doses >100 μg/mL in the absence and presence of metabolic activation in the dose range finding test as well as Tests 1 and 2. Significant reductions in the mitotic index were observed at >333 μg/mL in the absence of metabolic activation in the dose range finding test and at >450 μg/mL in the absence of metabolic activation in Test 2.

Mitomycin was dosed at 0.05 μg/mL as the positive control for cultures exposed for 3 hours, 0.2 μg/mL for cultures exposed for 24 hours and 0.1 μg/mL for cultures exposed for 28 hours in the absence of metabolic activation. Cyclophosphamide was dosed at 10 μg/mL as the positive control for cultures exposed for 3 hours in the presence of metabolic

activation. Both positive controls confirmed the sensitivity of the test system.

The test material did not induce any statistically significant increases in the frequency of cells with aberrations, or in the numbers of polyploid cells.

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

TEST FACILITY NOTOX (2007c)

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

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
EC Directive 2004/73/EC B.42 Skin Sensitisation (Local Lymph Node Assay)

Species/Strain Mouse/CBA strain
Vehicle *N,N*-Dimethylformamide
Remarks - Method No significant protocol deviations. A preliminary irritation study was conducted in order to select the highest concentration of the test substance to be used in the main study. Three groups of 5 animals were treated with 1 test substance concentration per group. One group of 5 animals was treated with vehicle. A test to confirm the reliability of the positive control substance (Alpha-Hexylcinnamaldehyde (HCA)) to predict the skin sensitisation potential using the LLNA test was conducted within the 6 months prior to the test using the notified chemical.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	613	1
25	969	1.6
50	2722	4.4
100	3876	6.3
Positive Control - HCA		
5	474	1.3
10	547	1.5
25	1980	5.5

Five females per group

Remarks - Results One animal in the 100% group displayed signs of toxicity such as significant loss of body weight, hunched posture, lethargy, hypothermia and ptosis, and was excluded from the data set for analysis.
These results indicate that the notified chemical could elicit a SI ≥3. The data showed a dose response and an EC3 value of 37.5% was calculated.
The positive control test found HCA to induce a Stimulation Index (SI) of 5.5 at 25% concentration, thus confirming the acceptability of HCA as a reliable positive control substance.

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

TEST FACILITY NOTOX B.V. (2008f)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical (>90% concentration)

METHOD: OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test (1992).

EC Directive 92/69/EEC C.4-C Biodegradation: Determination of the " Ready" Biodegradability: Carbon Dioxide Evolution Test Modified Sturm Test).

ISO 9439 “Water Quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium - carbon dioxide evolution test (1999).

Inoculum	Activated sludge from a domestic sewage treatment plant.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Total Organic Carbon (TOC) analysis
Remarks - Method	The notified chemical was tested in duplicate at 22 mg/L, corresponding to 12 mg TOC/L. The test media were continuously stirred during the test, to ensure optimal contact between the test substance and the test organisms.

A positive control (using sodium acetate as the reference substance at 40 mg/L, or TOC 12 mg /L) and a toxicity control test (containing the notified chemical 44.2 mg CO_2/L and sodium acetate 43.1 mg CO_2/L) were conducted.

The CO_2 produced was absorbed with barium hydroxide in the gas scrubbing bottle and precipitated out as barium carbonate. The amount of CO_2 produced was determined by titrating the remaining $Ba(OH)_2$ with 0.05 M standardized HCl.

The Total Organic Carbon (TOC) content was determined to be 54.72% by measuring a sample of the pure test substance. The $ThCO_2$ was therefore calculated to be 2.01 mg CO_2/mg of the chemical.

RESULTS

Notified Chemical		*Sodium Acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
2	8	2	14
5	23	12	58
19	55	14	61
29	65	29	70

Remarks - Results: All the criteria for the test validity were met.

More than 25% degradation occurred within 14 days (63% based on $ThCO_2$), therefore, the notified chemical is assumed not to inhibit microbial activity.

The notified chemical reached a biodegradation degree of 65% at the end of the test. It narrowly missed the 10-day window criterion. Therefore, it is considered not to be readily biodegradable according to the OECD test guideline.

CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	NOTOX B.V. (2007d)

C.1.2. Biodegradability in seawater

TEST SUBSTANCE	Notified chemical (>90% concentration)
METHOD	OECD TG 306: Biodegradability in seawater, Closed Bottle Method
Inoculum	No inoculum was added
Exposure Period	28 days
Auxiliary Solvent	Acetone
Analytical Monitoring	Total Organic Carbon (TOC) analysis. The biochemical oxygen demand (BOD) of the test substance is calculated as a percentage of the theoretical oxygen demand (ThOD).
Remarks - Method	The study was performed at a test concentration of 2.58 mg/L in duplicate (corresponding to ThOD 5.03 mg O_2/L) at 19.6 – 20.1˚C. Due to the poor solubility of the notified chemical in water, glass fibre filters were used as an inert carrier for the test substance. 100 µL of a test substance solution in acetone (77.3 mg/10mL) was applied to the filters. After evaporation of the solvent, the filters were placed in the test bottles. Seawater aged 7 days at room temperature after collection from a non-polluted site was decanted to remove possible particles and was used in the test. Nutrients were added presumably according to the method recommended in the Guideline. A blank control, a positive control (using sodium acetate as the reference substance at 2.5 mg/L or ThOD 4.05 mg/L) and a toxic control (containing the notified chemical at 2.58 mg/L and sodium acetate at 1.25 mg/L) were conducted in duplicate.

RESULTS

Notified Chemical		*Sodium Acetate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	36	7	80
14	43	14	89
21	50	21	87
28	56	28	93

Remarks - Results	All the criteria for the test validity were met. The degradation degree reached by the reference chemical in the toxicity control test has been calculated as 84% assuming the notified chemical reached the same degree of degradation as when tested alone. Comparing 84% with 93%, the notified chemical is not considered to inhibit the microbial activity to any significant extent at the concentration tested. The biodegradation level of the notified chemical after 28 days was 56%. Therefore, it could not be concluded as having a potential for biodegradation in the marine environment. Further studies are necessary to establish such a potential. Further degradation to 60% may be expected when prolonging the test period for 1-2 weeks.
CONCLUSION	Further studies are necessary to establish the potential for biodegradation in marine environment.

TEST FACILITY M-LAB AS (2005)

C.1.3. Bioaccumulation

Notified chemical (>90% concentration)

CONCLUSION A bioaccumulation test in fish has not been provided. The notified chemical has a mean molecular weight of < 1000 Da. and has very limited solubility in water. Therefore, the notified chemical is potentially bioavailable to aquatic organisms. However, its biodegradability in fresh and salty water reduces the significance of the potential for bioaccumulation.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish – Study 1

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD OECD TG 203 Fish, Acute Toxicity Test - Semi-static-96h (1993), modified to marine conditions.

OSPAR / PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 1995 (OSPAR, 1995).

Species	*Scophthalmus maximus* (Turbot)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	No analytical confirmation of the actual test concentrations
Remarks – Method	Following a range-finding test, turbot was exposed to the notified chemical at loading rates of 0, 32, 56, 100, 180 and 320 mg/L (water-accommodated fraction, WAF) according to OSPAR guidelines. The test was conducted at 15±1.5˚C, pH 7.7–8.4 and dissolved oxygen concentrations of > 83% air saturation value. Seven fish were used for each vessel. For the preparation of WAFs, a stock solution of 1000 mg/L was prepared in seawater (salinity: 30–34 g/L NaCl). After being agitated vigorously and allowed to settle for 4 hours, a cloudy off white solution was obtained. For each nominal concentration, the required amount of homogenised sample was added to 10 litres of diluted water. The test and control solutions were renewed after 48 hours of the tests.

RESULTS

Concentration mg/L Nominal(WAF)	*Number of Fish*	*Mortality*				
		1 h	*24 h*	*48 h*	*72 h*	*96 h*
0	7	0	0	0	0	0
32	7	0	0	0	0	0
56	7	0	0	0	0	0
100	7	0	0	0	0	0
180	7	0	0	0	0	4
320	7	0	5	7	7	7

LL50	172 mg/L (95% confidence limits 138 – 214 mg/L) at 96 hours (WAF)
NOEL	100 mg/L at 96 hours (WAF)
Remarks – Results	The 96 h LL50 value was determined by the Spearman-Karber method. The 95% confidence limits were calculated using ToxCalc version 5.0 “Comprehensive Toxicity Data Analysis and Database Software”.

Considering the low water solubility of the notified chemical (< 1 mg/L), and the fact that no mortality was observed in the 96 hours test at loading rates up to 100 mg/L, the notified chemical is not considered to be

harmful to turbot up to the limit of solubility in water. Mortalities observed at the 2 highest nominal concentrations may result from physical effects of the suspended particles of the notified chemical. However, it is not reported if particulate matter was present in the WAFs used for the test.

CONCLUSION The notified chemical is not harmful to turbot up to its limit of solubility in water

TEST FACILITY Chemex Environmental International Limited (2002a)

C.2.2. Acute toxicity to fish – Study 2

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD OECD TG 203 Fish, Acute Toxicity Test - Semi-static-96h (1993), modified to marine conditions.

OSPAR / PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 1995 (OSPAR, 1995).

Species	*Scophthalmus maximus* (Turbot)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	No analytical confirmation of the actual test concentrations
Remarks – Method	Following a range-finding test, turbot was exposed to the notified chemical loading rates of 0, 100, 180, 320, 560 and 1000 mg/L (water-accommodated fraction, WAF) according to OSPAR guidelines. The test was conducted at 15±1.5˚C, pH 7.9 – 8.4 and dissolved oxygen concentrations of > 78% air saturation value. Seven fish were used for each vessel. For the preparation of WAFs, a stock solution of 1000 mg/L was prepared in seawater (salinity: 29 – 35 g/L NaCl). After being agitated vigorously and allowed to settle for 4 hours, a cloudy white solution with undissolved sediment material was observed. For each nominal concentration, the required amount of homogenised sample was added to seawater, mixed for 20-24 h and then allowed to separate for 4 h. The supernatant was drawn and used for the test.

RESULTS

Concentration mg/L Nominal (WAF)	*Number of Fish*	*Mortality*				
		1 h	*24 h*	*48 h*	*72 h*	*96 h*
0	7	0	0	0	0	0
100	7	0	0	0	0	0
180	7	0	0	0	0	0
320	7	1	1	1	1	1
560	7	4	4	4	4	4
1000	7	7	7	7	7	7

LL50	493 mg/L (95% confidence limits 359 – 685 mg/L) at 96 hours (WAF)
NOEL	180 mg/L at 96 hours (WAF)
Remarks – Results	The 96 h LL50 value was determined by the Maximum Likelihood-Probit method. The 95% confidence limits are calculated using ToxCalc version 5.0 “Comprehensive Toxicity Data Analysis and Database Software”.

Considering the low water solubility of the notified chemical (< 1 mg/L), and the fact that no mortality was observed in the 96 hours test at loading rates up to 180 mg/L, the notified chemical is not considered to be harmful to turbot up to the limit of solubility in water. Mortalities observed at the higher nominal concentrations may result from physical

effects of the suspended particles of the notified chemical. However, it is not reported if particulate matter was present in the WAFs used for the test.

CONCLUSION The notified chemical is not harmful to turbot up to its limit of solubility in water

TEST FACILITY Chemex Environmental International Limited (2002b)

C.2.3. Acute toxicity to fish – Study 3

TEST SUBSTANCE Notified chemical (50%)

METHOD OECD TG 203 Fish, Acute Toxicity Test - Semi-static – 96h (1993), modified to marine conditions.

OSPAR / PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 1995 (OSPAR, 1995).

Species	*Scophthalmus maximus* (Turbot)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	No analytical confirmation of the actual test concentrations
Remarks – Method	Following a range-finding test, turbot was exposed to the test substance at loading rates of 0 (control), 560, 1000 and 1800 mg/L (water-accommodated fraction, WAF) according to OSPAR guidelines. The test was conducted at 15±1.5˚C, pH 7.6–8.4 and dissolved oxygen concentrations of 90% - 98% air saturation value. The number of animals tested was not reported.

Concentration mg/L	*Mortality*				
Nominal (WAF)	*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	0	0	0	0
560	0	0	0	0	0
1000	0	0	0	0	0
1800	0	0	0	0	0

LL50	> 1800 mg/L at 96 hours (WAF)
NOEL	1800 mg/L at 96 hours (WAF)
Remarks – Results	No mortality was observed at each of all the test levels. Therefore, the LL50 is determined to be > 1800 mg/L, the NOEL was determined to be 1800 mg/L.

The test substance is considered not harmful to turbot based on the test results.

CONCLUSION The test substance is not harmful to turbot

TEST FACILITY Chemex Environmental International Limited (2005a)

C.2.4. Acute toxicity to aquatic invertebrates – Study 1

TEST SUBSTANCE Notified chemical (>90% concentration)

METHOD ISO 14669 (1998): Water Quality-Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*)

OECD 2000: Guidance Document on aquatic toxicity testing of difficult substances and mixtures. OECD Environmental Health and Safety

Publications Series on Testing and Assessment No. 23. ENV/JM/MONO (2000)6.

Species	*Acartia tonsa* (marine crustacean)
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	TOC Analysis
Remarks - Method	*Acartia tonsa* was exposed to the notified chemical at loading rates 32, 56, 100, 180 and 320 mg/L (WAFs) in four vessels at 19.3 – 20.1˚C, pH 7.89 – 8.09. For preparation of the WAFs, the notified chemical was agitated in the test medium in the dark at 20°C for 22 h, and allowed to settle for 1 h. The WAFs were then drawn off by siphoning through a glass tube avoiding the settled material at the bottom of the flasks. Natural seawater (salinity adjusted by distilled water to 32 S) was used for preparation of the WAFs. A reference control using 3,5-dichlorophenol at 1 mg/L and a set of four blank controls were also performed in four replicates. 5-8 animals were used in each of the test and control vessels. The TOC analysis showed at the start of the test a value of 1.3 mg /L for the control seawater, 11.5 mg /L for the 32 mg/L WAF and 85.8 mg/L for the 320 mg/L WAF. The test substance was not fully dissolved at any of the loadings used for preparation of WAFs. Some of the material dispersed into the water phase resulted in slightly turbid solutions. The total numbers of immobilised and alive animals at all the test levels were analysed using probit analysis (Swedish Environmental Protection Agency software) to determine the LL50.

RESULTS

Concentration mg/L Nominal (WAF)	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
0	108	1	5
32	23	0	0
56	23	0	1
100	25	0	1
180	23	0	2
320	25	1	17

EL50	290 mg/L at 48 hours (95% confidence limits 207 – 547 mg/L) (WAF)
NOEL	100 mg/L at 48 hours (WAF)
Remarks - Results	The control mortality at 48 hours was 5%. The mortality in the WAFs exceeded this level at loading rates of 180 mg/L and 320 mg/L. Therefore, the NOEL has been determined to be 100 mg/L (WAF). The LL50 (48h) has been determined to be 290 mg/L (95% confidence limits 207 – 547 mg/L) (WAF). Based on the results and considering the low solubility in water, the notified chemical is not considered to be harmful to *Acartia tonsa* up to its solubility in water. The TOC analysis showed that a significant portion of the test substance partitioned to the water phase as dispersable matter. The lethal effects observed at higher levels (180 and 320 mg/L) are considered to result from the physical effects of the dispersed particles of the notified chemical in the test medium.

CONCLUSION	The notified chemical is not harmful to *Acartia tonsa* up to its limit of solubility in water.
TEST FACILITY	Norwegian Institute for Water Research (NIVA) (2002)

C.2.5. Acute toxicity to aquatic invertebrates – Study 2

TEST SUBSTANCE	Notified chemical (50%)
METHOD	ISO 14669 (1998): Water Quality-Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*) OECD 2000: Guidance Document on aquatic toxicity testing of difficult substances and mixtures. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 23. ENV/JM/MONO (2000)6.
Species	*Acartia tonsa* (marine crustacean)
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	TOC Analysis
Remarks - Method	*Acartia tonsa* was exposed to the test substance at loading rates 100, 180, 320, 560 and 1000 mg/L (WAFs) at 20±1˚C. For preparation of the WAFs, the test substance was agitated in the test medium for 23h in the dark at 21±1°C, allowed to settle for 1 h. The WAFs were then drawn off by siphoning through a glass tube avoiding the settled material at the bottom of the flasks. Natural seawater (salinity adjusted by distilled water to 32 S) was used for preparation of the WAFs. A reference control using 3,5-dichlorophenol at 1 mg/L and a set of four blank controls were also performed in four replicates. 5-8 animals were used in each of the test and control vessels. The TOC analysis showed at the start of the test a value of 1.2 mg /L for the control seawater, 2.1 mg /L for the 100 mg/L WAF and 7.5 mg/L for the 1000 mg/L WAF.

RESULTS

Concentration mg/L Nominal (WAF)	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
0	144	2	5
100	24	1	1
180	28	0	0
320	24	0	0
560	26	0	0
1000	24	0	0

EL50	> 1000 mg/L at 48 hours (WAF)
NOEL	1000 mg/L at 48 hours (WAF)
Remarks - Results	The control mortality was 3.5%. Since no mortality was observed at levels 180 mg/L – 1000 mg/L the NOEL has been determined to be 1000 mg/L (WAF). The LL50 (48h) has been determined to be > 1000 mg/L. The one mortality at 100 mg/L was not considered due to the toxicity of the test substance to *Acartia tonsa,* given no mortality was observed at all other higher levels. The TOC analysis showed that a small portion of the test substance partitioned to the water phase. Based on the results, the test substance is not considered to be harmful to *Acartia tonsa* up to the limit of its

solubility in water.

CONCLUSION The test substance is not harmful to *Acartia tonsa* up to the limit of its solubility in water.

TEST FACILITY Norwegian Institute for Water Research (NIVA) (2004a)

C.2.6. Algal growth inhibition test – Study 1

TEST SUBSTANCE The notified chemical (>90% concentration)

METHOD ISO 10253 (1998): Water Quality-Marine growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum.*

OECD 2000: Guidance Document on aquatic toxicity testing of difficult substances and mixtures. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 23. ENV/JM/MONO (2000)6.

Species	*Skeletonema costatum* (marine alga)
Exposure Period	72 hours
Concentration Range	18, 32, 56, 100, 180, 320, 560 and 1000 mg/L (WAF)
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	TOC Analysis for test concentration determination. The cell growth was monitored by daily counting of cell numbers using a Coulter Multisizer and measurement of *in vitro* chlorophyll fluorescence.
Remarks - Method	Test algae were exposed in three replicates to the WAFs of the notified chemical at a cell density of approximately 5×10^6 cells/L and 20.5±1°C under continuous illumination. A blank control was also conducted in 6 replicates. To prepare the WAFs, the notified chemical was agitated for 22.5 h in the dark at 20°C, and allowed to settle for 1 h. The WAFs were drawn with a pipette, avoiding the settled material at the bottom of the flasks. Some of the material dispersed into the water phase and resulted in slightly turbid solutions. The test medium used was ISO 10253 medium based on filtered (0.45 µm) natural seawater (salinity 33.7 S). Nutrients were added. The TOC analysis showed at the start of the test a value of 6.1 mg/L for the control, 11.1 mg/L for the 18 mg/L WAF, 32.1 mg/L for the 100 mg/L WAF and 313 mg/L for the 1000 mg/L WAF.

RESULTS

Growth *E_rL50* *mg/L at 48h (WAF)*	*Biomass* *E_bL50* *mg/L at 48 h (WAF)*
220 (95% confidence limits 82-104 mg/L)	93

Remarks - Results Although the inhibition of growth rate was only 5% or less at levels up to 56 mg/L, the statistical analysis (Dunnett's test) showed that the deviation from the controls was significant at all loading rates. Therefore, the NOEL cannot be established.

The 72 h E_rL50 was determined to be 220 mg/L using graphical interpolation in the plots of the data points. The E_bL50 was determined to be 93 mg/L (95% confidence limits 82–104 mg/L) using regression

model.

Based on the test results and the low water solubility, the notified chemical is not considered to be harmful to algae up to the limit of its solubility in water. The effects observed at all the test loading rates may be due to the physical effects of the dispersed particles of the notified chemical in the test medium.

CONCLUSION The notified chemical is not harmful to algae up to its limit of solubility in water.

TEST FACILITY Norwegian Institute for Water Research (NIVA) (2003)

C.2.7. Algal growth inhibition test – Study 2

TEST SUBSTANCE The notified chemical (50%)

METHOD ISO 10253 (1998): Water Quality-Marine growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum.*

OECD 2000: Guidance Document on aquatic toxicity testing of difficult substances and mixtures. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 23. ENV/JM/MONO (2000)6.

Species	*Skeletonema costatum* (marine alga)
Exposure Period	72 hours
Concentration Range	100, 180, 320, 560 and 1000 mg/L (WAF)
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	TOC Analysis for test concentration determination. The cell growth was monitored by daily counting of cell numbers using a Coulter Multisizer.
Remarks - Method	Test algae were exposed in three replicates to the WAFs of the test substance at a cell density of approximately 5×10^6 cells/L, pH 7.9 – 8.8 and 20.9 – 21.3°C under continuous illumination. A blank control was also conducted in 6 replicates. To prepare the WAFs, the notified chemical was agitated for 22.5 h in the dark at 20°C, and allowed to settle for 1 h. The WAFs were decanted to avoid the settled material at the bottom of the flasks. The test medium used was ISO 10253 medium based on filtered (0.45 µm) natural seawater (salinity 33.7 S). Nutrients were added. The TOC analysis showed at the start of the test a value of 6.0 mg/L for the control, 7.2 mg /L for the 100 mg/L WAF and 11.9 mg/L for the 1000 mg/L WAF.

RESULTS

Biomass (WAF)		*Growth (WAF)*	
E_bL50 *mg/L at 72 h*	*NOEL* *mg/L*	*E_rL50* *mg/L at 72 h*	*NOEL* *mg/L*
> 1000	1000	> 1000	1000

Remarks - Results Since no inhibition of the growth of *S. costatum* was observed at the highest loading rate tested, the loading causing 50% reduction of both the average growth rate (E_rL50) and biomass growth rate (E_bC50) can be determined to be > 1000 mg/L (WAF). The NOEC is determined to be

	1000 mg/L (WAF). The test substance (containing 50% of the notified chemical) is considered as not harmful to marine algae *Skeletonema costatum*.
CONCLUSION	The test substance is not harmful to algae.
TEST FACILITY	Norwegian Institute for Water Research (NIVA) (2004b)

C.2.8. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical (>90% concentration)
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test (1984). EC Directive 87/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test. ISO 8192, Water Quality - Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation (2007).
Inoculum	Activated sludge from a domestic sewage treatment plant.
Exposure Period	3 hours
Concentration Range	100 mg/L
Remarks – Method	The study was conducted by aerobically exposing the sludge micro-organisms to the notified chemical in three replicates at a nominal loading rate of 100 mg/L at 18.3 – 19.7˚C and pH 7.6 – 8.8. Reference control vessels using 3,5-dichlorophenol at 1.0, 3.2, 10 and 32 mg/L in singular and three blank control vessels were also set up.
RESULTS	
IC50	> 100 mg/L (nominal)
NOEC	100 mg/L (nominal)
Remarks – Results	No inhibition, but a slight stimulation of respiration rate of the sludge was recorded at 100 mg/L. Therefore, the notified chemical is not considered to be harmful to the sewage sludge micro-organisms.
CONCLUSION	The notified chemical is not harmful to sludge micro-organisms.
TEST FACILITY	NOTOX B.V. (2007e)

C.2.9. Acute toxicity to marine sediment organisms

TEST SUBSTANCE	Notified chemical (50%)
METHOD	PARCOM (1994) Paris Commission Guideline: A sediment bioassay using an amphipod *Corophium sp*. PARCOM (1995) Protocols on Methods for the Testing of Chemicals used in the Offshore Industry.
Species	*Corophium sp*
Exposure period	10 days
Remarks – Method	*Corophium sp* was exposed to the test substance in duplicate at levels of 0 (control), 625, 1250, 2500, 5000 and 10000 mg/kg wet sediment. Batches of 10 *Corophium sp* were used in each test vessel. A nominal 1000 mg/L solution was prepared in dilution water and inverted 10 times. A pale yellow suspension with undissolved material was obtained. After extended stirring, a colourless suspension with undissolved material was obtained. Test concentrations were prepared by addition of the appropriate amount of the test substance to a small

amount of dry sediment, prior to addition to the remaining quantity of wet sediment. The sediment used was collected from the aerobic layer (top 5-10 cm) and was sieved through a 500 µm sieve, washed, settled and stored refrigerated in the dark until the start of the test. The water content of the sediment was determined to be 20.08%. The dilution water used was natural seawater and the pH was adjusted to 8.0±0.5 prior to use.

RESULTS

Nominal Concentration (mg/kg)		*Number of test animals*	*Mortality*
Wet sediment	*Dry sediment*		
0	0	20	2
625	765	20	2
1250	1528	20	2
2500	3051	20	3
5000	6101	20	2
10000	12208	20	4

Remarks – Results

No mortality that significantly differs from the blank control has been observed up to the level of 6101 mg/kg (dry sediment). 20% of mortality was observed at level 12208 mg/kg (dry sediment). The 10-day LC50 for the test substance (containing 50% of the notified chemical) is estimated to be > 12208 mg/kg dry sediment, and the NOEC is determined to be 6101 mg/kg dry sediment.

Since the test substance contains 50% of the notified chemical, caution has to be taken in determining the toxicity of the notified chemical to the test species based on the test results.

CONCLUSION

The test substance is not harmful to *Corophium sp*.

TEST FACILITY

Chemex Environmental International Limited, Cambridge, England (2005b)

BIBLIOGRAPHY

Chemex Environmental International Limited (2002a) The Toxicity to Turbot (*Scopthalmus maximus)* of Radiagreen RA. Study No. ENV6097. Cambridge, England. 22 July 2002 (Unpublished report provided by notifier)

Chemex Environmental International Limited (2002b) The Toxicity to Turbot (*Scopthalmus maximus)* of Radiagreen RA (38-02-CD-SC). Study No. ENV6120. Cambridge, England. 28 August 2002 (Unpublished report provided by notifier)

Chemex Environmental International Limited (2005a) The Toxicity to Turbot (*Scopthalmus maximus)* of Radiagreen 0980/7831. Study No. ENV7153. Cambridge, England. 21 January 2005 (Unpublished report provided by notifier)

Chemex Environmental International Limited (2005b) The Toxicity to *Corophim sp.* of Radiagreen 0980/7831. Study No. ENV7154. Cambridge, England. 5 May 2005 (Unpublished report provided by notifier)

M-LAB AS (2005) Radiagreen RA (7831) Test for Biodegradability in Seawater. Study No. OT04-050-5. M-LAB AS. Stavanger, Norway. 6 January 2005 (Unpublished report provided by notifier)

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

Norwegian Institute for Water Research (NIVA) (2002) Test Report: 48 Hours Acute Toxicity of Radiagreen RA Towards *Acartia Tonsa*. NIVA Study No. G028/2. Oslo, Norway. 21 March 2002 (Unpublished report provided by notifier)

Norwegian Institute for Water Research (NIVA) (2003) Test Report: Radiagreen RA Growth Inhibition of the Marine Alga *Skeletonema Costatum*. NIVA Study No. G038/1. Oslo, Norway. 14 February 2003 (Unpublished report provided by notifier)

Norwegian Institute for Water Research (NIVA) (2004a) Test Report: Radiagreen 0980/7831 Acute Toxicity to the Marine Crustacean *Acartia Tonsa*. NIVA Study No. G067/2. Oslo, Norway. 10 December 2004 (Unpublished report provided by notifier)

Norwegian Institute for Water Research (NIVA) (2004b) Test Report: Radiagreen 0980/7831 Growth Inhibition of the Marine Alga *Skeletonema Costatum*. NIVA Study No. G067/1. Oslo, Norway. 26 November 2004 (Unpublished report provided by notifier)

NOTOX B.V. (2007a) Determination of Physico-Chemical Properties of Radiagreen RA. Project 485768. NOTOX B.V. Hertogenbosch, The Netherlands. 6 December 2007 (Unpublished Report provided by notifier).

NOTOX B.V. (2007b) Evaluation of the Mutagenic Activity of Radiagreen RA in the *Salmonella Typhimurium* Reverse Mutation Assay and the *Escherichia Coli* Reverse Mutation Assay (with Independent Repeat). Project 485792. NOTOX B.V. Hertogenbosch, The Netherlands. 12 September 2007 (Unpublished Report provided by notifier).

NOTOX B.V. (2007c) Evaluation of the Ability of Radiagreen RA to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes (with Repeated Experiment). Project 485793. NOTOX B.V. Hertogenbosch, The Netherlands. 11 October 2007 (Unpublished Report provided by notifier).

NOTOX B.V. (2007d) Determination of 'Ready' Biodegradability: Carbon Dioxide (CO_2) Evolution Test (Modifiered Sturm Test) of Radiagreen RA. Project 485794. NOTOX B.V. Hertogenbosch, The Netherlands. 4 October 2007 (Unpublished Report provided by notifier).

NOTOX B.V. (2007e) Activated Sludge Respiration Inhibition Test with Radiagreen RA. Project 485795. NOTOX B.V. Hertogenbosch, The Netherlands. 18 September 2007 (Unpublished Report provided by notifier).

NOTOX B.V. (2008a) Assessment of Acute Oral Toxicity with Radiagreen RA in the Rat (Acute Toxic Class Method). Project 485784. NOTOX B.V. Hertogenbosch, The Netherlands. 2 January 2008 (Unpublished Report provided by notifier).

NOTOX B.V. (2008b) Assessment of Acute Dermal Toxicity with Radiagreen RA in the Rat. Project 485785. NOTOX B.V. Hertogenbosch, The Netherlands. 2 January 2008 (Unpublished Report provided by notifier).

NOTOX B.V. (2008c) Primary Skin Irritation/Corrosion Study with Radiagreen RA in the Rabbit (4 Hour Semi-Occlusive Application). Project 48578. NOTOX B.V. Hertogenbosch, The Netherlands. 2 January 2008 (Unpublished Report provided by notifier).

NOTOX B.V. (2008d) Acute Eye Irritation/Corrosion Study with Radiagreen RA in the Rabbit Eye. Project 485787. NOTOX B.V. Hertogenbosch, The Netherlands. 2 January 2008 (Unpublished Report provided by notifier).

NOTOX B.V. (2008e) Repeated Dose 28-Oral Toxicity Study with Radiagreen RA by Daily Gavage in the Rat. Project 485789. NOTOX B.V. Hertogenbosch, The Netherlands. 28 March 2008 (Unpublished Report provided by notifier).

NOTOX B.V. (2008f) Assessment of Contact Hypersensitivity to Radiagreen RA in the Mouse (Local Lymph Node Assay). Project 485788. NOTOX B.V. Hertogenbosch, The Netherlands. 27 March 2008 (Unpublished Report provided by notifier).

NOTOX B.V. (2008g) Toxicokinetic Assessment of Radiagreen 7838. Project 485791. NOTOX B.V. Hertogenbosch, The Netherlands. 28 February 2008 (Unpublished Report provided by notifier).

NTC (National Transport Commission) 2007 Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 7th Edition, Commonwealth of Australia

OSPAR (1995) OSPAR / PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 1995.

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