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February 2009

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Fatty Amidoalkyl Betaine

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:

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Director
NICNAS

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FULL PUBLIC REPORT

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Dowell Schlumberger (Western) SA (ABN 51 376 230 339)
256 St. Georges Terrace,
Perth WA 6000

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: Chemical Name, Other Names, CAS Number, Molecular Formula, Structural Formula, Molecular Weight, Spectral Data, Purity, Impurities, Import volume.

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, Induction of Germ Cell Damage, *Daphnia sp*. Acute Immobilisation Test and Reproduction Test

NOTIFICATION IN OTHER COUNTRIES
EU (France, 2003)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Fatty Amidoalkyl Betaine

MOLECULAR WEIGHT
< 500 Da.

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

3. COMPOSITION

DEGREE OF PURITY > 70%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: White powder

Property	Value	Data Source/Justification
Melting Point	230.4°C	Measured
Boiling Point	Decomposes at 230.4°C	Measured
Density	1,080 kg/m^3 at 20°C	Measured
Vapour Pressure	3.24 x 10^{-7} kPa at 25°C	Measured
Surface Tension	45.49 mN/m at 19°C	Measured
Water Solubility	0.596 g/L at 20°C	Measured
Hydrolysis as a Function of pH	Expected to be stable to abiotic hydrolysis	A hydrolysis test was not considered relevant as the notified chemical contains no readily hydrolysable groups and is readily biodegradable.
Partition Coefficient (n-octanol/water)	log Pow = > 1.86 at 20°C	Measured

Adsorption/Desorption	log K_{oc} > 4.09 at 40°C	Measured
Dissociation Constant	Not determined	The notified chemical is an inner salt (zwitterion) with ionic characteristics and does not contain any additional ionisable groups.
Particle Size	Inhalable fraction (< 100 μm): < 14.1% Respirable fraction (< 10 μm): 0%	Measured
Flammability	Not flammable	Measured
Autoignition Temperature	> 400ºC	Measured
Explosive Properties	Not explosive	Measured

DISCUSSION OF PROPERTIES
The notified chemical is slightly soluble in water and is expected to have low reactivity. Water solubility is likely to be much lower in natural waters than was measured in distilled water, with possible precipitation of calcium salts. Measured and estimated data for partition coefficient and adsorption/desorption are unreliable and potentially misleading because the methods used are unsuitable for chemicals with ionic and/or surface active characteristics. The notified chemical is considered to be surface active as the surface tension is < 60 mN/m. For full details of tests on physical and chemical properties, please refer to Appendix A.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported in products at concentrations up to 40%.

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

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

PORT OF ENTRY
Fremantle

TRANSPORTATION AND PACKAGING
The notified chemical will be imported by sea in products at concentrations up to 40% in Tote Tanks (1000 L) or in steel/plastic drums (200 L), transferred into offshore stainless steel tanks (~ 1000 L) and shipped to offshore mining sites.

USE
The notified chemical will be used as a gelling agent in offshore oil and gas mining operations.

OPERATION DESCRIPTION
At the mining sites formulations containing the notified chemical (at ≤ 40%) will be pumped using a liquid additive system into a blender where it will be mixed with brine and gravel or sand to form a slurry. Once the appropriate blend has been achieved the slurry will be pumped down the well sites. After use, ≥ 80% of the fluid will flow back to the surface into waste tanks. The remainder will remain in the well.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

EXPOSURE DETAILS
The scenario with the highest probable dermal and ocular exposure will be during connection and disconnection of hoses to the import containers with the formulation containing the notified chemical (≤ 40%) to the liquid additive system. For worst-case estimates, this is assumed to occur daily (although in practice it may be less frequent). The dermal exposure during such processes has been described as a probable half-hand exposure (420 cm^2) and an exposure level of 0.04 mg/cm^2/day (assuming the notified chemical is present at a concentration of 40%) to an adult male worker (70 kg body weight (bw)) (EC, 2003). This gives a worst-case

dermal exposure of:

$$(0.04\ mg/cm^2/day \times 420\ cm^2 \times 100\%\ absorption)/70\ kg\ bw = 0.24\ mg/kg\ bw/day$$

The dermal exposures that are expected during mixing or pumping through a closed system (where the notified chemical will be diluted) are likely to be lower than this estimate considering impervious gloves are expected to be used by workers.

Ocular exposure may also occur incidentally during connection and disconnection of hoses. However, this is expected to be further minimised by the use of safety glasses.

Inhalation exposure to formulations containing the notified chemical is not expected due to the closed systems in use during transfer, blending and transport. In addition, the notifier states that adequate ventilation and a respirator will be provided to workers exposed to the notified chemical. Therefore inhalation exposure to the notified chemical is not expected.

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	low toxicity, LD50 > 2000 mg/kg bw
Rat, acute dermal	low toxicity, LD50 > 2000 mg/kg bw
Rabbit, skin irritation	moderately irritating
Rabbit, eye irritation	slightly irritating
Skin Sensitisation - LLNA	evidence of sensitisation.
Rat, oral repeat dose toxicity - 28 days.	NOEL 50 mg/kg bw/day
Genotoxicity - bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosome aberration	non genotoxic

Toxicokinetics, metabolism and distribution

Absorption of the notified chemical across biological membranes is likely given its low molecular weight (< 500 Da.) and its water solubility (0.596 g/L) (EC, 2003). As the chemical is surface active this may enhance the potential dermal uptake. There is evidence supporting predictions of absorption in the oral repeat dose toxicity study in rats. Absorption across the epithelium in the lungs would also be expected following inhalation of powder of the notified chemical.

Acute toxicity

The notified chemical was found to be of low acute oral toxicity in rats according to OECD TG 423 Acute Toxic Class Method (Huntingdon Life Sciences, 2003b). There were no mortalities or adverse findings reported in the study and the oral LD50 was determined to be > 2000 mg/kg bw.

The notified chemical was found to be of low toxicity in a rat acute dermal toxicity study similar to OECD TG 402 (see Appendix B for further details). No mortality occurred during the study. Dermal irritation was observed (erythema up to grade 3 and oedema up to grade 2) in all animals. Scabbing of the treatment sites persisted in 4/5 females until the end of the study (day 15). The acute dermal median LD50 was determined to be > 2000 mg/kg bw.

Similarly, low acute toxicity was observed via the oral route (LD50 = 4,900 - ~7,900 mg/kg bw) for a structurally related chemical (ECB, 2000).

Irritation and Sensitisation

The notified chemical was found to be moderately irritating to the skin of 3 male rabbits upon testing in accord with OECD TG 404 Acute Dermal Irritation/Corrosion (see Appendix B for further details). Erythema was observed in all animals ranging from very slight to moderate/severe. Very slight to slight oedema was also observed in all animals. Erythema and exfoliation persisted in 2 males to the end of the testing period on day 15. Due to the persistence of the erythema, these effects are sufficient for classification with R38 Irritating to skin

according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

The notified chemical was found to be slightly irritating to the rabbit eye in a test conducted in accord with OECD TG 405 Acute Eye Irritation/Corrosion (see Appendix B for further details). Very slight discharge, red-coloured conjunctival appearance and very slight chemosis were observed in all animals 1 hour following instillation. However, these effects had cleared completely by 72 hours following instillation. These effects were not sufficient for classification with R36 Irritating to eyes according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

The chemical with structural similarities was found to be moderately irritating to skin in one test and not irritating to skin in another. In the eye irritation test it was found to be highly irritating to the eye when used undiluted (confirmed in 2 tests) and moderately irritating to the eye at a concentration of 1% (confirmed in 2 tests) (ECB, 2000).

The notified chemical, dissolved in propylene glycol induced a lymphocyte proliferative response indicative of skin sensitisation at all tested concentrations (5%, 10%, 25%) according to a test conducted in accord with OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (see Appendix B for further details). The notified chemical was considered to have a high potential for sensitisation given the stimulation index for the notified chemical at 25% was 24.0, which was considerably higher than the stimulation index (16.6) of a known sensitiser Hexyl cinnamic aldehyde (HCA). Based on the results of the skin sensitisation test the notified chemical is classified R43 May cause sensitisation by skin contact according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

Repeated Dose Toxicity

In a 28-day oral gavage repeat dose toxicity study in rats, the notified chemical was found to have a no observed effect level (NOEL) of 50 mg/kg bw/day, on the basis of observed effects on weight gain, the stomach, mesenteric lymph nodes and thymus at higher doses (see Appendix B for details and discussion of the observed effects). Histopathological changes in the stomach including ulceration were observed in the stomach in one female treated with 150 mg/kg bw/day.

The effects in the stomach may be secondary adaptive responses to chemically-induced inflammation as the notified chemical is a moderate skin irritant and strong skin sensitiser. The reversibility of these effects was not investigated in the notified chemical. However, in a 28-day repeat dose oral toxicity study on a structurally related chemical, the effects observed in the high dose group were completely reversible following an observation period of 14 days. Therefore, the effects observed in the stomach of rats were considered to be reversible based on the reversibility of effects observed in an oral repeat dose study in a structurally related chemical.

The absence of toxicity at low doses was also indicated in a 90-day repeat dose oral toxicity study in rats on a structurally related chemical which produced no adverse effects in the low dose group and reversible adverse effects in the stomach of rats in the mid and high dose groups.

As such, the notified chemical is not considered classifiable according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

Genotoxicity

The notified chemical was found to be negative in a bacterial reverse mutation assay (Ames test), conducted both with and without metabolic activation according to OECD TG 471 (Huntingdon Life Sciences, 2003h). No evidence of reduction of the background lawn or cytotoxicity was observed during the test.

The notified chemical did not lead to structural aberrations in a chromosome aberration study in human peripheral blood lymphocyte cells, conducted according to OECD TG 473 (see Appendix B for further details). However, an increase in polyploidy at a concentration of 150 μg/mL was observed in Tests 1 and 2, reaching statistical significance in the presence of metabolic activation in Test 1 and in the presence and absence of metabolic activation in Test 2. Polyploidy was not evaluated at lower concentrations. A significant reduction in the mitotic index (35-56%) was also observed at 150 μg/mL.

An increased incidence of polyploid cells may give an indication of the potential of a chemical to induce aneuploidy. However, polyploidy alone does not indicate aneugenic potential and may simply indicate cell cycle perturbation; it is also commonly associated with increased cytotoxicity. However, in the absence of further

investigations regarding the chemicals potential to induce aneuploidy, the notified chemical would be considered to be a concern for mutagenicity.

Carcinogenicity/Developmental toxicity
The notified chemical has not been investigated for its carcinogenicity and developmental toxicity potential. Aneuploidy is associated with human embryonic loss, some birth defects and potential carcinogenicity (COM, 2000). Therefore, given the concern for genotoxic effects described above carcinogenic/developmental effects cannot be ruled out.

Health hazard classification
Based on the high stimulation index in the skin sensitisation – local lymph node assay, and the persistent erythema observed in the acute dermal irritation test, the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following classification:

R38 Irritating to skin
R43 May cause sensitisation by skin contact

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Local Effects
Dermal and ocular exposure to the notified chemical (≤ 40%) during the limited manual handling procedures, such as connection and disconnection of hoses, presents a risk of sensitisation and irritation. The primary risk is an allergic skin reaction, as indicated by the high lymphocyte proliferative response observed in the local lymph node assay. However, there is also a possibility of skin irritation and to a lesser extent eye irritation. The notifier states that PPE such as impervious gloves and safety goggles are expected to be worn by workers during handling of formulation containing the notified chemical which would minimise the risks of sensitisation and irritation to workers. Coveralls or appropriate long-sleeved protective clothing should also be worn by workers.

Systemic effects
Although prolonged exposure by the oral route is not anticipated, there may be some risk of systemic effects upon repeated exposure, given that dermal absorption is probable. The exposure estimate above and the NOEL of the notified chemical (50 mg/kg bw/day) give the following Margin of Exposure (MoE) value:

MoE = (50 mg/kg bw/day)/(0.24 mg/kg bw/day)
= 208

MOE greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. However, the potential for the chemical to induce aneuploidy and carcinogenic/developmental effects cannot be ruled out and no NOEL for these effects could be established. Therefore, the risk to workers is likely to only be acceptable when used under highly controlled conditions, and with the appropriate PPE. As the notifier has described the operations to be highly controlled, and good worker practices (including PPE such as impervious gloves) are in place during limited activities where worker handling is required, the risk of adverse effects is significantly reduced and is considered acceptable under the occupational settings described.

6.3.2. Public health
The public are not expected to be exposed to the notified chemical as its use will be confined to specific industrial sites. Therefore, the risk of the notified chemical to the health of the public is not considered to be unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical is imported and transferred to the well site as a formulated product. No releases are expected except as a result of spills.

RELEASE OF CHEMICAL FROM USE
The notified chemical is used in a closed system and is not expected to be released to the environment as a result of its use, except for accidental spills. At least 80% of the fluids containing the notified chemical will flow back to the surface and be collected in waste tanks before transport back to the mainland for disposal.

The remaining fraction of the notified chemical will remain in the well pending production, at which point some of it will return to the surface entrained in oil, gas and water fractions. These fractions will be mixed with those from other producers. Most of the remaining notified chemical will be retained in the aqueous fractions. This will be partially separated in a coalescer/hydrocyclone, with recovered water and entrained contaminants added to the water injection stream or recovered as slops. Slops will be stored pending transport back to the mainland for disposal. The oil and gas fractions will be exported to a refinery, where any entrained notified chemical will be destroyed during the refining process.

A small fraction of the notified chemical is expected to remain underground as part of the gelled matrix.

RELEASE OF CHEMICAL FROM DISPOSAL
The recovered fluids containing the notified chemical, including slops, will be disposed of by incineration after evaporation of excess water in settling ponds. Incineration will destroy the notified chemical.

7.1.2 Environmental fate

The notified chemical is water soluble (0.6 g/L) but is expected to have low mobility in the environment because of its surface activity and consequent affinity for surfaces. Residues that enter aquatic environments are expected to sorb to sediment and suspended solids, but will not persist as the notified chemical was readily biodegradable in standard tests in fresh and salt water, and underwent significant biodegradation in a second ready biodegradability test in salt water. A potential for bioaccumulation has been identified by the notifier based on calculated high values for the octanol-water partition coefficient, but the estimation method is inapplicable to surface active chemicals with ionic characteristics. Bioaccumulation in fish is considered unlikely based on the water solubility of the notified chemical. For the details of the environmental fate testing, please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

Aquatic exposure is expected to be limited to accidental spills and therefore a PEC has not been determined. Residues entering aquatic environments will disperse, partition to sediment and suspended solids, and degrade through biotic processes.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	EC50 = 0.406 mg/L	Very toxic
Daphnia Toxicity	EC50 = 33.6 mg/L	Harmful
Marine Copepod Toxicity	LC50 = 0.9 mg/L	Very toxic
Marine Amphipod Toxicity	LC50 = 168 mg/kg	-
Algal Toxicity (Freshwater)	EC50 = 85.4 mg/L	Harmful
Algal Toxicity (Marine)	EC50 0.22 mg/L	Very toxic
Inhibition of Bacterial Respiration	EC50 > 100 mg/L	Not harmful

Testing found the notified chemical to be very toxic to fish, marine copepods and marine diatoms.

7.2.1 Predicted No-Effect Concentration

As data are available for three trophic levels, the PNEC can be determined by application of an assessment factor of 100 to the most sensitive freshwater aquatic endpoint.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Fish toxicity	0.406	mg/L
Assessment Factor	100	
PNEC:	4.06	µg/L

7.3. Environmental risk assessment

The use of the notified chemical in off-shore drilling operations provides potential for releases to the marine environment. In practice, the proposed use-pattern and the disposal protocol for the spent fluids provide limited possibility of release of the chemical to the aquatic environment. Therefore, the risk to the environment from the introduction of the notified chemical is considered acceptable. However as a precaution the following environmental recommendation should be placed on the notification: No release of recovered fluids or produced water ("slops") to the aquatic environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification

Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The classification and labelling details are:

R38 Irritating to skin
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 irritation	2	Causes skin irritation
Skin sensitisation	1	May cause an allergic reaction
Acute hazards to the aquatic environment	1	Very toxic to aquatic life

Human health risk assessment

This risk to occupational health and safety is considered acceptable provided that the notified chemical is only used under controlled conditions by trained 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 its biodegradability and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification for the notified chemical:
  - R38 Irritating to the skin
  - R43 May cause sensitisation by skin contact

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc ≥ 20%: R43, R38
  - 1% ≤ Conc < 20%: R43

- The following safety phrases should appear on the MSDS and label for the notified chemical and products containing the notified chemical:
  - S24 Avoid contact with skin
  - S27 Take off immediately all contaminated clothing
  - S28 After contact with skin, wash immediately with plenty of water
  - S36 Wear suitable protective clothing
  - S37 Wear suitable gloves

Health Surveillance

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

Material Safety Data Sheet

- The MSDS provided by the notifier should be amended as follows:
  - the appropriate safety phrases should be added in section 2.
  - the notified chemical should be identified by its chemical name (as it is a Type 1 ingredient) in section 3.
  - The recommendation regarding recovered fluids or produced water should be added to section 13.
  - The recommendations regarding use of engineering controls, safe working practices and PPE should be added to sections 7 and 8.

CONTROL MEASURES
Occupational Health and Safety

- Employers should ensure that the facility is equipped such that operations involving the notified chemical are performed in a highly controlled manner. The following isolation and engineering controls should be in place to minimise occupational exposure to the notified chemical:
  - Prevention of leaks and spills
  - Automated processes

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
  - Avoid skin contact
  - Workers must have adequate education and training before handling the notified chemical
  - Avoid spills and splashing during use.
  - After exposure, any contaminated PPE should be thoroughly cleaned before re-use.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced and when diluted for use:
  - Impervious gloves and coveralls

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.

Environment

- No release of recovered fluids or produced water ("slops") to the aquatic environment

Disposal

- The notified chemical should be disposed of by incineration.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - the chemical is introduced in a solid form.

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a gelling agent for site-specific use in sand control or oil and gas projects, or is likely to change significantly;
  - the amount of chemical being introduced has increased from 100 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 products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 230.4°C

Method	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Differential scanning calorimetry
Test Facility	Huntingdon Life Sciences (2003a)

Boiling Point > 230.4°C at 101.3 kPa

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks	Notified chemical decomposed at 230.4 °C
Test Facility	Huntingdon Life Sciences (2003a)

Density 1,080 kg/m^3 at 20°C

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density.
Remarks	Pycnometer
Test Facility	Huntingdon Life Sciences (2003a)

Vapour Pressure 3.24×10^{-7} kPa at 25°C

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure.
Test Facility	Huntingdon Life Sciences (2003a)

Water Solubility 0.596 g/L at 20°C

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	Flask Method. The notified chemical was shaken with distilled water at 30°C for up to 3 days and equilibrated at 20°C for 24 hours before filtration and HPLC analysis. Note that dissolution in this test was more successful than in the fish and daphnid toxicity tests, suggesting that the notified chemical precipitates from aqueous solution as a calcium salt.
Test Facility	Huntingdon Life Sciences (2003a)

Hydrolysis as a Function of pH

A hydrolysis test was not considered relevant as the notified chemical contains no readily hydrolysable groups and is readily biodegradable.

Partition Coefficient (n-octanol/water) $\log P_{ow} > 1.86$ at 20°C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks	Flask Method. The result is unreliable as the notified chemical is surface active and likely to be enriched in the interface, but some relevant observations can be made. The test was conducted by mixing an octanol stock solution of the notified substance (76.25 mg/L) with equal volumes of octanol saturated water and octanol (or with two volumes or 0.5 volumes of the organic phase). The notified chemical could not be detected in the aqueous phase (LOD 0.97 mg/L) despite its water solubility. Concentrations measured in the organic phase were close to nominal. The partitioning to octanol with no retention in the aqueous phase potentially reflects emulgation of water into the organic phase, as noted in the report on bioaccumulation (Section C.1.4).

The test report also provided estimates for log P_{ow} in the order of 5, but these are considered unreliable because the methods used are inapplicable to substances with ionic characteristics.

Test Facility Huntingdon Life Sciences (2003a)

Adsorption/Desorption – screening test log K_{oc} > 4.09 at 40°C

Method OECD TG 106 Adsorption - Desorption HPLC Screening Method.

Remarks The notified chemical is likely to have a strong affinity for surfaces because of its surface activity, but the result is unreliable as the notified chemical has surface active and ionic properties. Surface active chemicals are likely to film within the column and disrupt any measurements.

Test Facility Huntingdon Life Sciences (2003a)

Dissociation Constant

The test was not conducted. The notified chemical is an inner salt (zwitterion) with ionic characteristics but does not contain any ionisable groups.

Particle Size

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
> 400	52.8
400 – 125	34.1
125 – 75	9.7
75 – 30	4.0
30 - 10	0.4
< 10	0.0

Remarks Sieve analysis

Test Facility Huntingdon Life Sciences (2003a)

Flammability

Method EC Directive 92/69/EEC A.10 Flammability (Solids).

Remarks Not highly flammable.

Test Facility Huntingdon Life Sciences (2003a)

Autoignition Temperature > 400°C

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.

Remarks The notified chemical failed to ignite under the test conditions.

Test Facility Huntingdon Life Sciences (2003a)

Explosive Properties

Method EC Directive 92/69/EEC A.14 Explosive Properties.

Remarks Not explosive

Test Facility Huntingdon Life Sciences (2003a)

Surface Tension 45.49mN/m at 19°C

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 92/69/EEC A.5 Surface Tension.

Remarks Concentration: 0.81 g/mL

Test Facility Huntingdon Life Sciences (2003a)

Oxidizing Properties

Method	EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks	Non-oxidising.
Test Facility	Huntingdon Life Sciences (2003a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test. EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
Species/Strain	Rat/CD Sprague-Dawley
Vehicle	Water (20% solution)
Type of dressing	Occlusive
Remarks - Method	Three males were slightly under the weight range recommended by the OECD TG402 at the start of the study. This deviation was not considered to significantly alter the outcome of the test. No further protocol deviations.

RESULTS

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

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Dermal irritation (Grade 1 to 3 erythema with or without Grade 1 or 2 oedema) was observed in all animals following removal of the dressings, resolving completely by day 7 in males and day 9 in females. In addition, desquamation (characterised by dryness/exfoliation) was seen in 4 males and all females from day 3, resolving in 3 males by day 12 and 2 females by day 14 and persisting in 1 male and 3 females until study termination on day 15. Spots and/or scabbing (confined to a small area of the dose site) were also observed in 1 male and 4 females from day 7, resolving in the male by day 9 and persisting in all the females until day 15.
Signs of Toxicity - Systemic	One female did not show any bodyweight increase on day 8 and day 15. A notably low bodyweight gain was seen in 1 female on day 8 and 1 female on day 15. All other animals were considered to have average bodyweight gains throughout the study.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Huntingdon Life Sciences (2003c)

B.2. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation).
Species/Strain	Rabbit/New Zealand White
Number of Animals	3 males
Vehicle	moistened with water
Observation Period	15 days
Type of Dressing	Semi-occlusive.
Remarks - Method	The temperature range was 15-23ºC rather than the recommended range of 17-23ºC. The batch number reported was incorrect. These deviations were not considered to significantly alter the outcome of the test. No further 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			
Erythema/Eschar	2.67	1.33	1.00	3.00	15 days	1
Oedema	1.00	1.33	1.00	2.00	15 days	1

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

Remarks - Results: Erythema was observed in all animals, ranging from very slight to moderate/severe. Very slight to slight oedema was observed in all animals. Signs of irritation persisted until Day 15 in 2 animals. In addition, loss of flexibility was observed in 2 animals 72 hours after treatment and in 1 animal on Day 8. Eschar formation or exfoliation was observed in 2 animals on Day 8 and Day 15.

CONCLUSION: The notified chemical is moderately irritating to the skin.

TEST FACILITY: Huntingdon Life Sciences (2003d)

B.3. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3
Observation Period: 72 hours
Remarks - Method: The temperature range was 15-23^{o}C rather than the recommended range of 17-23^{o}C. The initials of the Study Director were omitted from the study protocol affixed to each cage housing the animals. These deviations from protocol were not considered to affect the outcome of the test.

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	0.67	0.67	0.67	2	< 72 hours	0
Conjunctiva: chemosis	0	0	0	1	< 24 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.

Remarks - Results: A crimson-red conjunctival appearance, very-slight discharge and in 2 cases very-slight chemosis was observed 1 hour after instillation of the notified chemical. Injection of the conjunctival blood vessels was observed in all animals 24 and 48 hours. However, no effects were observed 72 hours after treatment.

CONCLUSION: The notified chemical is slightly irritating to the eye.

TEST FACILITY: Huntingdon Life Sciences (2003e)

B.4. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitization: Local Lymph Node Assay
Species/Strain	Mouse/CBA/Ca
Vehicle	Propylene glycol
Remarks - Method	Hexyl cinnamic aldehyde (HCA) was used as positive control. No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	83.3	-
5	499.3	6.0
10	804.3	9.7
25	1995.6	24.0
Positive Control		
10	562.4	1.8
25	4374.9	13.9
50	5225.7	16.6

Remarks - Results	Given that the stimulation index induced by the notified chemical at 25% was greater than the stimulation index of a known sensitiser (HCA) at 50%, the skin sensitisation potential is considered high.
CONCLUSION	There was evidence of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.
TEST FACILITY	Huntingdon Life Sciences (2003f)

B.5. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
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/Crl:CD (SD)IGS BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Corn oil
Remarks - Method	Blood cell morphology was assessed using the H1E Haematology Analyser and not by examination of the "Blood film" as recommended in the test guidelines. No recovery groups were included in the study and no justification was provided for their exclusion.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 per sex	0	0/10
low dose	5 per sex	50	0/10
mid dose	5 per sex	150	0/10
high dose	5 per sex	1000	0/10

Mortality and Time to Death

No mortality was observed during the study.

Clinical Observations

There were no treatment-related clinical findings during the study. No abnormalities were noted in neurobehavioural or functional assessments.

Significantly lower bodyweight gains were observed in animals of both sexes treated with 1000 mg/kg bw/day (20.5% for males and 26.3% for females). Slightly lower bodyweight gains were observed in animals treated with 50 mg/kg bw/day (9.7% for males and 12.4% for females) and females treated with 150 mg/kg bw/day showed a 15.6% decrease in bodyweight gain. However, the variation in bodyweights of animals treated with lower doses was not considered significant.

Decreased food consumption was observed in animals treated with 1000 mg/kg bw/day (14.0% for males and 10.8% for females). Food consumption was slightly decreased in both sexes treated with 50 mg/kg bw/day (9.1% for males and 6.2% for females) and by 9.2% in females treated with 150 mg/kg bw/day in association with the bodyweight variations observed in those groups.

Decreased food conversion efficiency was observed in animals treated with 1000 mg/kg bw/day (7.4% for males and 17.4% for females) but the values observed in other treated animals were comparable to controls.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

Statistically significant increases in aspartate amino-transferase levels (27.8% for males and 24.3% for females) and decreased glucose (25.1% for males and 17.3% for females) and cholesterol (33.9% for females, 12% for males (not significant)) values were observed in animals treated with 1000 mg/kg bw/day. In addition, females treated with 1000 mg/kg bw/day showed statistically significant decreases in mean total protein levels (4.5%).

Haematology

Statistically significant increases in prothombin time compared to controls were observed in males treated with 1000 mg/kg bw/day (4.8%)and 150 mg/kg bw/day (3.4%) and females treated with 1000 mg/kg bw/day (4.5%). Males treated with 1000 mg/kg bw/day also showed statistically significant increases in mean monocyte values (90.0%). Males treated with 1000 mg/kg bw/day also showed a lower platelet count, although this was not statistically significant. Statistically significant lower mean eosinophil values were observed in all treated males but these findings were not dose dependent.

Effects in Organs

Statistically significant decreased thymus weights (28.7%) were observed in males treated with 1000 mg/kg bw/day. Animals treated with 1000 and 150 mg/kg bw/day had lower relative mean heart weights than controls but the decrease in weights was not dose dependent. There were no treatment-related lesions and therefore the effects were not considered treatment-related. Females treated with 1000 mg/kg bw/day showed statistically significant increases in relative mean ovary weights compared to controls. However, absolute mean weights were within the control range and no treatment-related lesions were observed. Therefore the reduced relative weights were not considered related to treatment.

Stomach

A thickened forestomach was observed in 4 males and 3 females treated with 1000 mg/kg bw/day. A roughened forestomach was observed in 2 males and 4 females treated with 1000 mg/kg bw/day and forestomach depressions were observed in 2 males treated with 1000 mg/kg bw/day and 1 female treated with 150 mg/kg bw/day. The following histopathological effects were observed in the stomach:

Effect in Stomach	**Control**	**50 mg/kg bw/day**	**150 mg/kg bw/day**	**1000 mg/kg bw/day**
Non-glandular				
Ulceration	0	0	1 F	2 M, 2 F
Erosion	0	0	0	3 M, 2 F
Epithelial hyperplasia	0	0	1 F	5 M, 4 F
Hyperkeratosis	0	0	1 F	5 M, 4 F

Epithelial parakeratosis	0	0	1 F	1 M, 3 F
Subepithelial oedema	0	0	1 F	4 M, 3 F
Subepithelial inflammatory cells	0	0	1 F	4 M, 3 F
Glandular				
Dilated glands	0	0	0	1 M
Submucosal inflammatory cells	1 M	0	0	0
Submucosal oedema	1 M	0	0	0
Focal ectopic non-glandular epithelium	1 F	2 M, 1 F	0	0
Congestion	0	1 M	1 M	0

M = Male, F = Female

The effects observed in the non-glandular stomach of animals treated with 1000 mg/kg bw/day and the female treated with 150 mg/kg bw/day were considered to be adverse and treatment-related.

Mesenteric lymph nodes

Dilated sinuses of the mesenteric lymph nodes was observed in 4 males and 4 females treated with 1000 mg/kg bw/day and was considered related to the inflammation observed in the stomach.

Thymus

Minimal involution and atrophy of the thymus was observed in all 5 male rats treated with 1000 mg/kg bw/day. Involution and atrophy was also observed in 1 male from each of the groups treated with 150 mg/kg bw/day, 50 mg/kg bw/day as well as the control group.

Remarks – Results

The decreased bodyweight gain in animals treated with 1000 mg/kg bw/day was considered treatment-related given the accompanied decrease in food conversion efficiency as well as decreased food consumption. The slight decreases in bodyweight gain in animals treated with 50 mg/kg bw/day and females treated with 150 mg/kg bw/day were not accompanied by decreased food conversion efficiency and therefore were not considered to be related to treatment.

The increased aspartate amino-transferase levels and decreased glucose and cholesterol values observed in animals treated with 1000 mg/kg bw/day and the decreased mean total protein levels observed in females treated with 1000 mg/kg bw/day were not considered to be indicative of overt toxicity in the absence of effects in the liver.

The statistically significant increases in prothombin time observed in males treated with 1000 mg/kg bw/day and 150 mg/kg bw/day and females treated with 1000 mg/kg bw/day, and the statistically significant increase in mean monocyte values in males treated with 1000 mg/kg bw/day were considered related to treatment.

The effects seen in the stomach of animals of both sexes treated with 1000 mg/kg bw/day were considered to be treatment related. The effects observed in one female in the non-glandular stomach at 150 mg/kg bw/day were comparable to effects seen in females treated at the highest dose and were therefore considered related to treatment. These effects may be caused by local irritant and sensitisation properties of the notified chemical. However, the reversibility of these effects was not investigated.

Statistically significant decreases (28.7%) in thymus weights observed in males treated with 1000 mg/kg bw/day were accompanied by involution/atrophy in all males in that group. These were considered treatment-related adverse effects. The involution/atrophy of the thymus in males treated with 150 and 50 mg/kg bw/day as well as the males in the control group were not considered treatment-related.

CONCLUSION

The No Observed Effect Level (NOEL) was established as 50 mg/kg bw/day in this study, based on the histopathological effects observed in the stomach of one female treated with 150 mg/kg bw/day.

TEST FACILITY Huntingdon Life Sciences (2003g)

B.6. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test. EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Human (male volunteers)
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction
Vehicle	Culture medium
Remarks - Method	Mitomycin C (0.2 µg/mL in Test 1 and 0.1 µg/mL in Test 2) was used as positive control in the absence of metabolic activation. 10 µg/mL Cyclophosphamide was used as positive control in the presence of metabolic activation in Tests 1 and 2. No significant protocol deviations.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	12.5, 25, 50, 75*, 100*, 150*, 300	3 hrs	20 hrs
Test 2	6.25, 12.5*, 25*, 50, 75, 100, 125, 150*	20 hrs	20 hrs
Present			
Test 1	12.5, 25, 50, 75*, 100*, 150*, 300	3 hrs	20 hrs
Test 2	6.25, 12.5, 25*, 50, 75, 100*, 125, 150*, 175	3 hrs	20 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	≥ 312.5	≥ 150	None observed	Polyploidy
Test 2	-	-	None observed	Polyploidy
Present				
Test 1	≥ 312.5	≥ 150	None observed	Polyploidy
Test 2	-	≥ 150	None observed	Polyploidy

Remarks - Results	No significant increase in the percentage of cells with chromosomal aberrations was observed in the absence or presence of metabolic activation in any of the type of treatments. Also, no precipitation of the notified chemical was seen. However, an increase in polyploidy at a concentration of 150 µg/mL was observed in Tests 1 and 2, reaching statistical significance in the presence of metabolic activation in Test 1 and in the presence and absence of metabolic activation in Test 2. Polyploidy was not evaluated at lower concentrations. In Test 1, at a concentration of 150 µg/mL the mitotic index was reduced by 35% in the absence of metabolic activation and 40% in the presence of metabolic activation. Similarly, in Test 2, at a concentration of 150 µg/mL the mitotic index was reduced by 56% in the absence of metabolic activation and 45% in the presence of metabolic activation.
CONCLUSION	The notified chemical did not lead to structural aberrations in human lymphocytes treated in vitro under the conditions of the test. However, the observation of polyploidy at 150 µg/mL indicate that the notified chemical may have the potential to cause numerical aberrations.
TEST FACILITY	Huntingdon Life Sciences (2003i)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test. EC Directive 92/69/EEC C.4-C Biodegradation: Determination of the " Ready" Biodegradability: Carbon Dioxide Evolution Test
Inoculum	Activated sludge (Oakley sewage treatment works)
Exposure Period	29 days
Auxiliary Solvent	None
Analytical Monitoring	CO_2
Remarks - Method	A preliminary solubility trial achieved aqueous dispersions containing 978 mg/L of the notified chemical in ultrapure water, using sonication and vigorous shaking in silylised glassware.
RESULTS	Cumulative CO_2 production in the controls (78.7 and 82.5 mg CO_2) was within the acceptable range for this assay system. The degradation of sodium benzoate was rapid and had achieved 64% of its T CO_2 after 6 days and 78% after 29 days. The degradation of sodium benzoate was also rapid in the presence of the test substance and had achieved 61% of its T CO_2 after 6 days.

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*
2	1	2	19
4	16	4	44
5	26	5	57
6	35	6	64
7	42	7	68
8	47	8	69
11	55	11	73
12	59	12	75
13	62	13	75
15	67	15	77
21	72	21	78
25	74	25	78
28	76	28	78
29	77	29	78

Remarks – Results	A biodegradation plateau was not considered to have been reached by the end of the test.
CONCLUSION	The notified chemical can be classed as readily biodegradable.
TEST FACILITY	Huntingdon Life Sciences (2003j)

C.1.2. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 306 1992. Biodegradation in seawater. OECD guideline for testing of chemicals 27 pp. and OPPTS Method 835.3160
Inoculum	Natural seawater collected off the Suffolk, UK coast, ca. 2 m deep (TVC: total viable count of micro-organisms was performed the day of sampling.)
Exposure Period	28 days

Auxiliary Solvent	None
Analytical Monitoring	Oxygen measurement, nitrite-N and nitrate-N
Remarks – Method	Preliminary solubility trials found that an adequate stock solution could not be established in the marine mineral salts medium (containing 36 g/L $CaCl_2.2H_2O$). The test substance was added directly to empty BOD bottles so as to achieve a nominal test concentration of 2 mg/L. Substances containing nitrogen that are degradable may also be susceptible to biodegradation by nitrifying bacteria which produce oxidised forms of nitrogen (e.g. nitrite and/or nitrate) and in so doing consume dissolved oxygen. This reaction interferes with the assay for organotrophic biodegradation, so analysing samples for levels of nitrite and nitrate is used to establish the extent of nitrification.

RESULTS

Nitrification of the test substance did not occur, thus the level of biodegradation was calculated using its ThODammonia value (2.67 mgO_2/mg).
No biodegradation inhibition was observed after 5 days exposure of the micro-organisms to a mixture of test substance and sodium benzoate.

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*
5	30	5	76
7	31	7	76
11	39	11	88
14	17	14	87
18	8	18	93
21	26	21	86
25	41	25	92
28	41	28	92

Remarks – Results

Analysis on day 14, 18 and 21 indicated that levels of oxygen consumption equated to a level ranging between 0.11 and 0.97 mgO_2/mg or 4% and 36% of the ThOD, respectively. This variation was thought to be related to difficulties obtaining adequately dissolved fractions of the test substance in the seawater at the start of the test. However, on day 25 and 28, the extent of the test substance biodegradation had achieved similar, consistent levels to those observed earlier in the test.

The test substance is not considered to have achieved the level of biodegradation under this test condition (> 60%). However, owing to the stringency of this type of test, substances that fail to achieve the required level of degradation are not necessarily non-biodegradable. Biodegradation may occur under more favourable conditions, such as exposure to a larger and more diverse mixture of micro-organisms in a system more suited to an assessment of degradation of a poorly soluble substance.

CONCLUSION

The notified chemical cannot be classed as readily biodegradable.

TEST FACILITY

Huntington Life Sciences (2003k)

C.1.3. Ready biodegradability

METHOD

OECD TG 306 1992. Biodegradation in seawater. OECD guideline for testing of chemicals.

Inoculum	Natural seawater collected at Alvøen ferjekai, Bergen-Norway (depth 3 meters)
Exposure Period	28 days

Auxiliary Solvent	None
Analytical Monitoring	Oxygen measurement
Remarks – Method	1.21 mg/L of test substance was inoculated in seawater, giving a chemical oxygen demand of 2.49 mg O_2/L seawater.

RESULTS

Microbial activity was satisfactory by reaching > 60% degradation within 28 days. The inhibitory test gave 75% degradability, showing that when sodium benzoate and test substance are added together into the test vessels the degradation for the chemicals are not inhibited, i.e. there was no interference between the two components. No O_2 consumption took place in the abiotic test.

Test substance		*Sodium benzoate*	
Day	*% degradation*	*Day*	*% degradation*
7	40	7	65
14	60	14	78
21	78	21	77
28	73	28	72

Remarks – Results

CONCLUSION

The notified chemical can be classed as ready biodegradable.

TEST FACILITY

M-I Production Chemicals (2004a)

C.1.4. Bioaccumulation

A potential for bioaccumulation has been identified by the notifier based on calculated high values for the octanol-water partition coefficient, but the estimation method is inapplicable to surface active chemicals with ionic characteristics. Bioaccumulation in fish is considered unlikely based on the water solubility of the notified chemical. However, in view of the small molecular size of the test substance (molecular weight < 500 Da.) the notifier has indicated that a bioaccumulation potential cannot be excluded.

A report on bioaccumulation (M-I Production Chemicals, 2004b) notes that the notified chemical is soluble in pure water, and insoluble in octanol, but that it emulgates water into octanol. It is concluded that the notified chemical has a low potential for bioaccumulation (log P_{ow} < 0) because of high hydrophilicity and low lipophilicity.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 203 Fish, Acute Toxicity Test – semi-static.
EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - semi static.

Species	*Oncorhynchus mykiss* (Rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	150 – 200 mg/L $CaCO_3$
Analytical Monitoring	HPLC
Remarks – Method	Ultrasound treatment and prolonged stirring were employed to assist in the dissolution of the test substance in the 100 mg/L stock solution, but particles remained visible, necessitating filtration before preparation of the test media. All glassware was siliconised (with

dichlorooctamethyltetrasiloxane) to minimise adsorption of the test substance.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1h*	*24h*	*48h*	*72h*	*96h*
0	0	7	0	0	0	0	0
0.427	0.303	7	0	0	0	0	0
0.939	0.478	7	0	0	0	2	6
2.07	1.35	7	0	0	7	7	7
4.55	3	7	0	6	7	7	7
10	7.23	7	0	7	7	7	7

LC50	2.25 mg/L at 24 hours. 0.804 mg/L at 48 hours. 0.616 mg/L at 72 hours. 0.406 mg/L at 96 hours.
NOEC	0.303 mg/L at 96 hours.
Remarks – Results	The measured concentrations were less than nominal at the start of the test and declined in the 24 hours between renewals, with the losses especially pronounced at lower test concentrations. The authors suggest that the losses may reflect adsorption onto or uptake by the fish.

CONCLUSION The notified chemical is toxic to *Oncorhynchus mykiss*

TEST FACILITY Huntingdon Life Sciences (2003l)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – static.
EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - static.

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	268 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Ultrasound treatment and prolonged stirring were employed to assist in the dissolution of the test substance in the 100 mg/L stock solution, but particles remained visible, necessitating filtration through a cellulose nitrate membrane before preparation of the test media. Vacuum filtration was needed as gravity flow was too slow. All glassware was siliconised to minimise adsorption of the test substance. Solubility trials in the test media found 44% of the test substance at the nominal concentration of 100 mg/L but could not detect any test substance at a nominal 4.3 mg/L.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	5 daphnids per replicate (4 repl.)	0	0
4.27	1.64	5 daphnids per replicate (4 repl.)	0	0
9.39	3.75	5 daphnids per replicate (4 repl.)	0	0
20.7	7.57	5 daphnids per replicate (4 repl.)	0	0
45.5	14.6	5 daphnids per replicate (4 repl.)	0	0
100	35.6	5 daphnids per replicate (4 repl.)	0	11

LC50	> 35.6 mg/L at 24 hours 33.6 mg/L at 48 hours
NOEC	14.6 mg/L at 48 hours
Remarks – Results	The LC50 was determined using Stephan's method. Measured concentrations fell well short of nominal at the start of the test but were maintained through the test period.

CONCLUSION The notified chemical is harmful to *Daphnia magna*.

TEST FACILITY Huntingdon Life Sciences (2003m)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test.
EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species	*Selenastrum capricornutum*
Exposure Period	72 hours
Concentration Range Nominal	0, 4.27, 9.39, 20.7, 45.5 and 100 mg/L
Concentration Range Actual	0, 4.12, 8.87, 19.8, 42.9 and 85.4 mg/L
Auxiliary Solvent	None
Water Hardness	Not mentioned
Analytical Monitoring	HPLC
Remarks – Method	Ultrasound treatment and prolonged stirring were employed to assist in the dissolution of the test substance in the 100 mg/L stock solution, but particles remained visible, necessitating filtration through a cellulose nitrate membrane before preparation of the test media. All glassware was siliconised to minimise adsorption of the test substance.

RESULTS

Biomass		*Growth*	
E_bC50	*NOEC*	*E_rC50*	*NOEC*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
57.6	42.9	85.4	42.9

Remarks – Results	Measured concentrations fell a little short of nominal at the start of the test but were maintained through the test period. The better dissolution in this test compared with the fish and daphnid tests may reflect the low

	concentration of calcium ions typically present in algal culture media.
CONCLUSION	The notified chemical is harmful to *Selenastrum capricornutum*.
TEST FACILITY	Huntingdon Life Sciences (2003n)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 209 Activated Sludge, Respiration Inhibition Test. EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test.
Inoculum	Activated sludge from Oakley sewage treatment works (predominantly domestic waste)
Exposure Period	3 hours
Concentration Range Nominal	0, 1, 10 and 100 mg/L
Remarks – Method	3,5-DCP was used as reference substance. The notified chemical was tested as a dispersion, as preliminary trials found it to be insufficiently soluble to allow preparation of a suitable aqueous stock solution.
RESULTS	Sludge respiration rates were progressively reduced in the presence of increasing concentrations of 3,5-DCP. The three-hour EC50 for 3,5-DCP was calculated by the Moving Average method to be ca 17.9 mg/L. The specific respiration rate of control cultures established at the start and end of the test was 30.5 $mgO_2/g/h$. The test substance was considered to have had no inhibitory effect on the respiration rate of activated sludge at any of the concentrations employed in the test.
IC50	> 100 mg/L
NOEC	≥ 100 mg/L
Remarks – Results	A suitable oxygen consumption rate measurement could not be obtained from the mixture containing the test substance at a nominal concentration of 10 mg/L owing to technical difficulties that occurred during this measurement. When this problem was rectified, the dissolved oxygen concentration in the mixture had decreased to 2.8 mg $O_2/g/h$. Any measurement that was taken was considered to be unreliable because it was not obtained from a linear portion of the oxygen consumption rate. As no effects were observed in three mixtures containing the test substance at 100 mg/l, this did not affect the integrity of the test.
CONCLUSION	The notified chemical is not harmful to microbial activities.
TEST FACILITY	Huntingdon Life Sciences (2003o)

C.2.5. Acute toxicity to the marine diatom *Skeletonema costatum*

TEST SUBSTANCE	Notified chemical
METHOD	ISO/DP 10253: Nyholm, N. and Kusk, K.O. 1990. Growth inhibition toxicity test with the diatoms *Phaeodactylum tricornutum* and *Skeletonema costatum*. Paris Commission, toxicity test with marine unicellular algae, technical support document for the ISO DP 10253 standard.
Remarks – Method	Reference substance: 3,5-dichlorophenol 97% Species: *Skeletonema costatum* Media: Natural seawater (collected Alvøen ferjekai, Bergen depth = 3,

	salinity = 31‰) Replicates: three of each test concentration, 6 of control. Concentration (nominal): 0, 0.23, 0.37, 0.64, 1.15 and 2.12 mg/L The diatoms were exposed to water accommodated fractions, but the preparation of these is not described.
RESULTS	72 hours, growth rate EC50 = 0.22 mg/l
Remarks – Results	The response of the test substance on *Skeletonema costatum* followed a sigmoid curve. The growth rate in blank ranged from 2.16 to 2.96, which is typical for the organism. Growth inhibition for 1.5 mg/l of 3,5-dichlorophenol was 58%, which is within the range (20 -80 %) for validation of the test. Due to the nature of the number, confidence intervals for some of the results were not possible to obtain.
CONCLUSION	The notified chemical is very toxic to *Skeletonema costatum*
TEST FACILITY	M-I Production Chemicals (2003c)

C.2.6. Acute toxicity to the sediment reworker *Corophium volutator*

TEST SUBSTANCE	Notified chemical
METHOD	PARCOM 1995: A sediment bioassay using an amphipod *Corophium* sp. Protocols on methods for testing of chemicals used in the offshore oil industry.
Remarks – Method	Species: *Corophium volutator* (collected at Fløksand and acclimated in 5 L beakers containing aerated seawater with detritus from the collection site. Only healthy, adult animals, longer than 5 mm measured by eye, were used.) Media: Natural seawater (collected Alvøen ferjekai, Bergen depth = 3, salinity = 33‰); sediment collected at the same time and site as Corophium. Replicates: two of each test concentration, four of control and reference (10 animals in each vessels). The report does not clearly state which reference substance was used. Concentration (nominal): 0, 126, 262, 527 and 1011 mg/kg dry weight sediment Dry sediment was spiked with the test substance and mixed into wet sediment.
RESULTS	10 days LC50 = 168 mg/kg dry weight sediment
Remarks – Results	The response of the test substance on *Corophium volutator* followed a sigmoid curve. It appears that the test result is expressed in terms of the initial concentration in the dry sediment, before mixing with the wet sediment. The transformed LC50 is reported as 2.2 mg/kg.
CONCLUSION	The notified chemical is relatively toxic to *Corophium volutator*.
TEST FACILITY	M-I Production Chemicals (2003d)

C.2.7. Acute toxicity to the marine copepod *Acartia tonsa*

TEST SUBSTANCE	Notified chemical
METHOD	Thompson R.S. 1990. UK ISO proposal to TC147/SC5/WG/2. Water quality-determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea).
Remarks – Method	Reference substance: 3,5-dichlorophenol 97% Species: *Acartia tonsa* (supplier: Marinbiologisk laboratorium, Helsingør

	– Denmark, at the start of the test, the animals were ca. 25 days old) Media: Natural seawater (0.45 µm filtered followed by an rapid heating to app. 80ºC and stored. Collected Alvøen ferjekai, Bergen depth = 3, salinity = 32‰) Replicates: four of each test concentration, four of control and reference (5 animals in each vessels). Concentration (nominal): 0, 0.5, 0.8, 1.6, 3.3 and 5 mg/L. The preparation of the test media is not described in the test report.
RESULTS	48 hours LC50 = 0.9 mg/l
Remarks – Results	The test is acceptable as 32% mortality was observed in the reference substance (between 20-80%) and a low mortality (0%) was shown in the control group.
CONCLUSION	The notified chemical is very toxic to *Acartia tonsa*.
TEST FACILITY	M-I Production Chemicals (2004e)

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